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# CONTENTS

# Effects of Ten Dietary Management Programs on Performance of Silkworm Hybrids

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**Abstract:** The purpose of this experiment was to investigation on different delay times in starting of feeding in different instars, and their effects on the performance and uniformity of silkworm larvae. Silkworm egg production stages, egg washing, disinfecting, maintenance of silkworm eggs, microscopic tests in order to removing of contaminated samples against pebrin pathogen, first to fifth larval instars rearing, cocoon production framework and cocoon recording was conducted based on standard guidelines. Ten dietary management programs were used as ten treatments. Performance records analyzed using generalized linear models procedure. All the measured indices was compared between different treatments based completely randomized design model. From obtained results, it has showed that among studied methods, the highest level of best cocoon number belonged to 5th treatment (80.75), and 3rd treatment (44.70) remained at lower level than other methods ($P>0.05$). The highest level of best cocoon weight belonged to 5th treatment (80.75 gr), and 3rd treatment (44.70 gr) remained at lower level than other methods ($P>0.05$). The highest level of larva weight (5th day of 5th instar) belonged to 3rd treatment (3.48 days), and 9th treatment (3.24 days) remained at lower level than other methods ($P<0.05$). Among studied methods, the highest level of female cocoon weight belonged to 1st treatment (2.13 gr), and 2nd treatment (1.90 gr) remained at lower level than other methods ($P<0.05$). The highest female cocoon shell weight belonged to 1st treatment (0.42 gr), and 2nd treatment (0.36 gr) remained at lower level than other methods ($P<0.05$). Among studied methods, the highest level of female cocoon shell percentage belonged to 9th treatment (24.18%), and 2nd treatment (18.94%) remained at lower level than other methods ($P>0.05$). The highest level of male cocoon weight belonged to 6th treatment (1.69 gr), and 2nd treatment (1.56 gr) remained at lower level than other methods ($P<0.05$).
[Mohammad Molaei, Moeinoddin Mavvajpour, Afsane Merat, Alireza Seidavi. Effects of Ten Dietary Management Programs on Performance of Silkworm Hybrids. Life Science Journal. 2011;8(2):465-475] (ISSN:1097-8135). http://www.lifesciencesite.com.

**Keywords:** Silkworm; instar; feeding; schedule, performance; hybrid

## 1. Introduction

Silkworm rearing has huge history in world. Based on the obtained evidences, this activity seems to started and originated from China and has been transferred to other countries. The valuable product of this job natural silk, according to its structural characteristics, despite lower production than other natural and synthetic fibers, causing many cultural, social, and economical exchanges, in the history has provided. Accordingly, addressing the status of this industry can help us to utilize and develop this valuable industry in domestic and foreign relations in the future (Bizhannia and Seidavi, 2004).

Silk formed based on natural protein fibers. These very fine fibers are long and delicate. Yarn length extracted from a cocoon is around 900-1300 meters and it having between 15-20 microns diameter. These fibers have large natural brightness. Silk yarn strength is between 3.6-4.4 gram/denier and have high elasticity capacity. Silk fibers absorb moisture well. The excellent properties of the silk has caused people in different countries from thousands of years ago rearing silkworm an thus a lot of researchers and scientists to study and develop this industry for preparing silk yarn, silk fabrics and silk dyeing economically and scientifically (Seidavi et al., 2006a).

One of the major problems of sericulture and silk cocoon production is the lack of uniformity when rearing silkworm larvae, hence there are small and large larvae simultaneously, and larvae have different instars together. Since there are different nutritional requirements based on larva instars, the incidence of this matter causing larvae cannot supply all their nutritional requirements and hence larvae will be uniform. In addition, since smaller larvae are more sensitive against pathogens, rapid spread of pathogens in the environment will damage large and small larvae (Motahari et al., 2008a; Motahari et al., 2008b; Seidavi et al., 2006b). This set of factors ultimately produced a sharp reduction in the silk production. The purpose of this experiment was to investigation on different delay times in starting of feeding in different instars, and their effects on the performance and uniformity of silkworm larvae.

## 2. Material and Methods

The experiment was conducted during 2010-2011. The larvae hatched and reared based on ESCAP (1993). Silkworm egg production stages, egg washing, disinfecting, maintenance of silkworm eggs, microscopic tests in order to removing of contaminated samples against pebrin pathogen, first to fifth larval instars rearing, cocoon production framework and cocoon recording was conducted based on standard guidelines and protocols, especially ESCAP (1993). Commercial hybrid silkworm egg was prepared from Iran Silkworm Research Center (ISRC).

Ten treatments were treatment 1 (control): start time of feeding for each of the first to fifth larvae instars were standard and without delay (immediately after larvae molting and readiness for feeding); treatment 2: start time of feeding for each of the first, second and third larvae instars were 24 hours late and delay (24 hours after larvae molting and readiness for feeding) and start time of feeding for each of the fourth and fifth larvae instars were 36 hours late (36 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 3: start time of feeding for first larvae instar were 24 hours late and delay (24 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 4: start time of feeding for second larvae instar were 24 hours late and delay (24 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 5: start time of feeding for third larvae instar were 24 hours late and delay (24 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 6: start time of feeding for fourth larvae instar were 36 hours late and delay (36 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 7: start time of feeding for fifth larvae instar were 36 hours late and delay (36 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 8: start time of feeding for each of the first, second, and third larvae instars were 24 hours late and delay (24 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; treatment 9: start time of feeding for each of the first, second, and third larvae instars were 12 hours late and delay (12 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach; and treatment 10: start time of feeding for each of the fourth, and fifth larvae instars were 36 hours late and delay (24 hours after larvae molting and readiness for feeding) in comparison with to the conventional and standard approach.

Fifty two studied traits included best cocoon number, best cocoon alive pupae number, best cocoon alive pupae percentage, best cocoon dead pupae number, middle cocoon number, middle cocoon alive pupae number, middle cocoon alive pupae percentage, low cocoon number, low cocoon alive pupae number, low cocoon dead pupae number, double cocoon number, double cocoon alive pupae number, double cocoon alive pupae percentage, double cocoon dead pupae number, cocoon number per one liter, cocoon weight per one liter, best cocoon weight, middle cocoon weight, low cocoon weight, double cocoon weight, 1st larval duration, 1st feeding duration, 1st molting duration, 2nd larval duration, 2nd feeding duration, 2nd molting duration, 3rd larval duration, 3rd feeding duration, 3rd molting duration, 4th larval duration, 4th feeding duration, 4th molting duration, 5th larval duration, 5th feeding duration, mounting duration, young larval duration, grown (mature) larvae duration, larva weight (5th day of 5th instar), 10 larvae weight (5th day of 5th instar), female cocoon weight, female cocoon shell weight, female cocoon shell percentage, coefficient of variations for female cocoon weight, coefficient of variations for female cocoon shell weight, coefficient of variations for female cocoon shell percentage, male cocoon weight, male cocoon shell weight, male cocoon shell percentage, coefficient of variations for male cocoon weight, coefficient of variations for male cocoon shell weight, coefficient of variations for male cocoon shell percentage.

It was used rice straw as cocoon making position (framework) in cocoon spinning stage separately for each replication. After completing of the pupa development (7 days after onset spinning of cocoons), it was collected total produced cocoons. Then, it was sorted and classified all cocoons based on appearance, hardness softness, and cleanliness levels of cortex and outer cortex into four categories including good, moderate, low and double cocoons. Health situation of the cocoon pupae and the disease and mortality of pupae have been studied and it was calculated the percentage of pupa vitality for each replication separately. Also good (male and female) and double cocoon weight in each replication was recorded. All recording steps was performed on the eighth day after the onset of cocoon spinning. Production records analyzed by statistical software SPSS (1999) using generalized linear models procedure (GLM), and after ensuring of data normality, the averages was compared using Tukey test. All the measured indices was compared between

different treatments based completely randomized design model (CRD).

## 3. Results

Obtained results are summarized in Tables 1-9.

**Best cocoon number**

From obtained results, it has showed that amount of best cocoon number in ten studied methods included between 44.70-80.75. Among studied methods, the highest level of best cocoon number belonged to 5th treatment (80.75), and 3rd treatment (44.70) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Best cocoon alive pupae number**

From obtained results, it is showed that amount of best cocoon alive pupae number in ten studied methods included between 71.25-80.25. Among studied methods, the highest level of best cocoon alive pupae number belonged to 5th treatment (80.25), and 1st treatment (71.25) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Best cocoon alive pupae percentage**

From obtained results, it is showed that amount of best cocoon alive pupae percentage in ten studied methods included between 95.42-99.40%. Among studied methods, the highest level of best cocoon alive pupae percentage belonged to 5th treatment (99.40%), and 1st treatment (95.42%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P>0.05).

**Best cocoon dead pupae number**

From obtained results, it is showed that amount of best cocoon dead pupae number in ten studied methods included between 0.50-3.50. Among studied methods, the highest level of best cocoon dead pupae number belonged to 1st treatment (3.50), and 5th treatment (0.50) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Middle cocoon number**

From obtained results, it is showed that amount of middle cocoon number in ten studied methods included between 6.25-11.00. Among studied methods, the highest level of middle cocoon number belonged to 6th treatment (11.00), and 3rd treatment (6.25) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Middle cocoon alive pupae number**

From obtained results, it is showed that amount of middle cocoon alive pupae number in ten studied methods included between 4.00-10.00. Among studied methods, the highest level of middle cocoon alive pupae number belonged to 6th treatment (10.00), and 3rd treatment (4.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Middle cocoon alive pupae percentage**

From obtained results, it is showed that amount of middle cocoon alive pupae percentage in ten studied methods included between 65.00-97.50%. Among studied methods, the highest level of middle cocoon alive pupae percentage belonged to 2nd treatment (97.50%), and 3rd treatment (65.00%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Low cocoon number**

From obtained results, it is showed that amount of low cocoon number in ten studied methods included between 0.00-1.50. Among studied methods, the highest level of low cocoon number belonged to 4th treatment (1.50), and 1st and 10th treatments (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Low cocoon alive pupae number**

From obtained results, it is showed that amount of low cocoon alive pupae number in ten studied methods included between 0.00-0.50. Among studied methods, the highest level of low cocoon alive pupae number belonged to 6th treatment (0.50), and most of treatments (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Low cocoon dead pupae number**

From obtained results, it is showed that amount of low cocoon dead pupae number in ten studied methods included between 0.00-1.25. Among studied methods, the highest level of low cocoon dead pupae number belonged to 4th treatment (1.25), and 1st and 10th treatment (0.00) remained at lower level than other methods. Other methods were

between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Double cocoon number**

From obtained results, it is showed that amount of double cocoon number in ten studied methods included between 0.75-2.50. Among studied methods, the highest level of double cocoon number belonged to 5th and 7th treatments (2.50), and 3rd treatment (0.75) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Double cocoon alive pupae number**

From obtained results, it is showed that amount of double cocoon alive pupae number in ten studied methods included between 1.50-4.75. Among studied methods, the highest level of double cocoon alive pupae number belonged to 7th treatments (4.75), and 3rd and 10th treatments (1.50) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Double cocoon alive pupae percentage**

From obtained results, it is showed that amount of double cocoon alive pupae percentage in ten studied methods included between 50.00-100.00%. Among studied methods, the highest level of double cocoon alive pupae percentage belonged to 2nd and 10th treatment (100.00%), and 3rd treatment (50.00%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Double cocoon dead pupae number**

From obtained results, it is showed that amount of double cocoon dead pupae number in ten studied methods included between 0.00-0.50. Among studied methods, the highest level of double cocoon dead pupae number belonged to some treatments (0.50), and some other treatments (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Cocoon number per one liter**

From obtained results, it is showed that amount of cocoon number per one liter in ten studied methods included between 103.00-123.00. Among studied methods, the highest level of cocoon number per one liter belonged to 7th treatment (123.00), and 3rd treatment (103.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Cocoon weight per one liter**

From obtained results, it is showed that amount of cocoon weight per one liter in ten studied methods included between 190.64-216.75 gr. Among studied methods, the highest level of cocoon weight per one liter belonged to 9th treatment (216.75 gr), and 3rd treatment (190.64 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Best cocoon weigh**

From obtained results, it is showed that amount of best cocoon weight in ten studied methods included between 44.70-80.75 gr. Among studied methods, the highest level of best cocoon weight belonged to 5th treatment (80.75 gr), and 3rd treatment (44.70 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Middle cocoon weight**

From obtained results, it is showed that amount of middle cocoon weight in ten studied methods included between 82.57-146.37 gr. Among studied methods, the highest level of middle cocoon weight belonged to 5th treatment (146.37 gr), and 3rd treatment (82.57 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Low cocoon weight**

From obtained results, it is showed that amount of low cocoon weight in ten studied methods included between 10.28-18.84 gr. Among studied methods, the highest level of low cocoon weight belonged to 6th treatment (18.84 gr), and 3rd treatment (10.28 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Double cocoon weight**

From obtained results, it is showed that amount of double cocoon weight in ten studied methods included between 0.00-2.18 gr. Among studied methods, the highest level of double cocoon weight belonged to 4th treatment (2.18 gr), and 10th treatment (0.00 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between

studied methods for this trait were not significant (P>0.05).

**1st larval duration**

From obtained results, it is showed that amount of 1st larval duration in ten studied methods included between 4.07-5.08 gr. Among studied methods, the highest level of 1st larval duration belonged to 8th treatment (5.08 gr), and some other treatments (4.07 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**1st feeding duration**

From obtained results, it is showed that amount of 1st feeding duration in ten studied methods included between 3.07-3.23 days. Among studied methods, the highest level of 1st feeding duration belonged to 2nd treatment (3.23 days), and 4th treatment (3.07 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**1st molting duration**

From obtained results, it is showed that amount of 1st molting duration in ten studied methods included between 1.00-2.00 days. Among studied methods, the highest level of 1st molting duration belonged to 4th treatment (2.00 days), and most of treatments (1.00 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**2nd larval duration**

From obtained results, it is showed that amount of 2nd larval duration in ten studied methods included between 2.10-3.05 days. Among studied methods, the highest level of 2nd larval duration belonged to 3rd treatment (3.05 days), and 10th treatment (2.10 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**2nd feeding duration**

From obtained results, it is showed that amount of 2nd feeding duration in ten studied methods included between 2.10-3.05 days. Among studied methods, the highest level of 2nd molting duration belonged to 3rd treatment (3.05 days), and 9th treatment (2.10 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**2nd molting duration**

From obtained results, it is showed that amount of 2nd molting duration in ten studied methods included between 0.18-2.00 days. Among studied methods, the highest level of 2nd molting duration belonged to 8th treatment (2.00 days), and 4th treatment (0.18 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**3rd larval duration**

From obtained results, it is showed that amount of 3rd larval duration in ten studied methods included between 4.01-5.01 days. Among studied methods, the highest level of 3rd larval duration belonged to 2nd treatment (5.01 days), and 8th treatment (4.01 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**3rd feeding duration**

From obtained results, it is showed that amount of 3rd feeding duration in ten studied methods included between 3.01-4.01 days. Among studied methods, the highest level of 3rd feeding duration belonged to 4th treatment (4.01 days), and 8th treatment (3.01 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**3rd molting duration**

From obtained results, it is showed that amount of 3rd molting duration in ten studied methods included between 44.70-2.00 days. Among studied methods, the highest level of 3rd molting duration belonged to 10th treatment (2.00 days), and 1st treatment (1.00 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**4th larval duration**

From obtained results, it is showed that amount of 4th larval duration in ten studied methods included between 5.10-6.17 days. Among studied methods, the highest level of 4th larval duration belonged to 2nd treatment (6.17 days), and 4th treatment (5.10 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences

between studied methods for this trait were significant (P<0.05).

**4th feeding duration**

From obtained results, it is showed that amount of 4th feeding duration in ten studied methods included between 4.00-4.01 days. Among studied methods, the highest level of 4th feeding duration belonged to most of treatments (4.01 days), and 7th treatment (4.00 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**4th molting duration**

From obtained results, it is showed that amount of 4th molting duration in ten studied methods included between 1.09-2.16 days. Among studied methods, the highest level of 4th molting duration belonged to 2nd treatment (2.16 days), and 4th treatment (1.09 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**5th larval duration**

From obtained results, it is showed that amount of v in ten studied methods included between 44.70-80.75 days. Among studied methods, the highest level of 5th larval duration belonged to 5th treatment (80.75 days), and 3rd treatment (44.70 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**5th feeding duration**

From obtained results, it is showed that amount of 5th feeding duration in ten studied methods included between 7.00-7.23 days. Among studied methods, the highest level of 5th feeding duration belonged to 4th treatment (7.23 days), and 7th treatment (7.00 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Mounting duration**

From obtained results, it is showed that amount of mounting duration in ten studied methods included between 0.08-0.21 days. Among studied methods, the highest level of mounting duration belonged to some of treatments (0.21 days), and 1st treatment (0.08 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Young larval duration**

From obtained results, it is showed that amount of young larval duration in ten studied methods included between 12.10-15.04 days. Among studied methods, the highest level of young larval duration belonged to 2nd treatment (15.04 days), and 1st treatment (12.10 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Grown (mature) larvae duration**

From obtained results, it is showed that amount of grown (mature) larvae duration in ten studied methods included between 12.24-13.23 days. Among studied methods, the highest level of grown (mature) larvae duration belonged to 5th treatment (12.24 days), and some treatments (13.23 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Larva weight (5th day of 5th instar)**

From obtained results, it is showed that amount of larva weight (5th day of 5th instar) in ten studied methods included between 3.24-3.48 days. Among studied methods, the highest level of larva weight (5th day of 5th instar) belonged to 3rd treatment (3.48 days), and 9th treatment (3.24 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**10 larvae weight (5th day of 5th instar)**

From obtained results, it is showed that amount of 10 larvae weight (5th day of 5th instar) in ten studied methods included between 3.24-3.48 gr. Among studied methods, the highest level of larva weight (5th day of 5th instar) belonged to 3rd treatment (3.48 gr), and 9th treatment (3.24 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Female cocoon weight**

From obtained results, it is showed that amount of female cocoon weight in ten studied methods included between 1.90-2.13 gr. Among studied methods, the highest level of female cocoon weight belonged to 1st treatment (2.13 gr), and 2nd treatment (1.90 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Female cocoon shell weight**

From obtained results, it is showed that amount of female cocoon shell weight in ten studied methods included between 0.36-0.42 gr. Among studied methods, the highest level of female cocoon shell weight belonged to 1st treatment (0.42 gr), and 2nd treatment (0.36 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Female cocoon shell percentage**

From obtained results, it is showed that amount of female cocoon shell percentage in ten studied methods included between 18.94-24.18%. Among studied methods, the highest level of female cocoon shell percentage belonged to 9th treatment (24.18%), and 2nd treatment (18.94%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Coefficient of variations for female cocoon weight**

From obtained results, it is showed that amount of coefficient of variations for female cocoon weight in ten studied methods included between 7.85-11.98%. Among studied methods, the highest level of coefficient of variations for female cocoon weight belonged to 9th treatment (11.98%), and 4th treatment (7.85%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Coefficient of variations for female cocoon shell weight**

From obtained results, it is showed that amount of coefficient of variations for female cocoon shell weight in ten studied methods included between 8.85-21.22%. Among studied methods, the highest level of coefficient of variations for female cocoon shell weight belonged to 10th treatment (21.22%), and 6th treatment (8.85%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Coefficient of variations for female cocoon shell percentage**

From obtained results, it is showed that amount of coefficient of variations for female cocoon shell percentage in ten studied methods included between 5.65-60.75%. Among studied methods, the highest level of coefficient of variations for female cocoon shell percentage belonged to 9th treatment (60.75%), and 2nd treatment (5.65%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Male cocoon weight**

From obtained results, it is showed that amount of male cocoon weight in ten studied methods included between 1.56-1.69 gr. Among studied methods, the highest level of male cocoon weight belonged to 6th treatment (1.69 gr), and 2nd treatment (1.56 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Male cocoon shell weight**

From obtained results, it is showed that amount of male cocoon shell weight in ten studied methods included between 0.35-0.40 gr. Among studied methods, the highest level of male cocoon shell weight belonged to some treatments (0.40 gr), and 2nd treatment (0.35 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

**Male Cocoon shell percentage**

From obtained results, it is showed that amount of male Cocoon shell percentage in ten studied methods included between 22.75-24.41%. Among studied methods, the highest level of male Cocoon shell percentage belonged to 2nd treatment (24.41%), and 7th treatment (22.75%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Coefficient of variations for male cocoon weight**

From obtained results, it is showed that amount of coefficient of variations for male cocoon weight in ten studied methods included between 7.15-10.29%. Among studied methods, the highest level of coefficient of variations for male cocoon weight belonged to 7th treatment (10.29%), and 2nd treatment (7.15%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant (P>0.05).

**Table 1**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Best Cocoon Number | Best Cocoon Alive Pupae Number | Best Cocoon Alive Pupae Percentage, % | Best Cocoon Dead Pupae Number | Middle Cocoon Number | Middle Cocoon Alive Pupae Number |
| 1 | 74.75±3.30$^{a}$ | 71.25±2.59$^{a}$ | 95.42±0.95$^{b}$ | 3.50±0.87$^{a}$ | 9.25±1.55$^{a}$ | 7.75±1.44$^{ab}$ |
| 2 | 76.00±4.60$^{a}$ | 74.00±4.10$^{a}$ | 97.46±0.77$^{ab}$ | 2.00±0.71$^{ab}$ | 9.25±1.65$^{a}$ | 9.00±1.63$^{a}$ |
| 3 | 44.75±4.82$^{b}$ | 43.50±4.35$^{b}$ | 97.41±1.49$^{ab}$ | 1.25±0.75$^{ab}$ | 6.25±0.48$^{a}$ | 4.00±0.71$^{b}$ |
| 4 | 74.25±3.73$^{a}$ | 73.25±4.13$^{a}$ | 98.56±0.63$^{ab}$ | 1.00±0.41$^{ab}$ | 10.75±1.70$^{a}$ | 8.75±1.44$^{ab}$ |
| 5 | 80.75±1.44$^{a}$ | 80.25±1.18$^{a}$ | 99.40±0.35$^{b}$ | 0.50±0.29$^{b}$ | 9.50±2.33$^{a}$ | 7.75±2.32$^{ab}$ |
| 6 | 76.50±4.97$^{a}$ | 73.75±4.50$^{a}$ | 96.51±1.02$^{ab}$ | 2.75±0.85$^{ab}$ | 11.00±1.73$^{a}$ | 10.00±0.82$^{ab}$ |
| 7 | 78.50±2.40$^{a}$ | 75.50±1.94$^{a}$ | 96.24±1.16$^{ab}$ | 3.00±1.00$^{ab}$ | 7.50±0.87$^{a}$ | 6.00±0.41$^{ab}$ |
| 8 | 75.50±2.50$^{a}$ | 74.25±2.95$^{a}$ | 98.28±0.93$^{ab}$ | 1.25±0.63$^{ab}$ | 8.25±1.38$^{a}$ | 7.50±1.76$^{ab}$ |
| 9 | 76.00±4.26$^{a}$ | 73.50±3.88$^{a}$ | 96.77±0.58$^{ab}$ | 2.50±0.50$^{ab}$ | 8.00±0.58$^{a}$ | 7.00±0.41$^{ab}$ |
| 10 | 70.50±5.17$^{a}$ | 68.25±4.73$^{a}$ | 97.01±2.20$^{ab}$ | 2.25±1.65$^{ab}$ | 10.50±3.23$^{a}$ | 9.75±3.64$^{a}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 2**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Middle Cocoon Alive Pupae Percentage, % | Middle Cocoon Dead Pupae Number | Low Cocoon Number | Low Cocoon Alive Pupae Number | Low Cocoon Dead Pupae Number | Double Cocoon Number |
| 1 | 83.32±4.32$^{ab}$ | 1.50±0.50$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 1.25±0.47$^{a}$ |
| 2 | 97.50±2.50$^{a}$ | 0.25±0.25$^{a}$ | 0.75±0.48$^{ab}$ | 0.00±0.00$^{a}$ | 0.75±0.48$^{a}$ | 1.50±0.28$^{a}$ |
| 3 | 65.00±2.25$^{b}$ | 2.25±0.85$^{a}$ | 0.75±0.25$^{ab}$ | 0.00±0.00$^{a}$ | 0.75±0.25$^{a}$ | 0.75±0.47$^{a}$ |
| 4 | 83.13±8.81$^{ab}$ | 2.00±1.08$^{a}$ | 1.50±0.65$^{a}$ | 0.25±0.25$^{a}$ | 1.25±0.48$^{a}$ | 1.50±0.50$^{a}$ |
| 5 | 77.72±13.33$^{ab}$ | 1.75±0.75$^{a}$ | 1.00±0.41$^{ab}$ | 0.00±0.00$^{a}$ | 1.00±0.41$^{a}$ | 2.50±0.86$^{a}$ |
| 6 | 93.75±6.25$^{ab}$ | 1.00±1.00$^{a}$ | 0.75±0.47$^{ab}$ | 0.50±0.50$^{a}$ | 0.25±0.25$^{a}$ | 2.00±1.08$^{a}$ |
| 7 | 81.79±7.03$^{ab}$ | 1.50±0.65$^{a}$ | 1.00±0.71$^{ab}$ | 0.00±0.00$^{a}$ | 1.00±0.71$^{a}$ | 2.50±0.50$^{a}$ |
| 8 | 89.29±10.71$^{ab}$ | 0.75±0.75$^{a}$ | 0.50±0.50$^{ab}$ | 0.00±0.00$^{a}$ | 0.50±0.50$^{a}$ | 1.75±0.25$^{a}$ |
| 9 | 88.10±4.60$^{ab}$ | 1.00±0.41$^{a}$ | 1.25±0.25$^{ab}$ | 0.00±0.00$^{a}$ | 1.25±0.25$^{a}$ | 1.50±0.64$^{a}$ |
| 10 | 87.50±12.50$^{ab}$ | 0.75±0.75$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 1.00±0.640$^{a}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 3**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Double Cocoon Alive Pupae Number | Double Cocoon Alive Pupae Percentage | Double Cocoon Dead Pupae Number | Cocoon Number per one Litr | Cocoon Weight per one Litr | Best Cocoon Weight |
| | - | % | - | - | gr | gr |
| 1 | 2.50±0.96$^{a}$ | 75.00±25.00$^{a}$ | 0.00±0.00$^{a}$ | 113.50±1.71$^{ab}$ | 211.36±3.86$^{a}$ | 142.08±4.88$^{a}$ |
| 2 | 3.00±0.58$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 121.00±2.65$^{ab}$ | 205.14±2.82$^{a}$ | 129.10±8.52$^{a}$ |
| 3 | 1.50±0.96$^{a}$ | 50.00±8.87$^{a}$ | 0.00±0.00$^{a}$ | 103.00±4.43$^{c}$ | 190.64±8.35$^{b}$ | 82.57±9.20$^{b}$ |
| 4 | 2.50±0.87$^{a}$ | 62.50±21.65$^{a}$ | 0.50±0.29$^{a}$ | 115.50±1.50$^{ab}$ | 209.28±2.45$^{a}$ | 134.07±5.95$^{a}$ |
| 5 | 4.50±1.55$^{a}$ | 67.71±22.85$^{a}$ | 0.50±0.29$^{a}$ | 118.25±0.63$^{ab}$ | 214.17±2.14$^{a}$ | 146.37±1.81$^{a}$ |
| 6 | 3.75±2.25$^{a}$ | 62.50±23.94$^{a}$ | 0.25±0.25$^{a}$ | 115.50±0.96$^{ab}$ | 210.62±3.37$^{a}$ | 139.70±8.46$^{a}$ |
| 7 | 4.75±1.11$^{a}$ | 93.75±6.25$^{a}$ | 0.25±0.25$^{a}$ | 123.00±1.91$^{a}$ | 212.79±2.52$^{a}$ | 135.81±4.15$^{a}$ |
| 8 | 3.50±0.50$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 117.502.06$^{ab}$ | 204.86±3.46$^{a}$ | 131.79±4.17$^{a}$ |
| 9 | 2.75±1.38$^{a}$ | 62.50±3.94$^{a}$ | 0.25±0.25$^{a}$ | 119.50±2.87$^{ab}$ | 216.75±4.37$^{a}$ | 135.78±6.61$^{a}$ |
| 10 | 1.50±0.65$^{a}$ | 56.25±21.35$^{a}$ | 0.50±0.29$^{a}$ | 117.50±2.63$^{ab}$ | 209.97±4.92$^{a}$ | 124.32±8.89$^{a}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 4**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Middle Cocoon Weight | Low Cocoon Weight | Double Cocoon Weight | 1st Larval Duration | 1st Feeding Duration | 1st Molting Duration |
| | gr | gr | gr | day | day | day |
| 1 | 14.85±3.25$^{a}$ | 0.00±0.00$^{a}$ | 4.74±1.77$^{a}$ | 4.08±0.01$^{e}$ | 3.08±0.01$^{c}$ | 1.00±0.00$^{c}$ |
| 2 | 13.75±2.56$^{a}$ | 1.25±0.79$^{a}$ | 5.00±1.10$^{a}$ | 5.08±0.00$^{a}$ | 3.23±0.00$^{a}$ | 1.09±0.00$^{b}$ |
| 3 | 10.28±0.78$^{b}$ | 1.33±0.47$^{a}$ | 2.85±1.72$^{a}$ | 4.23±0.00$^{d}$ | 3.23±0.00$^{a}$ | 1.00±0.00$^{c}$ |
| 4 | 18.40±2.62$^{a}$ | 2.18±1.21$^{a}$ | 5.54±1.85$^{a}$ | 5.07±0.00$^{b}$ | 3.07±0.00$^{c}$ | 2.00±0.01$^{a}$ |
| 5 | 15.00±3.78$^{a}$ | 2.04±0.86$^{a}$ | 9.31±3.20$^{a}$ | 4.07±0.00$^{e}$ | 3.07±0.00$^{c}$ | 1.00±0.00$^{c}$ |
| 6 | 18.84±2.65$^{a}$ | 1.50±0.91$^{a}$ | 6.95±3.80$^{a}$ | 4.07±0.00$^{e}$ | 3.07±0.00$^{c}$ | 1.00±0.00$^{c}$ |
| 7 | 12.49±1.41$^{a}$ | 1.69±1.15$^{a}$ | 8.91±2.08$^{a}$ | 4.07±0.00$^{e}$ | 3.07±0.00$^{c}$ | 1.00±0.00$^{c}$ |
| 8 | 13.12±1.90$^{a}$ | 0.76±0.76$^{a}$ | 6.13±0.91$^{a}$ | 5.08±0.00$^{a}$ | 3.23±0.00$^{a}$ | 1.09±0.00$^{b}$ |
| 9 | 14.34±1.73$^{a}$ | 2.07±0.62$^{a}$ | 5.23±2.30$^{a}$ | 4.15±0.00$^{d}$ | 3.15±0.00$^{b}$ | 1.00±0.00$^{c}$ |
| 10 | 16.70±5.17$^{a}$ | 0.00±0.00$^{a}$ | 3.54±1.29$^{a}$ | 4.07±0.00$^{e}$ | 3.07±0.00$^{c}$ | 1.00±0.00$^{c}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 5**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | 2nd Larval Duration | 2nd Feeding Duration | 2nd Molting Duration | 3rd Larval Duration | 3rd Feeding Duration | 3rd Molting Duration |
| | day | day | day | day | day | day |
| 1 | 3.16±0.01$^{c}$ | 2.21±0.01$^{c}$ | 0.19±0.00$^{d}$ | 4.10±0.01$^{bc}$ | 3.10±0.01$^{b}$ | 1.00±0.00$^{b}$ |
| 2 | 5.1±0.01$^{a}$ | 3.01±0.01$^{b}$ | 2.00±0.01$^{a}$ | 5.01±0.01$^{a}$ | 3.01±0.01$^{c}$ | 2.00±0.01$^{a}$ |
| 3 | 4.1±0.01$^{b}$ | 3.05±0.01$^{a}$ | 0.20±0.00$^{c}$ | 4.10±0.01$^{bc}$ | 3.10±0.01$^{b}$ | 1.00±0.00$^{b}$ |
| 4 | 3.16±0.01$^{c}$ | 2.22±0.01$^{c}$ | 0.18±0.00$^{e}$ | 5.01±0.01$^{a}$ | 4.01±0.01$^{a}$ | 1.00±0.00$^{b}$ |
| 5 | 4.16±0.01$^{b}$ | 2.21±0.01$^{c}$ | 0.19±0.00$^{d}$ | 4.10±0.01$^{bc}$ | 3.10±0.01$^{b}$ | 1.00±0.00$^{b}$ |
| 6 | 3.91±0.25$^{b}$ | 2.21±0.01$^{c}$ | 0.19±0.00$^{d}$ | 4.35±0.25$^{b}$ | 3.10±0.01$^{b}$ | 1.25±0.25$^{b}$ |
| 7 | 3.15±0.00$^{c}$ | 2.20±0.00$^{c}$ | 0.19±0.00$^{d}$ | 4.09±0.00$^{c}$ | 3.09±0.00$^{b}$ | 1.00±0.00$^{b}$ |
| 8 | 5.01±0.01$^{a}$ | 3.01±0.01$^{b}$ | 2.00±0.01$^{a}$ | 4.01±0.01$^{c}$ | 3.01±0.01$^{c}$ | 1.00±0.00$^{b}$ |
| 9 | 4.01±0.01$^{b}$ | 2.10±0.01$^{d}$ | 1.15±0.00$^{b}$ | 4.10±0.01$^{bc}$ | 3.10±0.01$^{b}$ | 1.00±0.00$^{b}$ |
| 10 | 3.16±0.01$^{c}$ | 2.21±0.01$^{c}$ | 0.19±0.00$^{d}$ | 5.10±0.01$^{a}$ | 3.10±0.01$^{b}$ | 2.00±0.01$^{a}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 6**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | 4th Larval Duration | 4th Feeding Duration | 4th Molting Duration | 5th Larval Duration | 5th Feeding Duration | Mounting Duration |
| | day | day | day | day | day | day |
| 1 | 5.16±0.01$^{b}$ | 4.01±0.01$^{a}$ | 1.15±0.00$^{b}$ | 7.11±0.02$^{b}$ | 7.03±0.02$^{a}$ | 0.08±0.00$^{c}$ |
| 2 | 6.17±0.02$^{a}$ | 4.01±0.01$^{a}$ | 2.16±0.01$^{a}$ | 7.02±0.02$^{c}$ | 6.11±0.02$^{bc}$ | 0.15±0.00$^{b}$ |
| 3 | 5.16±0.01$^{b}$ | 4.01±0.01$^{a}$ | 1.15±0.00$^{b}$ | 7.08±0.02$^{b}$ | 6.11±0.02$^{bc}$ | 0.21±0.00$^{a}$ |
| 4 | 5.10±0.01$^{c}$ | 4.01±0.01$^{a}$ | 1.09±0.00$^{c}$ | 7.23±0.02$^{a}$ | 7.02±0.02$^{a}$ | 0.21±0.00$^{a}$ |
| 5 | 5.16±0.01$^{b}$ | 4.01±0.01$^{a}$ | 1.15±0.00$^{b}$ | 7.08±0.02$^{b}$ | 6.11±0.02$^{bc}$ | 0.21±0.00$^{a}$ |
| 6 | 5.16±0.01$^{a}$ | 4.01±0.01$^{a}$ | 1.12±0.04$^{bc}$ | 7.08±0.02$^{b}$ | 6.34±0.23$^{b}$ | 0.18±0.03$^{ab}$ |
| 7 | 6.15±0.00$^{a}$ | 4.00±0.00$^{a}$ | 2.15±0.00$^{a}$ | 7.00±0.00$^{c}$ | 6.09±0.00$^{bc}$ | 0.15±0.00$^{b}$ |
| 8 | 5.16±0.01$^{b}$ | 4.01±0.01$^{a}$ | 1.15±0.00$^{b}$ | 7.08±0.02$^{b}$ | 6.11±0.02$^{bc}$ | 0.21±0.00$^{a}$ |
| 9 | 5.16±0.01$^{b}$ | 4.01±0.01$^{a}$ | 1.15±0.00$^{a}$ | 7.11±0.02$^{b}$ | 7.03±0.02$^{a}$ | 0.08±0.00$^{c}$ |
| 10 | 6.17±0.02$^{a}$ | 4.01±0.01$^{a}$ | 2.16±0.01$^{b}$ | 7.07±0.02$^{b}$ | 6.11±0.02$^{bc}$ | 0.20±0.00$^{a}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 7**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Young Larval Duration | Grown (Mature) Larvae Duration | Larva Weight (5th Day of 5th Instar) | 10 Larvae Weight (5th Day of 5th Instar) | Female Cocoon Weight | Female Cocoon Shell Weight |
| | day | day | gr | gr | gr | gr |
| 1 | 12.10±0.03$^{e}$ | 13.03±0.03$^{a}$ | 3.39±0.06$^{ab}$ | 3.39±0.06$^{ab}$ | 2.13±0.01$^{a}$ | 0.42±0.00$^{a}$ |
| 2 | 15.04±0.04$^{a}$ | 13.18±0.03$^{a}$ | 3.36±0.06$^{ab}$ | 3.36±0.06$^{ab}$ | 1.90±0.02$^{e}$ | 0.36±0.00$^{e}$ |
| 3 | 13.11±0.03$^{e}$ | 12.24±0.03$^{c}$ | 3.48±0.07$^{a}$ | 3.48±0.07$^{a}$ | 2.07±0.04$^{abc}$ | 0.41±0.01$^{abc}$ |
| 4 | 13.25±0.03$^{c}$ | 13.09±0.03$^{a}$ | 3.27±0.03 | 3.27±0.03$^{ab}$ | 2.04±0.02$^{cde}$ | 0.41±0.00$^{abc}$ |
| 5 | 13.10±0.03$^{d}$ | 12.24±0.03$^{c}$ | 3.37±0.14$^{ab}$ | 3.37±0.14$^{ab}$ | 2.00±0.03$^{cde}$ | 0.39±0.00$^{cd}$ |
| 6 | 13.10±0.03$^{d}$ | 12.44±0.19$^{b}$ | 3.35±0.04$^{ab}$ | 3.35±0.04$^{ab}$ | 2.06±0.03$^{abc}$ | 0.41±0.00$^{c}$ |
| 7 | 12.07±0.00$^{e}$ | 13.15±0.00$^{a}$ | 3.24±0.06$^{ab}$ | 3.24±0.06$^{ab}$ | 1.97±0.02$^{de}$ | 0.39±0.00 |
| 8 | 14.11±0.04$^{b}$ | 12.24±0.03$^{c}$ | 3.39±0.04$^{ab}$ | 3.39±0.04$^{ab}$ | 2.00±0.02$^{cde}$ | 0.39±0.00$^{bcd}$ |
| 9 | 13.03±0.03$^{d}$ | 13.03±0.03$^{a}$ | 3.24±0.08$^{ab}$ | 3.24±0.08$^{ab}$ | 1.98±0.05$^{cde}$ | 0.41±0.01$^{abc}$ |
| 10 | 13.10±0.03$^{e}$ | 13.23±0.03$^{a}$ | 3.26±0.11$^{ab}$ | 3.26±0.11$^{ab}$ | 2.00±0.03$^{bcd}$ | 0.40±0.01$^{abcd}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 8**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Female Cocoon Shell Percentage | Coefficient of Variations for Female Cocoon Weight | Coefficient of Variations for Female Cocoon Shell Weight | Coefficient of Variations for Female Cocoon Shell Percentage | Male Cocoon Weight | Male Cocoon Shell Weight |
| | % | % | % | % | gr | gr |
| 1 | 19.88±0.06$^{ab}$ | 9.33±0.88$^{ab}$ | 9.88±0.97$^{a}$ | 7.60±1.25$^{a}$ | 1.68±0.01$^{a}$ | 0.40±0.01$^{a}$ |
| 2 | 18.94±0.09$^{ab}$ | 8.97±0.80$^{ab}$ | 11.09±0.93$^{a}$ | 5.65±0.68$^{a}$ | 1.56±0.01$^{d}$ | 0.35±0.01$^{d}$ |
| 3 | 19.93±0.16$^{ab}$ | 9.60±0.71$^{ab}$ | 10.32±0.79$^{a}$ | 7.79±0.46$^{a}$ | 1.60±0.01$^{cd}$ | 0.38±0.02$^{bc}$ |
| 4 | 20.11±0.10$^{ab}$ | 7.85±0.71$^{b}$ | 8.29±0.18$^{a}$ | 6.32±0.28$^{a}$ | 1.67±0.02$^{ab}$ | 0.40±0.01$^{ab}$ |
| 5 | 19.70±0.10$^{ab}$ | 11.92±0.83$^{a}$ | 13.09±0.36$^{a}$ | 9.34±0.90$^{a}$ | 1.61±0.04$^{bc}$ | 0.37±0.02$^{cd}$ |
| 6 | 19.91±0.12$^{ab}$ | 8.42±0.67$^{ab}$ | 8.85±0.89$^{a}$ | 6.88±0.42$^{a}$ | 1.69±0.02$^{a}$ | 0.40±0.01$^{ab}$ |
| 7 | 19.87±0.17$^{ab}$ | 9.07±0.72$^{ab}$ | 10.47±1.02$^{a}$ | 7.62±0.79$^{a}$ | 1.57±0.02$^{de}$ | 0.38±0.01$^{c}$ |
| 8 | 19.75±0.16$^{ab}$ | 8.37±0.77$^{ab}$ | 10.09±0.83$^{a}$ | 8.50±1.19$^{a}$ | 1.59±0.03$^{cd}$ | 0.37±0.01$^{cd}$ |
| 9 | 24.18±4.17$^{a}$ | 11.98±2.83$^{a}$ | 17.61±8.24$^{a}$ | 60.75±54.62$^{a}$ | 1.63±0.03$^{abc}$ | 0.38±0.02$^{bc}$ |
| 10 | 20.31±0.62$^{ab}$ | 10.04±0.72$^{ab}$ | 21.22±9.67$^{a}$ | 18.89±9.52$^{a}$ | 1.58±0.02$^{de}$ | 0.38±0.01$^{bc}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Table 9**- Mean comparison (±SEM) of productive parameters in studied treatments*

| Treatment | Parameters | | | |
|---|---|---|---|---|
| | Male Cocoon Shell Percentage | Coefficient of Variations for Male Cocoon Weight | Coefficient of Variations for Male Cocoon Shell Weight | Coefficient of Variations for Male Cocoon Shell Percentage |
| | % | % | % | % |
| 1 | 23.93±0.23$^{ab}$ | 10.09±1.16$^{a}$ | 12.76±1.46$^{abc}$ | 9.68±1.16$^{ab}$ |
| 2 | 22.75±0.18$^{d}$ | 7.15±0.56$^{a}$ | 9.38±1.60$^{cd}$ | 6.65±1.20$^{b}$ |
| 3 | 23.94±0.30$^{ab}$ | 10.34±0.76$^{a}$ | 14.72±2.03$^{a}$ | 10.64±3.29$^{ab}$ |
| 4 | 23.78±0.19$^{abc}$ | 7.08±0.87$^{a}$ | 9.16±0.70$^{ab}$ | 8.00±0.51$^{ab}$ |
| 5 | 22.98±0.42$^{cd}$ | 10.06±1.28$^{a}$ | 13.26±0.98$^{ab}$ | 10.14±1.31$^{ab}$ |
| 6 | 23.61±0.27$^{abcd}$ | 7.59±0.74$^{a}$ | 8.31±0.55$^{d}$ | 7.89±0.59$^{ab}$ |
| 7 | 24.41±0.54$^{a}$ | 10.29±1.98$^{a}$ | 11.21±0.27$^{abcd}$ | 17.30±8.52$^{a}$ |
| 8 | 23.31±0.22$^{bcd}$ | 8.07±0.16$^{a}$ | 11.63±0.37$^{abcd}$ | 9.21±0.81$^{ab}$ |
| 9 | 23.61±0.15$^{abcd}$ | 9.29±1.40$^{a}$ | 10.32±0.60$^{bcd}$ | 9.14±0.98$^{ab}$ |
| 10 | 24.10±0.16$^{ab}$ | 7.76±0.97$^{a}$ | 9.70±1.17$^{bcd}$ | 8.02±1.44$^{ab}$ |

*Means in each column followed by the same letters are not significantly different at α=0.05

**Coefficient of variations for male cocoon shell weight**

From obtained results, it is showed that amount of coefficient of variations for male cocoon shell weight in ten studied methods included between 8.31-14.72%. Among studied methods, the highest level of coefficient of variations for male cocoon shell weight belonged to 3rd treatment (14.72%), and 6th treatment (8.31%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P>0.05).

**Coefficient of variations for male cocoon shell percentage**

From obtained results, it is showed that amount of coefficient of variations for male cocoon shell percentage in ten studied methods included between 6.65-17.30%. Among studied methods, the highest level of coefficient of variations for male cocoon shell percentage belonged to 7th treatment (17.30%), and 2nd treatment (6.65%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant (P<0.05).

## 4. Discussions

Starting of silkworm feeding after hatching or after molting at different instars, depending on breed and environment temperature is different. According to studies done by the researchers, larvae just about 41 minutes after hatch are starting to feeding. In the second instar, larvae are feeding 99 minutes after molting. This delay increase as instars increases, so larvae at the fifth instar are eating 167 minutes after the molting. Feeding start time is depended and based on different factors such as larvae garlic or hungry, environmental conditions, larval appetite etc. Silkworm has little mobility after feeding and keeps up its head and chest, and is pale and drawn its body and skin. Their skin is loose after digestion of mulberry consumption and larvae starts to crawl which is a sign as hungry.

Resistance is a quantitative trait with incessant distribution and affected by major genes and minor genes. It was demonstrated that silkworm resistance controlled by double dominance gene on un-sexual chromosomes. If there is random mates in successive generations of silkworm population, natural selection resulted to major genes and modifier genes.

To date various studies has been conducted on nutrition pattern and hunger tolerance levels of various insects (Simpson, 1981; Simpson, 1982; Simpson, 1983; Simpson, 1995; Simpson and Abisgold, 1985; Simpson and Ludlow, 1986; Simpson and Raubenheimer, 1993; Reynolds et al, 1986; Barton and Raubenheimer, 2003; Bernays and Singer, 1998; Bernays and Woods, 2000; Cohen et al, 1988;) but so far very few reports have been published regarding silkworm feeding patterns (Hamamura, 1959, Hamamura et al, 1962; Ueda and Suzuki, 1967; Hirao et al, 1976; Hirao et al, 1978; Hirao and Yamaoka, 1981).

Sericulture is a labor intensive industry with its agricultural part of mulberry cultivation, silkworm egg production and silkworm rearing as well as its industrial sector of cocoon processing, silk reeling and weaving. The silkworm rearing is a traditional industry in Asia and the life of many people is depended on it. Hence improvement of rearing management can increase its income and attraction among farmers. Defining future scenarios for agricultural production and deriving management programs of feeding improvement for these scenarios is a useful tool in developing rearing strategies that are robust to changes in markets. Therefore, it is necessary to study the effect of other constraints of production system on various rearing methods of important traits in silkworm hybrids.

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# Effect of Knowledge, Attitude and Constraints on Postharvest losses among plantain farmers and wholesalers in south-western Nigeria

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**Abstract:** Postharvest losses have been a constraining factor in plantain production such that increase in yield brought about by advances in technologies through research did not make any significant impact on the economy of small scale farmers. The study examined the influence knowledge, attitude and constraints on postharvest losses among farmers and wholesalers in south-western Nigeria. A combination of multistage random sampling and Snowball techniques were used to select farmers and wholesalers respectively. Primary data was collected through pre-tested structured questionnaire and analysed using frequency counts, percentages and t-test. The result shows a significant relationship between knowledge and constraints to postharvest activities and postharvest losses among farmers and wholesalers. Similarly, significant differences were recorded in the attitude ($t = 4.04$, $p < 0.05$) and knowledge ($t = 2.23$, $p < 0.05$) and postharvest losses ($t = 3.98$, $p < 0.05$) among the respondents, while no significant differences was observed in the constraints they faced with ($t = 1.26$, $p < 0.05$). The result shows that there is need for an improved knowledge on the postharvest activities, an improvement in the constraints faced by them to reduce the post harvest losses incurred.

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***Keywords:*** postharvest activities, postharvest losses, attitude, knowledge and constraints.

## 1. Introduction

Plantain belongs to the family *Musaceae*. It descended from a wild ancestor *Musa balbisiana* (Samson, 1980). In sub-saharan Africa, plantain provides up to 25% of the required food energy to 70 million people (Swennen, 1990). Plantain constitutes an important source of revenue for the backyard producers and large scale farmers. This crop was one of the first to be domesticated as it requires no specialised tool for harvest or propagation (Chuckwu, 1996). The long association between man and this crop is also indicated by the many forms in which it is consumed. Plantain could be consumed in the unripe, ripe, and overripe forms, when raw or cooked. In Nigeria, it is grown in the southern states in the so called plantain belt by small peasant farmers in traditional farming system from backyard gardens to pure stand field (Noupadja, 1995; Akinyemi and Tijani Eniola, 2000). Nigeria is the largest producer of plantain in West Africa with annual production of about 2.4 million metric tonnes mostly from the plantain growing states which include; Edo, Delta, Osun, Ondo, Rivers, Cross-rivers, Akwa-Ibom, Imo, Abia, Anambra, Oyo, Lagos, and Enugu states.

Food preservation remains a major challenge in developing countries including Nigeria. The capacity to preserve food is directly related to the level of technological development. While affluent and industrialized countries are more concerned with the adverse health effects of excessive food or nutrient intake, leading to obesity, arteriosclerosis and hypervitaminosis, developing nations continue to grapple with food shortages and nutritional deficiency diseases. The perishability of plantain like other crops gives rise to the need to preserve it. One of the ways of ensuring good food preservation is through efficient postharvest handling.

Postharvest handling of crops is of great importance in food production. This is because it is one of the determinants of the quantum of the profit that the rural farmers will make on their harvested crops (Chukwu 1996). While research has shown that increased production is possible, it has however been discovered that the increase in crop yield brought about by the advances in technologies during the last decade did not make any significant impact on the economy of the small-scale farmers. This is because the increase is lost due in part to poor postharvest handling of the crops produced (Arowojolu, 2000).

Management of postharvest losses is therefore essential if these losses are to be minimised. Plantain is chosen for this study because of its perishable nature. Plantain is among the major food crops (Frison, 1997) that require proper postharvest handling. Also, it is in high demand by both the high and ″low income earners in the society and worldwide. Plantain is of high commercial value, available in the rural and urban areas, commands

usage diversity and is an important source of revenue for small farm holders (Dorosh, 1988; Tshiunza, et al, 2001).

The south western agro-ecological zone of Nigeria is the dominant zone for the production of plantain. The zone has heavy rainfall of 1200 – 1500mm, and well drained ground for plantain production. The production of plantain is scattered in farms all over rural areas in the zone and have to be gathered together before being transported to the ultimate consumers (Eboh and Ogbazi, 1990). Plantain is a seasonal crop, highly perishable, high in moisture content, and characterized by high postharvest losses (Olorunda and Aworh, 1988).

Aworh (2004) stated that the postharvest losses of fruits run into billions of Naira annually, while Khang (2003) opined that the losses not only affect output but reduce farmers' income all over the world. Postharvest loss has been a bane to food security in Nigeria. Even though increased yield has been found to be possible (Arowojolu, 2000), postharvest losses have prevented the effect of the increase to be felt on the income of the small-scale farmers. Many of the technologies wherein farmers invest time and money for higher yield are nullified by postharvest losses (Chukwu, 1996). Nigeria the largest producer of plantain in West Africa consumes all her production with nothing for export because of the decline in production in recent times. Postharvest loss has been found to be responsible for the decline in production of plantain in association with other factors such as poor road networks, constraints to postharvest activities, low soil fertility and non-maximisation of the processing potentials of plantain (Ladapo, 2010).

Eradication or reduction of postharvest losses is therefore important to bring about increased food security and reduce suffering to both rural and urban households. In order to ensure that every Nigerian has an unimpeded access to enough food for healthy living throughout the year, and that farmers have adequate financial reward for their efforts on the farm, special attention has to be paid to the postharvest handling of fruits like plantain that have high perishability. This is in order to identify the determinants of the losses in effort at reducing them to the barest minimum. The general objective of the study is to identify the determinants of post harvest losses of plantain among farmers and wholesalers in south-western Nigeria.

**Materials and Methods**

This study was carried out in South Western Nigeria using a multi-stage sampling technique. The first stage involved the selection of three states namely: Edo, Osun, and Ondo out of the eight in the south western ecological zone of Nigeria. The states were selected because of the high production of plantain. The second stage involved the selection of the Agricultural Development Programme (ADP) zones in each of the 3 states. Ten percent of the ADP zones were randomly selected from each state, to give one zone per state, making a total of 3 ADP zones. The third stage involved the random selection of 10% of the blocks to give five blocks from the forty-six in the selected zones. The fourth stage involved the random selection of one cell from each of the selected blocks to give 5 cells. Each cell has an average of one hundred and twenty farmers. The fifth stage involved the random selection of 40 percent of the farmers in the selected cells to give two hundred and fifty farmers (out of 600) which formed the sample size for farmers. There were no registered wholesalers in the selected states. The snowball technique; in which the researcher identified some wholesalers who have the required information and helped to identify other wholesalers was used. A total of one hundred and twenty wholesalers were identified, out of which ninety were randomly selected for the study using the systematic random technique.

Data were collected using structured interview schedule that included list of five postharvest activities, from which farmers and wholesalers indicated those they practiced, while a knowledge test on postharvest activities was provided on a 2 point scale of True (2) and false (1) containing eleven items on postharvest activities. Attitude to postharvest activities were determined with a Likert scale of SA, A, U, D and SD were containing developed 10 statements. Data were analysed through the use frequency and percentages, Pearson Product Moment Correlation and t-test.

**2. Result**

Table 2 shows the personal characteristics of plantain farmers and wholesalers, while Table 3 presents the post harvest activities among plantain farmers and wholesalers and Table 4 reveals the constraints of plantain farmers and wholesalers to postharvest activities. Table 5 and 6 presents the attitude of plantain farmers and wholesalers to postharvest activities and knowledge of postharvest activities of plantain by farmers and wholesalers respectively; while tables 7 and 8 shows the correlation analysis of post harvest losses and attitude of respondents and t-test statistics of difference in attitude, knowledge, constraints and post harvest losses of farmers and wholesalers respectively.

Table 1: Sampling procedure for the plantain farmers

| States | Edo | Osun | Ondo | Total |
|---|---|---|---|---|
| No. of zones in each state | 3 | 2 | 3 | 8 |
| No. of zones randomly selected (10%) | 1 | 1 | 1 | 3 |
| No. of blocks in each zone | 18 | 10 | 18 | 46 |
| No. of blocks randomly selected (10%) | 2 | 1 | 2 | 5 |
| No. of cells in the blocks selected (8 cells = 1 block) | 16 | 8 | 16 | 40 |
| No. of cells randomly selected (10%) | 2 | 1 | 2 | 5 |
| No. of farmers/cell at an average of 120 | 240 | 120 | 240 | 600 |
| No of randomly selected respondents | 96(40%) | 60 (50%) | 94 (40%) | 250 |

Table 2: Personal characteristics of plantain farmers and wholesalers

| Demographic characteristics | Farmers | Wholesalers |
|---|---|---|
| Variable | | |
| *Age (years)* | | |
| 31 – 40 | 22 (8.8) | 23 (25.5) |
| 41 – 50 | 60 (24) | 50 (55.6) |
| 51 – 60 | 142 (56.8) | 17 (18.9) |
| 61 – 70 | 26 (10.4) | ---- |
| Mean | 52.6 | 44.8 |
| *Sex* | | |
| Male | 204 (81.6) | 25 (27.8) |
| Female | 46 (18.4) | 65 (72.2) |
| *Marital Status* | | |
| Married | 232(92.8) | 84(93.3) |
| Widowed | 18 (7.2) | 6 (6.6) |
| *Level of Education* | | |
| No formal Education | 162 (64.8) | 14 (15.6) |
| Adult Education | 32 (12.8) | 19 (21.1) |
| Primary Sch. Leaving Cert | 20 (8.6) | 44 (48.9) |
| Attempted School Cert. | 36 (14.4) | 13 (14.4) |
| *Family Size* | | |
| 1 – 4 | 63 (25.2) | 35 (38.9) |
| 5 – 8 | 132 (52.8) | 46 (51.1) |
| 9 – 12 | 55 (22.0) | 9 (10.0) |
| $\bar{x}$ | 8.6 | 5 |

*Percentage in parenthesis.

Table 3: Post harvest activities among plantain farmers and wholesalers

| Activity | Farmers | Wholesalers |
|---|---|---|
| Storage mode using Traditional methods | 250 (100) | 90 (100) |
| Processing | 210 (84.0) | 64 (71.1) |
| Marketing | 250 (100) | 90 (100) |
| (a) farm-gate | 158 (68.2) | 61(67.8) |
| (b) urban market | 42 (36.8) | 90(100) |
| (c) on the farm | 42 (36.8) | 20(22.2) |
| Sorting | | |
| Sorting by number | 200(80.0) | 72(80.0) |
| by bruises | 198 (79.2) | 72(78.9) |
| by stage of ripeness | 186(74.1) | 83(92.2) |
| Transport involvement | 36 (14.4) | 90(100) |
| non-involvement | 214 (85.5) | -- |
| | 214(85.6) | -- |

* Percentages in parentheses.

**Table 4: Constraints of plantain farmers and wholesalers to postharvest activities**

| Constraints to various post-harvest activities | Farmers | Wholesalers |
|---|---|---|
| Transportation and Marketing Bad roads | 244 (97.6) | 77 (85.6) |
| High cost of transportation | 245 (98.0) | 90 (100) |
| Poor state of vehicles | 234 (93.6) | 81 (90.0) |
| Storage and Processing Lack of technological know-how | 232 (92.8) | 12 (13.3) |
| Lack of knowledge of improved storage/processing equipment | 240 (96.0) | 87 (96.7) |
| Non-affordability of the equipment | 242 (96.8) | 81 (90.0) |
| Lack of infrastructural facilities | 242 (96.8) | 82 (91.1) |
| High cost of maintenance | 186 (86.0) | 87 (96.7) |
| Government Policy | 214 (85.6) | 41 (45.6) |
| Social and Political instability | 188 (75.2) | 66 (73.3) |
| Poor hygiene of warehouses/packs | 62 (24.8) | 68 (75.6) |

*Percentages in parentheses

Table 5: Attitude of plantain farmers and wholesalers to postharvest activities

| Attitude | Farmers Mean | Wholesalers Mean |
|---|---|---|
| Post harvest handling of plantain is not necessary. | 1.98 | 3.03 |
| Post harvest handling of plantain is a waste of time. | 2.79 | 3.80 |
| Post harvest handling of plantain is expensive | 2.61 | 2.90 |
| It is necessary to float harvested banana in water immediately after harvesting | 2.40 | --- |
| Processing is additional labour cost | 2.23 | 3.70 |
| Storage is additional labour cost | 2.24 | 2.30 |
| High cost of processing equip. prevent respondents from processing Plantain and Banana | 2.74 | 4.20 |
| Long distance of market to farms encourage sales at the farm gate. | 2.11 | 4.1 |
| Fruits infected with disease have no markets value | 2.42 | 0.99 |
| It is important to maintain strict hygiene or sanitation in the plantain/banana pack houses to minimize infection. | 2.06 | 1.70 |
| Dehandling bunches will allow for max transportation of plantain/banana. | 2.57 | 2.75 |
| Overall Mean attitude scores | 2.45 | 3.03 |

Figures in parenthesis are percentages

**Table 6: Knowledge of postharvest activities of plantain by farmers and wholesalers**

| **Items** | **Farmers** | **Wholesalers** |
|---|---|---|
| Locating a cottage industry close to your farm/close to where you buy will encourage you to process plantain. | 58 (23.2) | 46 (51.1) |
| Do you think sorting of diseased/damaged plantain is a waste of time. | 98 (39.2) | 25 (27.8) |
| Damaged fruits are not useful. | 176(70.4) | 81(90.0) |
| Packaging your fruits properly will prevent mechanical damage to your plantain. | 200 (80.0) | 89 (96.7) |
| Do you know that you should not pack ripe and unripe plantain together | 188 (75.2) | 81 (90.0) |
| Selling your fruits at the farm gate attracts more income than in the local markets. | 166(67.0) | 67 (74.4) |
| Both mature and immature fruits should be harvested/purchased. | 94 (38.0) | 4 (13.3) |
| Processing enhance better storage. | 166(67.0) | 79 (63.3) |
| Processing of food crops is essential | 238 (95.2) | 90 (100) |
| Use of bad roads contribute to loss of plantain | 242 (96.8) | 90 (100) |
| Breakdown of vehicles causing delay in getting to destination encourage deteriorating of plantain | 242 (96.8) | 90 (100) |
| It is financially more rewarding to sell in the urban markets than in rural markets. | 226 (90.4) | 8 (26.6) |
| Bruised plantain can be waxed to prevent further deterioration | 238 (95.2) | 90 (100) |
| You should prevent plantain from getting bruised | 240 (96.0) | 90 (100) |

Percentages in parentheses.

Table 7: Correlation analysis of post harvest losses and attitude of respondents

| | Variables | r | df | p | Decision |
|---|---|---|---|---|---|
| Attitude | farmers | 0.14 | 248 | 0.13 | Not Significant |
| | wholesalers | 0.022 | 88 | 0.73 | Not Significant |
| Constraints | farmers | 0.62 | 248 | 0.01 | Significant |
| | wholesalers | 0.75 | 88 | 0.04 | Significant |
| Knowledge | farmers | -0.34 | 248 | 0.00 | Significant |
| | wholesalers | -0.22 | 88 | 0.03 | Significant |

Table 8: t-test statistics of difference in attitude, knowledge, constraints and post harvest losses of farmers and wholesalers

| Variables | Groups | N | Mean | SEM | MD | t | df | p |
|---|---|---|---|---|---|---|---|---|
| Attitude | Wholesalers | 90 | 18.21 | 1.18 | 5.8 | 4.04 | 414 | 0.00 |
| | Farmers | 250 | 13.70 | 0.88 | | | | |
| Knowledge | Wholesalers | 90 | 14.22 | 0.91 | 0.42 | 2.23 | 414 | 0.00 |
| | Farmers | 250 | 13.32 | 0.56 | | | | |
| Constraints | Wholesalers | 90 | 14.51 | 0.94 | 0.91 | 2.26 | 414 | 0.00 |
| | Farmers | 250 | 14.59 | 0.94 | | | | |
| Postharvest | Wholesalers | 90 | 360.77 | 38.02 | 161.71 | 3.98 | 414 | 0.00 |
| loss | Farmers | 250 | 158.98 | 14.21 | | | | |

## 3. Discussion

The table below reveals the age distribution, sex, marital status, level of education and family sizes of the farmers and wholesalers. The farmers had mean age of 52.6 ± 7.5 years, 81.6% were males and 64.8% had no formal education. Seventy-two percent of the wholesalers were females with mean age of 44.8 ± 6.7 years and 48.9% had primary school certificate. The age distribution among the farmers agree with Onanamadu (2000) and Akinsorotan (2004) which confirmed that majority of the farmers in the rural areas were within the age bracket of 41 – 60 years. Large percentage (81.6%) of the farmers were men while greater percentage (72.2%) of the wholesalers were women. This implies that there are define roles for the different gender in postharvest handling of plantain (Ladapo 2010). These results are in agreement with that of Igben (1998) and Ajayi (2000) who opined that relatively younger women are likely to be more dynamic and willing to take risks associated with plantain marketing activities with the hope of improving their living standard. Ninety-three and seven percent of both farmers and wholesalers were married and divorced respectively. The wholesalers were found to be relatively more educated with smaller family size than the farmers with an average family size of 9. Many of the families (64.8%) had no formal education while 49.1% of the wholesalers had primary school leaving certificate.

From Table 3, both farmers and wholesalers engage in similar postharvest activity which include storage, processing, marketing, sorting and transportation. While all farmers and wholesalers engage in storing plantain using the traditional method of placing or raised platform and covering only 84 percentage of the farmers and 71.1 percent of the wholesalers process plantain, this reveals that more of the farmers process plantain. Also all the farmer and wholesalers engaged in sorting as a post harvest activity. The parameters for sorting are however different while the size and the number of fingers is the most important for the wholesalers. While all the wholesalers (100%) also engage in transporting plantain only a small fraction (14.4%) of the farmers are involved in transporting plantain. The reasons adduced by the farmers are high cost of transportation.

Both farmers and wholesalers engaged in marketing of plantain while most of the farmers sell at the farmgate (63.2%), the wholesalers sell at the urban (100%) while (67.8%) also sell at the farm gate. Some of the farmers (36.8%) also sell on the farm when the need arises. Both farmers and wholesalers identified similar constraints to post harvest activities as revealed by Table 4. Majority of the farmers and wholesalers identified high cost of transportation (98%, 1000%) bad roads (97.6%, 85.6%) lack of knowledge of improved storage equipment (96.6%, 96.7%) and lack of infrastructural facilities (96.8%, 91.1%) . Poor hygiene and warehouse packs has identified as a constraints by seventy-five point six percent of the wholesalers only twenty-four point eight percent of the farmers see it as a constraints. However lack of technical know how pm storage was observed as a constraint by 92.8 % of the farmers and only 13.3 percent of the wholesalers. This may be as a result of the fact that more farmers process plantain (Table 3). Constraint was found to have a positive

correlation with postharvest which implies an increase in the constraint will bring about a corresponding decrease in postharvest losses which will result in increase income for the farmers.

The responses of the farmers to twelve attitudinal statements with mean score of 2.45 is shown in Table 5. Many of the farmers score lower mean values when compared with the overall mean except for statements Post harvest handling of plantain is a waste of time, post harvest handling of plantain is expensive; high cost of processing equipment prevents respondents from processing plantain and banana and dehandling bunches will allow for max transportation of plantain/banana. The overall distribution showed that 57.6% and 35.6% of farmers and wholesalers have unfavourable attitude towards postharvest handling respectively. The unfavourable attitude of farmers may be as a result of their poor educational background, inadequate information on the value addition potentials of plantain. However, the wholesalers showed more favourable attitude to postharvest activities than the farmers. This may also be as a result of their better educational background when compared with the farmers. Dubois (2008) reported similar findings among banana farmers. Responses of both farmers and wholesalers indicating their level of knowledge of various post harvest activities of plantain is shown in Table 6. Majority (54.4%) of the farmers and wholesalers (53.5%) have low knowledge of various improved post harvest activities. The farmers are however found to be rich in indigenous knowledge (locally developed skills) which is used in storing ripening and processing of plantain. This agrees with the findings of Ekunwe and Atalor, (2007).

Table 7 reveals the relationship between the respondents' attitude to postharvest activities and the postharvest losses they incur. There exist no significant relationship between the farmers' attitude to improved postharvest activities and the losses they incur. A correlation value of $r = 0.14$; $p = 0.13$ was obtained. Thus, the attitude of farmers does not significantly dictate the level of losses they incur. Similarly the result for wholesalers in their attitude to improved postharvest activities and the losses incurred by them. A value of $r = 0.22$; $p = 0.73$ was obtained. Thus, the attitude of respondents was not significantly correlated with the postharvest losses they incur. A positive and significant correlation of $r = 0.62$; $p < 0.05$ was observed for the farmers. This r value indicates a strong positive correlation between the constraints faced by the farmers and the postharvest losses they incur. This implies that the more the constraints, the higher the losses. Thus, farmers that have more constraints to contend will incur more losses. A positive and significant correlation was also observed for the wholesalers between their constraints to improved postharvest activities and the losses incurred by them. A correlation value of $r = 0.75$; $p < 0.05$ was significant and high. This implies that the more the constraints encountered by the wholesalers, the more the losses they incur, just like the farmers. The relationship in the case of the wholesalers is however stronger. The table further reveals a significant relationship between knowledge of post harvest activities and the postharvest losses incurred by the farmers with a value of $r = -0.34$ ($p < 0.05$). The result implies that an increase in the knowledge of farmers on postharvest activities will bring about a decrease in the post harvest losses incurred, that is, the more knowledgeable they are on effective storage of plantain and modern processing technologies, the lower the losses they will incur. The table also reveals a significant relationship between knowledge of post harvest activities and post harvest losses incurred by the wholesalers. A correlation coefficient of $r = -0.22$; $p < 0.05$ was obtained. This implies that wholesalers with low knowledge of improved postharvest activities were unable to process plantain into various utility forms that would enhance the shelf life, hence the high losses incurred. Thus, the more knowledgeable respondents are, the more they are able to reduce losses. Even though wholesalers have a higher mean knowledge score than the farmers, they yet record higher losses than the farmers. This could be because the wholesalers are always on the move from one place to another and therefore have no time to utilise their knowledge of the various product potentials of plantain Ladapo (2010).

In Table 8, significant differences were recorded in the attitude, knowledge, and the postharvest losses incurred among plantain farmers and wholesalers in the study area with t=4.04; 2.23; and 3.98 respectively at $p = 0.00$. However, no significant difference was observed in the constraints faced by the farmers and wholesalers with $t = 1.26$ (Table 5.19). The higher means of attitude and knowledge of the wholesalers was a reflection of the relatively higher educational attainment of the wholesalers. However, the lower mean losses of farmers (90 bunches/annum) compared while those of the wholesalers (251.71 bunches/annum). implies that the postharvest losses incurred by the wholesalers are more than those of the farmers**.**

**Conclusion**

A major conclusion in this paper is that while knowledge of postharvest activities of farmers and wholesalers to and constraints faced by them are positively correlated to postharvest losses, their

attitude is not. Also, there is significant difference in the attitude, and knowledge. There is therefore the need to develop scientific evidence that will ensure reduced postharvest losses, improve knowledge and attitude through the provision of adult literacy and home-economics classes on the processing potentials of plantain. Constraints faced by the respondents should also be looked into with a view to improving on them, thereby reducing postharvest losses greatly. It will also increase income of farmers/wholesalers, encourage farmers to increase plantain production and subsequently bring about food security.

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# Incidence of Nosocomial Infection with Nasal Continuous Positive Air Way Pressure Versus Mechanical Ventilation During Treatment of Respiratory Distress in Preterm Neonates

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**Abstract: Objective**: to determine the incidence of nosocomial infections in preterm infants with respiratory distress, if treatment with continuous positive air way pressure (CPAP) compared to treatment with mechanical ventilation (MV). **Patients and Methods**: Sixty premature neonates admitted to the intensive care unit in Al Galaa Teaching Hospital, in their first day of life suffering from respiratory distress, the infants were divided into two groups, 1st group include 30 patients supported by CPAP and the 2nd group include 30 patients who were supported by mechanical ventilation. Blood cultures and early endotracheal cultures were taken in the 1st day of life from the sixty neonates in both groups then another late endotracheal culture was taken from them in the 5th day of life. **Results**: 36.67% of patients in the MV group had +ve blood culture and 63.33% had no growth, while in the CPAP group 16.67% had +ve blood culture and 83.33% showed no growth. Early endotracheal cultures showed +ve growth in 63.33% in the MV groups a 23.33% in the CPAP group. (P=0.002), on the other hand late endotracheal cultures showed +ve growth in 36.67% in the MV group and 16.67% in the CPAP group. Klebsiella was the most frequent organism in all +ve cultures. **Conclusion**: The incidence of positive infection in blood cultures and endotracheal cultures is higher in the MV group than in the CPAP group. The incidence of klebsiella among the whole population in the two studied groups was higher in MV group more than in the CPAP group in all the cultures. Within the cases having positive cultures, MV patients needed longer duration on ventilation than patients on CPAP (whether the cultures were taken from the blood or endotracheal).

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**Keywords:** Premature infant, Respiratory distress, cultures, mechanical ventilation, continuous positive air way pressure.

## 1. Introduction

Respiratory distress syndrome (RDS) is the most common cause of respiratory failure and requirement for mechanical ventilation (MV) of newborns. In developing countries, despite facilities for respiratory care of newborn infants, RDS mortality rate and percentage of complications still remain high in comparison to the developed countries (Marraro, 2003).

Mechanical ventilation is a widely used supportive technique in the intensive care units (Greenough *et al.*, 2004). Several forms of external support for respiration have long been described to assist the failing ventilatory pump, and access to lower air ways through tracheostomy or endotracheal tubes had constituted a major advance in the management of patients with respiratory distress. However noninvasive ventilation (NIV) techniques, using patient ventilator interfaces in the form of facial masks, have been designed. (Brochard, 2003). Spontaneous breathing can be supported (CPAP, pressure of volume support ventilation) or ventilation can be totally or partially controlled (volume and pressure controlled ventilation, synchronized intermittent mandatory ventilation) (Mehta *et al.*, 2004). The early application of nasal continuous positive air way : pressure (NCPAP) reduces the need for subsequent endotracheal intubations, mechanical ventilation, and surfactant therapy. (Merran *et al.*, 2004 and; Subramaniam *et al.*, 2005).

Nosocomial infections are the most common complications encountered in the neonatal intensive care unit (NICU). They are associated with high mortality and prolonged duration of hospitalization in the survivors, contributing to an increased cost of health care (Srinivasan *et al.*, 1998).

The aim of this study was to determine the incidence of nosocomial infections in preterm infants with respiratory distress, if treatment with CPAP compared to treatment with mechanical ventilation.

## 2. Material and Methods

The study was conducted on 60 premature neonates admitted in the intensive care unit in Al Galaa Teaching Hospital, all were admitted in their first day of life and all were suffering from

respiratory distress. The patients were divided into two groups, 1st group include 30 patients supported by nasal continuous positive air way pressure (Hamilton Arabella Active Nasal CPAP system blender on bubble CPAP circuit) and the 2nd group include 30 patients who were supported by mechanical ventilation (The Bear Cub 750 vs infant ventilator made in USA).

All the neonates were subjected to full history taking including: maternal data and medical records of these neonates were reviewed for the mode of delivery, Apgar score, birth weight (BW), gestational age and thorough physical examination.

**Investigations included:**

1. Investigations conducted according to the need of every case:
   - CBC, CRP, serum electrolyte, kidney function, liver function and blood gases.
   - Imaging studies as ultrasonography, echocardiography, skeletal survey and CT scan.
2. Blood cultures and early endotrachial cultures were taken in the 1st day of life from the sixty neonates in both groups of the study,and then another late endotracheal cultures were taken from them in the 5th day of life. Blood cultures were performed using BACTEC Peds blood culture bottles and the BACTEC- 9050 instrument. In our study, we used new sterile endotracheal suction catheter during the technique of sampling. All the cases in our study were under routine care of physiotherapy and suctioning through the endotracheal tube every 3 hours when needed, the suction catheter was changed every time, as we used new sterile one.

All of patients were closely followed up during their period of stay for the progress of the condition including:

- Initial assessment of the original disease and initial cause of admission.
- The outcome, complications and recording the results.

**Statistical analysis**:

Data were entered and analyzed using the Statistical Package for Social Science (SPSS); version 12. Nominal data were expressed as frequency and percentage. Numerical data were expressed as means and standard deviations and were compared using student's t test. Associations were tested using Pearson's correlations. P value less than 0.05 were considered significant.

## 3. Results

On analyzing the data of the present study we observed that the mean gestational age in the CPAP group was $33.96 \pm 1.2$ while in the MV group it was $33.36 \pm 1.4$ with no statistically significant differences between both study groups, also mean body weight on admission and on dischange ($1.66 \pm 0.37$ and $1.77 \pm 0.22$ on CPAP group; $1.78 \pm 0.34$ and $1.88 \pm 0.26$ on MV group) showed no statistically significant differences between both group. The mean Apgar score at 1st min was statistically significant higher in the CPAP group (5.1 $\pm$ 1.09) than in MV group (4.4 $\pm$ 1.03) while there is no statistically significant differences between the two group as regards Apgar score at 5th min. ($7.96 \pm 0.49$ versus $7.83 \pm 0.59$ on MV group).

The numbers of siblings, order of delivery in multiple pregnancies and the mode of delivery showed no statistically significant differences between the two groups.

The blood culture results of CPAP group showed that 83.33% had no growth while 16.67% had +ve culture (klebsiella 6.67%, staph. aureus 3.33%, and citrobacter3.33% and strept. viridans 3.33%). The MV group showed that 63.33 had no growth and 36.67% had +ve culture (klebsiella 16.67, staph. aureus 13.33, strept. viridans 3.33% and candida 3.33%). The +ve blood cultures results were higher in MV group than in CPAP group, but this comparison is statistically insignificant (Table 1).

**Table (1) presentation of the blood culture results in both groups of study**

| | Blood culture results | | | | | | Chi-squre | |
|---|---|---|---|---|---|---|---|---|
| | CPAP | | MV | | Total | | X2 | P-value |
| | N | % | N | % | N | % | | |
| No growth | 25 | 83.33 | 19 | 63.33 | 44 | 73.33 | 3.068 | 0.08 |
| Growth | 5 | 16.67 | 11 | 36.67 | 16 | 26.67 | 3.068 | 0.08 |
| - Klebsiella | 2 | 6.67 | 5 | 16.67 | 7 | 11.67 | | |
| - Staph.aureus | 1 | 3.33 | 4 | 13.33 | 5 | 8.33 | | |
| - Citrobater | 1 | 3.33 | 0 | 0 | 1 | 3.33 | | |
| -Strept.viridans | 1 | 3.33 | 1 | 3.33 | 2 | 6.67 | | |
| - Candida | 0 | 0 | 1 | 3.33 | 1 | 3.33 | | |

The results of early endotrachial cultures (in the 1st day of life) in CPAP group showed no growth on 76.67% and growth on 23.33% of patients (klebsielle 16.67%, coagulase negative staphylococci 3.33% and pseudomnus 3.33%). While MV group showed no growth on 36.67% and growth on 63.33 (klebsiella 43.33%, coagulas negative staphylococci 3.33%, Pseudomonus 3.33%, Acinetobacter 10% and strept. viridans 3.33%). The number of patients showing +ve early endotracheal cultures results is higher in MV group than in CPAP group with statistically highly significant value (Table 2).

**Table (2): The results of early endotracheal cultures (in the 1st day of life) in both groups of study.**

| | Early Endotracheal cultures | | | | | | Chi-squre | |
|---|---|---|---|---|---|---|---|---|
| | CPAP | | MV | | Total | | X2 | P-value |
| | N | % | N | % | N | % | | |
| No growth | 23 | 76.67 | 11 | 36.67 | 34 | 56.67 | 9.774 | 0.002* |
| Growth | 7 | 23.33 | 19 | 63.33 | 26 | 43.33 | 9.774 | 0.002* |
| - Klebsiella | 5 | 16.67 | 13 | 43.33 | 18 | 30.00 | | |
| - CO NS | 1 | 3.33 | 1 | 3.33 | 2 | 3.33 | | |
| - Pseudomonus | 1 | 3.33 | 1 | 3.33 | 2 | 3.33 | | |
| - Acinetobacter | 0 | 0.00 | 3 | 10.00 | 3 | 5.00 | | |
| - Strept. Viridans | 0 | 0.00 | 1 | 3.33 | 1 | 1.67 | | |

The results of late endotracheal cultures (in the 5th days of life) in CPAP group showed no growth on 80% and +ve growth on 16.67 of the patients (klebsiella 10%, CONS 3.33% and strept. viridans 3.33%) while in MV group 63.33% of patients showed no growth and 36.67% showed +ve cultures (klebsiella 23.33%, CONS 3.33%, Pseudomonus 3.33%, Strept. viridans 3.33% and Staph. aureus 3.33%). The results of +ve late endotracheal cultures was higher in the MV group than in the CPAP group but the comparison is statistically insignificant (Table 3).

**Table (3): Presentation of the results of late endotracheal cultures (in the 5th day of life) in both groups of study.**

| | Late Endotracheal cultures | | | | | | Chi-squre | |
|---|---|---|---|---|---|---|---|---|
| | CPAP | | MV | | Total | | X2 | P-value |
| | N | % | N | % | N | % | | |
| No growth | 24 | 80.00 | 19 | 63.33 | 43 | 71.67 | 2.815 | 0.093 |
| Growth | 5 | 16.67 | 11 | 36.67 | 16 | 26.67 | 2.815 | 0.093 |
| - Klebsiella | 3 | 10 | 7 | 23.33 | 10 | 16.67 | | |
| - CO NS | 1 | 3.33 | 1 | 3.33 | 2 | 3.33 | | |
| - Pseudomonus | 0 | 0.00 | 1 | 3.33 | 1 | 1.67 | | |
| - Strept. Viridans | 1 | 3.33 | 1 | 3.33 | 2 | 3.33 | | |
| - Staph.aureus | 0 | 0.00 | 1 | 3.33 | 1 | 1.67 | | |

NB: one case of CPAP group died before late endotracheal culture was taken.

Table (4): showed the incidence of klebsiella results among the whole population in the two studied groups where it is higher in MV group than the CPAP group in all the cultures but this comparison is statistically significant only in early endotracheal cultures in 1st day of life.

**Table (4): The incidence of Klebsiella results among the whole population in the two studied groups.**

| Klebsiella | CPAP/M.V | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CPAP | | MV | | Total | | Chisquare | |
| | N | % | N | % | N | % | X2 | P-value |
| Blood culture | 2 | 6.67 | 5 | 16.67 | 7 | 11.67 | 1.456 | 0.228 |
| Early Endotracheal culture | 5 | 16.67 | 13 | 43.33 | 18 | 30.00 | 5.079 | 0.024* |
| Late Endotracheal culture | 3 | 10.34 | 7 | 23.33 | 10 | 16.95 | 1.920 | 0.166 |

Comparison between the non infected cases as regard blood cultures, early endotracheal cultures and late endotracheal cultures and their fate in both groups of study showed that the discharged cases in CPAP group are statistically higher than in MV group as regard no growth results in their blood cultures and early endotracheal cultures (p-value: 0.022 and 0.037 respectively), while it is not significant in the late endotracheal cultures (p-value 0.113) (table 5).

**Table (5): Comparison between the non infected cases as regard blood cultures, early endotracheal cultures and late endotracheal cultures and their fate in both groups of study.**

| No growth | FATE | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CPAP | | | | MV | | | | Chi-Square | |
| | Discharged | | Died | | Discharged | | Died | | | |
| | N | % | N | % | N | % | N | % | $X^2$ | P-value |
| Blood cultures | 22 | 88.00 | 3 | 12.00 | 11 | 57.89 | 8 | 42.11 | 5.218 | 0.022* |
| Early Endotracheal culture (1st day) | 20 | 86.96 | 3 | 13.04 | 6 | 54.55 | 5 | 45.45 | 4.344 | 0.037* |
| Late Endotracheal culture (5th day) | 22 | 91.67 | 2 | 8.33 | 14 | 73.68 | 5 | 26.32 | 2.516 | 0.113 |

On the other hand comparison between the infected cases as regard blood culture, early endotracheal cultures and late endotracheal cultures and their fate in both groups of the study showed that the died cases are higher among MV group than in the CPAP group but this difference is not statistically significant among the cases having +ve growth (Table 6).

**Table (6): Comparison between the infected cases as regard cultures, early endotracheal cultures and late endotracheal cultures and their fate in both groups of study.**

| +ve growth | FATE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CPAP | | | | MV | | | | Fisher's exact test |
| | Discharged | | Died | | Discharged | | Died | | |
| | N | % | N | % | N | % | N | % | |
| Blood cultures | 6 | 80 | 1 | 20 | 6 | 54.55 | 5 | 45.45 | 0.346 |
| Early Endotracheal culture (1st day) | 6 | 85.71 | 1 | 14.29 | 11 | 57.89 | 8 | 42.11 | 0.199 |
| Late Endotracheal culture (5th day) | 4 | 80 | 1 | 20 | 3 | 27.27 | 8 | 72.73 | 0.077 |

Our results also showed that MV group needed more time on ventilation than the CPAP group and this comparison is statistically significant whether the patients have negative or positive cultures results (Table 7).

**Table (7) Comparison between the duration on ventilation in relation to the results of cultures in both groups of the study**

| Cultures | | Duration on Ventilation | | | T-test | |
|---|---|---|---|---|---|---|
| | | N | Mean | SD | T | P-value |
| -ve blood culture | CPAP<br>MV | 25<br>19 | 7.360<br>13.895 | 3.581<br>14.259 | -2.209 | 0.033* |
| +ve Blood culture | CPAP<br>MV | 5<br>11 | 7.600<br>12.818 | 2.702<br>6.080 | -2.377 | 0.032* |
| -ve Early Endotracheal culture | CPAP<br>MV | 23<br>11 | 7.565<br>17.000 | 3.628<br>18.493 | -2.390 | 0.233* |
| +ve Early Endotracheal culture | CPAP<br>MV | 7<br>19 | 6.857<br>11.474 | 2.734<br>4.742 | -2.412 | 0.024* |
| -ve late Endotracheal culture | CPAP<br>MV | 24<br>19 | 6.958<br>10.632 | 2.274<br>5.387 | -3.024 | 0.004* |
| +ve late Endotracheal culture | CPAP<br>MV | 5<br>11 | 10.200<br>18.455 | 6.380<br>17.575 | -1.004 | 0.332 |

P value < 0.05 were considered significant

**4. Discussion**

Neonatal respiratory failure consists of several different disease entities, with different pathophysiologies. During the past 30 years technological advances have drastically altered both the diagnostic and therapeutic approaches to newborns requiring mechanical assistance. Treatments have become both patient-and disease-specific. The clinician has numerous choices among the noninvasive and invasive ventilatory treatments that are currently in use (Donn *et al.,* 2003). Concerns about the damaging effects, and expense of conventional mechanical ventilation have led neonatologists to seek new methods of respiratory support for the preterm infant such as non-invasive respiratory support (Millar *et al.,* 2004).

Our study was designed to determine the incidence of nosocomial infections in preterm infants with respiratory distress, if treatment with CPAP compared to treatment with mechanical ventilation.

In our study, the incidence of positive infection in blood cultures, early endotracheal cultures and late endotracheal cultures were higher in MV group more than in the CPAP group. But these comparisons were ststically significant only in the early endotracheal culture. Aurangzeb and Hameed (2003), found that neonatal sepsis is mainly caused by gram-negative organisms, which are developing resistance to commonly used antibiotics. Early onset neonatal sepsis (EOS, occurring in the first 72 hours of life) remains an important cause of illness and death among very low birth weight preterm infants. It was previously reported a change in the distribution of pathogens associated with EOS from predominantly gram-positive to primarily gram-negative organism (Stool *et al.*, 2005).

In our study, blood cultures results in CPAP group were negative in 25 cases, of which 5 patients (20%) showed in early ET cultures colonization by pathogenic gram negative bacilli, 4 cases infected by klebsiella and one by pseudomonus. Two cases had klepbsiella, one had staphylococcus aureus, one had citrobacter and one had strept. viridans in blood cultures that was not isolated from ET cultures, suggesting another site for entry of microorganism to blood stream. That means blood cultures were positive in 5 cases that showing no or a different microorganism in early ET cultures.

In MV group; blood cultures results were negative in 19 patients, of them 11 were colonized or infected by different microorganisms in early ET cultures (7 cases infected by klebsiella, 3 cases by A cinetobactere and one by strept. viridans). Klebsiella was isolated from blood cultures of 5 cases. 4 of them did not have klebsiella in early ET cultures and one had blood stream infection, in a patient with klebsiella in the respiratory tract. Staphylococcus aureus was isolated from blood cultures of 4 cases, all of them did not have staphylococcus aureus in early ET cultures. Strept. viridans and candida were isolated from blood cultures of one case, which did not show candida in the respiratory tract. The total number of cases of the blood stream infection was 11, only one of them showed the same microorganisms in both blood and early ET cultures.

Sanghvi and Tudehope (1997), found that, Gram-negative bacilli (GNB) and coagulase negative staphylococci (CONS) were the most common causes of early onset sepsis and late onset sepsis respectively. Mullett and his colleagues (1998), found that the risk for infection associated with presence of a central venous catheter is the same for each day of exposure (i.e., the same risk on day 5 of presence of the line as on day 30), but the risk assoicted with ventilatory support increases overtiem. Candida sepsis is associated with prolonged antibiotic use before the first episode of noscocomial sepsis and not with birth weight group.

Galanakis *et al.,* (2002) , found that, gram-negative bacilli, coagulase- negative staphylococci and streptococci were the most common pathogens: 42%, 34% and 17% respectively. Premature rupture of membranes was the main risk factor for early-onset sepsis and respiratory distress syndrome was the main risk factor for late-onset sepsis. Device use was the major risk factor for acquiring ventilator-assoicated pneumonia, central venous catheter related blood stream infection and urinary catheter associated urinary tract infection (Van der Kooi, 2007).

In our study, the incidence of Klebsiella results among the whole population in the two studied groups were higher in MV group more than in the CPAP group in all the cultures but this comparison was statistically significant only in early endotracheal cultures in 1$^{st}$ day of life.

Abdel-Hady *et al.* (2008), found that extended spectrum beta-lactamase producing Klebsiella Pneumoniae is an important cause of nosocomial infections in neonatal intensive care units. The organism gains access to the body either by direct inoculation through breached epithelial surfaces or following aspiration of oropharyngeal organisms (Umeho *et al.,* 2006). Pena *et al.* (2001), demonstrated that klebsiella pneumonia bacteraemia occurring in an epidemic ICU setting is mainly catheter-related.

Klebsiella outbreaks mainly affected premature neonates with intravenous catheters, mechanical ventilation, or both. The high mortality rate was notable. Resistance to multiple antibiotics,

but mainly to broad-spectrum beta-lactam antibiotics, was observed (Ayan *et al.*, 2003).

In our study we found that the number of infected cases decrease between early endotracheal cultures results (in the 1st day of life) and the late endotracheal cultures results (in the 5th day of life) in both groups of the study.

In comparing the relationship between the blood cultures results versus the fate of patients between both of the study groups in our study, we found that death among MV group is higher than death among CPAP group. These differences were not statistically significant among cases having positive cultures results but statistically significant in cases having negative blood cultures results. On the other hand comparison of the relationship between the early and late endotracheal cultures results versus the fate of patients between both of the study groups we found that death among MV group is higher than death among CPAP group, but these differences were not statistically significant among the cases having positive or negative cultures results.

Benjamin *et al.*, (2004), found that among premature infants, much of mortality experienced in gram-negative rod bacteremia (GNR) is due to infection with pseudomonas rather than enteric GNR. race, the need for mechanical ventilation, and younger post conception age when the blood culture was obtained were also strongly associated with mortality.

In our study we found that in patients having negative cultures results, MV patients needed longer duration on ventilation than patients on CPAP. These comparison were statistically significant whether the cultures were taken from the blood, early endotracheal or late endotracheal. Also, within cases having positive cultures results, MV patients needed longer duration on ventilation than patients on CPAP. These comparisons were statistically significant whether the cultures were taken from the blood, or early endotracheal but not with late endotracheal.

Kneyber *et al.* (2005), observed that, in ventilated infants a low occurrence of concurrent bacterial pulmonary infection, but infants with positive cultures needed prolonged ventilatory support. Couto *et al.* (2006), decided that invasive device use and duration of use continue to greatly influence the development of nosocomial infection in NICUs.

Within the same group, the results of blood and early endotracheal cultures weather positive or negative did not have a statistically significant difference on the duration of ventilation. Cases with positive late endotrocheal cultures expressed statistically significant longer duration on ventilation than cases with negative late endotrocheal cultures only in MV group. Also in our study we found that, the patients who died in both groups of the study needed more days on the ventilation than the patients who had been discharged. These comparisons were statistically significant in MV group.

Stoll *et al.* (2002), found that late-onset sepsis remains an important risk factor for death among VLBW preterm infants and for prolonged hospital stay among VLBW survivors.

In conclusion, the present study revealed that nosocomial infections are one of the common complications encountered in the neonatal intensive care unit. The incidence of positive infection in blood cultures and endotracheal cultures results were higher in the MV group than in the CPAP group. The incidence of klebsiella results among the whole population in the two studied groups were also higher in MV group than in the CPAP group in all the cultures within the cases having positive culture results, MV patients needed longer duration on ventilation than patients on CPAP contributing to an increased cost of health care.

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# Perceived Impact of Education on Poverty Reduction in Rural Areas of Iran

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**Abstract:** Education and learning are widely recognized as essential to processes of development and poverty reduction. In many developing countries, issues of educational access, equity, and quality have been identified as prerequisites to the achievement of development goals. The objective of this study is, through reviewing the available evidences and analyses in the role of education in rural poverty reduction, to identify weaknesses pertinent to basic education achieving poverty reduction and to come out with some conclusions that can be taken into consideration in planning successful basic education for poverty reduction. The findings through focus group groups indicated that there are some rural structural barriers in educational system on rural poverty reduction.
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**Keywords:** Education, poverty reduction, rural development

## Introduction

One of the main issues in rural development debates is how to tackle rural poverty. More than 70 percent of the world's poor are to be found in rural areas where hunger, literacy and low school achievement are common. Education for a large number of people in rural areas is crucial for achieving sustainable development (UNESCO, 2002). As the majority of the world's poor, illiterate and undernourished live in rural areas, it is a major challenge to ensure their access to quality education. The lack of learning opportunities is both a cause and an effect of rural poverty. Hence, education and training strategies need to be integrated within all aspects of sustainable rural development, through plans of action that are interdisciplinary (Gomes & Câmara, 2004).

The constraints to developing the rural areas as well as the problems of this critical sector have come to loom very large (Oyeranti, 2005). Education is widely recognized as essential to processes of poverty reduction. Education, needless to say, is a priority sector in every well-meaning society. Burtch (2006) referred to it as a major force in economic, intellectual, social and cultural empowerment. Its value in bringing about character and attitudinal change ranks as important as its ability to reshape human potentials for desired development (Jaiyeoba, 2007).

Education is one of the mechanisms to empower people to take part in poverty reduction. It was launched as a key strategy of rural development. Increased the education is a means to achieve development to resolve the rural problems (Lasker, Weiss, & Miller, 2001). Education may directly influence rural agricultural productivity via one or more of the routes described above (Weir, 1999). Education may increase the probability of success in each of these endeavours and, in so doing, diversify household income sources to reduce risk and improve economic security. Since farming is the primary activity in rural Iran, this paper will focus on the part played by eudcation in poverty reduction (Aref, 2011). World Bank studies also demonstrate education raises the production of farmers (Hegtvedt-Willson, 1984).

A rural community cannot foster development without an educated people. Businesses, large or small, are unlikely to choose to invest in rural areas if skilled or trainable human resources are unavailable. Similarly, a community cannot retain educated people without an attractive economic environment (Atchoarena & Gasperini, 2003). Education in rural development can support and uphold local culture, tradition, knowledge and skill, and create pride in community heritage (Lacy, Battig, Moore, & Noakes, 2002).

The paper stresses that education in rural areas is the foundation for both poverty reduction. Although, education has economic and noneconomic benefits to educated individuals and to the social as a whole, this study intended to focuses on the aspect of economic benefit of education to rural areas for poverty reduction. It reviews some critical issues that are related to education in the context of poverty reduction in Iran. The main purpose of the study was to investigate the perceived contributions of education to poverty reduction.

**Literature review**

It is a general belief that education plays a vital means in achieving rural development. In many countries, education has provided a dependable leverage for rural development. Raji (2004) described education as both a social and private good. It is an investment that is capable of yielding benefits that have some externalities (Jaiyeoba, 2007). Education has emerged as an essential prerequisite for reducing poverty and living conditions of rural people (Abdulahi, 2008). The rural poor face three fundamental problems: (i) few opportunities for productive employment in agricultural or nonagricultural activities; (ii) inadequate nutrition, poor health services and absence of educational opportunities; and (iii) lack of sufficient levels of organization needed to lobby effectively for rural interests (Abdulahi, 2008).

Recent research shows that improvements in education boost local development prospects (Echeverría, 1998). Education has a desirable controlling influence over development of the rual individual, community, and society, leading to reduced poverty, income and controlled unemployment (Navaratnam, 1986). Education is a phenomenon of affluent contemporary societies is a particularly difficult concept in rural communities in developing countries to grasp (Fägerlind & Saha, 1986). Much of the theoretical debate about the role of education in poverty reduction has focused upon whether education is productive in an economic sense.

There is much evidence that levels of education amongst the population are highly correlated with levels of economic development (Oxaal, 1997). Helliwell and Putnam (1999) found that education is correlated with social capital: trust and social participation. However, only recently have studies attempted to determine whether education exerts a causal influence on poverty reduction (Riddell, 2006). Education is a critical part of poverty reduction. Individuals who have had some education are better farmers and more capable of finding off-farm employment. The rural sector also benefits from the overall development of the national economy and the alleviation of poverty, in which basic education is essential (Moulton, 2001).

From this perspective, it is evident that education has significantly contributed to the mobilization and distribution of human capital by creating opportunities for people. In rural areas of low-income countries, the problem of access to education is acute and, in order to take on the enormous challenges involved in providing education for all, a more holistic view of education is needed. In particular, the issue of educational development in rural areas cannot be properly addressed without mentioning the upheavals that have occurred in the agricultural milieu (Atchoarena & Gasperini, 2003).

Education issues are central to rural poverty reduction in the rural area of Asia. Hence, it is important for government to understand that rural educational system also face barriers that can hinder its progress in responding and recognizing the priorities of rural communities (Aref, 2011). Involving rural communities in the education planning requires facing and tackling a number of challenges (Moulton, 2001). These issues include:

-Rural schools are farther apart, requiring many children to walk long distances or pay for transportation and to lose valuable time in walking that could otherwise be spent helping at home.

-Relatively weak extension services in some countries.

-Low levels of basic education and agricultural education among farmers.

-Inadequate initial training and continuing education for rural people.

-Long distances, poor roads, and inadequate shipping vehicles make it difficult to get building materials, furniture, equipment, and textbooks to rural schools.

-Even where a primary school is accessible, there may be no secondary school within commuting distance.

-While urban parents and communities sometimes play an active oversight role in their schools, this rarely happens in rural communities, where parents are less skilled at holding officials accountable, reviewing financial statements, and even feeling confident that they can ask questions.

-Communication between ministry offices and schools is difficult, so school principals and teachers get little if any guidance from a professional support network.

-The curriculum may not be relevant to rural communities.

-Support services for remote rural schools are not always fully institutionalized. The ministry often lacks the resources to help these links function as channels of support (Flor, Hazelman, & McLean, 2006; Moulton, 2001).

**Methodology**

The population of the study includes staff of local education and school teachers, in Abadeh Tashk, Shiraz. This study is based on quantitative methodology to investigate the barriers of education related to poverty reduction. To achieve the objectives of this study, the researcher uses quantitative method. Focus group discussion was performed to collect data from local residents. Focus group is probably the most widely used technique of

gathering qualitative data (Grover & Vriens, 2006). Focus group was conducted in a group setting and was used for obtaining a better understanding of participants' attitudes (Aref, 2010). All respondents were male. They ranged in age from 25 -51 years. The researcher explained to them the objectives of the study and what questions would be asked. For this study, pertinent articles and reports on critical issues of education in poverty reduction also are reviewed.

**Result**

The aim of this study was to demonstrate the contribution of rural education to poverty reduction in rural areas. Information for this study was gathered from school teachers in 12 villages in Abadeh Tashk, Shiraz. A qualitative analysis was undertaken to determine viewed the impacts of education on poverty reduction and also barriers of rural education on poverty reduction. According to the collected baseline data, there were overall 96 participants with an average of 35 years old, 61% were male and 39% were female. They were chosen because of their engagement in educational programs. The questions were asked about to contribution of education in poverty reduction and barriers of education. In terms of education on poverty reduction; they believe that rural education does not have important role in their villages especially on poor people.

The respondents referred to variety barriers of education for poverty reduction in their villages. The below items were provided from the focus group discussions.

-Immigrations of rural educated to urban areas as main obstacles for rural poverty reduction. In fact the educational system is the cases of this issue in rural areas of Iran.

-Lack of educational resource and curriculum.

-The participants mentioned to lack of suitable skill and knowledge as one barrier of education for rural poverty reduction.

-Lack of access to secondary school or high school for for majority of rural peolpe.

-Lack of capacity of local educational organizations; especially local organization was behind the failure investment for poverty reduction.

-Lack of participation rural educated people in process of poverty reduction.

-Lack of rural involvement; especially women in process of rural development.

The findings of this study are consist with the findings of Flor, et al. (2006) and Moulton (2001); that they belived the rural areas of Asia have some barreirs of poverty reduction throghu rural educational system. Based on the findings; although education has an positive impacts on rural poverty reduction, but as poverty is a multidimensional; rural educational system cannot solve the poverty problems.

**Conclusion**

The purpose of this article has been to demonstrate the contribution of education to poverty reduction in Iran. Education and learning are widely recognized as essential to processes of development and poverty reduction. In many developing countries, issues of educational access, equity, and quality have been identified as prerequisites to the achievement of development goals. Education helps to alleviate poverty by affecting labor productivity and via other paths of social benefit. It is therefore a vital development goal (Flor et al., 2006). The findings indicated the lack of local organizational capacity as main barriers related poverty. Hence, Education contributing to rural development must be locally controlled, practical, applied, problem-posing, and focused on functional specialization. Although; education has an important role in poverty reduction but as poverty is multidimensional; education cannot solve all problems. This suggests that poverty reduction efforts must be multi–targeted and are expected to show wide and diverse dimensions. Solutions to rural poverty have to straddle different disciplines and must encompass economic, social, political and institutional factors.

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# Socio-economic constraints to sunflower production in Bojanala farming community of the North-West province, South Africa

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Abstract: This paper examined the socio-economic constraints to sunflower production in Bojanala farming community of the North-West province, South Africa. Simple Random sampling was carried out to select 150 farmers from a list of 257 farmers. Primary data based on 2006/2007 and 2007/2008 growing seasons were obtain by use of the questionnaires. Data were collected on socio-economic, output levels, inputs costs measured in rand; and key role players and analyze with SPPS using percentages and double log function of the linear multiple regression. Results of the analysis show that, very few young people below 30 years of age are engage in sunflower production in the Bojanala Region. On gender, 69.5% of all the sunflower producers were male, 51% of the farmers had household size of 4 to 6 children, while 59.8% were married, and 58.5% were with less than three dependants. Farmers with educational levels from standard 8 to 10 constitute 34.10%. Also, 59.7% had 1.1-1.5 tons as output per hectare. Sunflower farmers who had access to the extension services constitute 70.7%. Significant determinant of the socio-economic constraints include number of plantings per year, storage costs, price, income, access to market and farm size.

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**Keywords:** socio-economic characteristics, sunflower, South Africa, output, extension contact

## 1. Introduction

Despite its relatively small share of the total GDP, primary agriculture is an important sector in the South African economy. It remains a significant provider of employment, especially in the rural areas, and a major earner of foreign exchange (NDA 2007). The value of commercial agricultural production in South Africa was R78 billion in 2006, while its contribution to GDP was approximately R35 billion. The primary agricultural sector has grown by an average of approximately 11,8 % per annum since 1970, while the economy as a whole has grown by 14,9 % per annum over the same period, resulting in a decline of agriculture's share of the GDP from 7,1 % in 1965 to 2,3 % in 2006 (NDA 2007). Agriculture's strong indirect role in the economy is a function of backward and forward linkages to other sectors. Purchases of goods such as fertilisers, chemicals and implements form backward linkages with the manufacturing sector, while forward linkages are established through the supply of raw materials to the manufacturing industry. About 68 % of agricultural output is used as intermediate products in the sector. Agriculture is therefore a crucial sector and an important engine of growth for the rest of the economy (NDA 2007). In the North-West province, agriculture remains a major source of income for the livelihood of approximately 65% of the rural population (North-West Department of Agriculture, Conservation and Environment, 2005). Sunflower is the second most important agricultural field crops produced in the North-West province after maize.

It is an important agricultural contributor to the provincial economic development of the North-West province. In addition, sunflower contributes to the household food security of farmers and its production is often affected among other factors by output levels and market prices of the commodity. Sunflower is marketed in the form of refined oil for domestic and industrial cooking, baking and animal feed. According to the National Department of Agriculture (2004), sunflower variety whose seed contain high oil content is the most important source of oil for human consumption in South Africa as compared to other oil seed crops. However, sunflower production is a complex and demanding business with numerous pressures in terms of socio-economic and financial factors, weather, and technological advancement. A prime concern in crop cultivation has always been water availability which limits yield potential in dry land agriculture of semiarid region such as the North-West province. Sunflower is an annual crop, highly drought tolerant and commonly grown as a dry land crop that produces satisfactory results when other crops are damaged seriously. This drought tolerant nature of sunflower is attributed to its deep rooted nature that enables it to increase the effective use of stored soil

water as oppose to other dry land crops. This drought tolerance makes it a better alternative to other dry land crops produced in the province which are more susceptible to drought. The current increase in the demand for alternative energy such as bio-fuel has resulted to a sharp increase in the prices of sunflower oilseeds. Emerging and medium scale sunflower producers in the North-West province provide a great window of opportunities for job creation in this sector. The price per ton of sunflower has dramatically increased from approximately R1800, 00 per ton in 2005 to over R4500, 00 per ton based on forward contracts in 2008.This act as an incentive for emerging sunflower growers to expand production and increase output levels. However, sunflower production and output levels has not grown as fast as its demand. What can be the reason for the slow expansion in production and slow increase in output levels of sunflower in the North-West province?

Costs of sunflower production and the risk of production with respect to drought in North-West province and South Africa in general is still relatively high as compared to their sunflower producers who are subsidized in Europe, America and Argentina. This result to unfair trade competition and drastically reduced the profit margin obtain by South African sunflower growers. Based on the current market price per ton of sunflower, the profit margin that can be obtained from sunflower production is higher relative to other dry land crops. Though the production of sunflower has been increasing over the years, its demand is rising than its production due to higher home and international market demand. According to SAGIS (2006), the importation of sunflower products into South Africa has risen from zero tonnage in 1999/2000 to a total of 34700 million tons in 2004/2005 and was expected to reach 60000 million tons by May 2006 within this six year period. Therefore addressing socio-economic constraints that prevents sunflower production in the North-West Province of South Africa can help reduce the production deficit in the country. Because of the importance of sunflower in terms of its uses and income generating potential, there is therefore a need to look into the reasons why its expansion in production in spite of land availability, technological advancement, infrastructural availability, market liberalization is slower than the demand for the product.

This study is concerned with sunflower production in the Bojanala farming community of the North-West province, especially those emerging farmers involved with commercial production of sunflower. It will assist in the identification of socio-economic constraints faced by sunflower farmers in the study area. Findings would provide information to policy makers on ways to assist sunflower producers increase production in the study area. The challenge for this study is to identify socio-economic factors which are preventing sunflower producers in Bojanala Region from meeting the surge in demand despite the excess milling capacity that exist and the encouraging price per ton at the market. Again, it will also help policy-makers, government and sunflower producers associations to design appropriate policy measures and programmes with the view of uplifting and improving the competitiveness of the sunflower production sector in South Africa. Despite the fact that the production level of sunflower in South Africa is increasing, the rate of increase is far lagging behind the rate of demand for its products. South Africa has the potential (in the form of land availability and technology) to take advantage of this surge in demand. However, producers of sunflower have not been able to take this advantage. The objective of the study is to identify and critically analyze the limiting socio-economic factors faced by sunflower producers in the Bojanala farming community of the North-West province.

## 2. Materials and Methods

The Bojanala Region is found in the North Eastern part of the North-West province. This region is made up of five local municipalities, namely, Madibeng, Moretele, Moses Kotane, Kgetleng River and Rustenburg. The area lies between 25 and 28 degrees longitude East of the Greenwich Meridian and between 27 and 30 degrees latitudes south of the Equator (Mabe, 2005). The region is bordered in north by the Northern Province and in the east by Gauteng province. In the west it is bordered by the Central Region and to the south by the Southern Region of which all are parts of the North-West province. A list of small scale emerging sunflower producers was obtained from the Extension Officer in the study area. Simple Random sampling was carried out to select 150 farmers from a list of 257 farmers. Primary data based on 2006/2007 and 2007/2008 growing seasons were obtain by use of the questionnaires. Data were collected on socio-economic, output levels, inputs costs measured in rand; and key role players and analyze with SPPS using percentages and double log function of the linear multiple regression.

$$\ln Y = \log A + a_1 \ln CS + a_2 SF + a_{3\ln} EPL + a_4 \ln MC + a_5 \ln TI + a_6 \ln SC + a_7 \ln LS + a_8 \ln MP + a_{10} \ln EXT + a_{11} \ln ACCM + a_{12} \ln CRED + e$$

Where:

Ln = Natural log

Y = Output of sunflower in tones per hectare
A = Constant which is the minimum output
$a_i$ = Estimates of the elasticities or regression coefficients
CS = Costs of sunflower seeds.
SF = Sex of farmers
EPL = Employment
MC = Machinery costs per hectare
TI = Total income per year
SC = Storage costs of output
LS = Land size under sunflower production
MP = Market selling price/ton of sunflower
EXT = Use of extension services
ACCM = Access to market
CRED = Access to credit
E = Error term

## 3. Results

The results on the socio-economic characteristics of emerging sunflower farmers covers their personal characteristics while Table 1 presents the regression estimates of effects of significant variables on sunflower output per hectare.

### 3.1 Socio-economic characteristics of emerging sunflower farmers

Results of the analysis show that, farmers who were less than 30 years of age and above 70 years constitute 9.8% and 6.1% respectively. The result is an indication that, very few young people below 30 years of age are engage in sunflower production in the Bojanala Region. On gender, 69.5% of all the sunflower producers were male while 30.5% were female farmers indicating a male dominated . About 51% of the farmers had household size of 4 to 6 children, while 59.8% were married, 58.5% were with less than three dependants. Farmers with educational levels from standard 8 to 10 constitute 34.10%. Farmers with educational levels from standard 1 to 7 constitute 26.8%. In terms of land ownership 91.5% do not own the land on which they farm while 8.5% agreed that they own the land on which they farm. Also, 59.7% had 1.1-1.5 tons as output per hectare. Sunflower farmers who had access to the extension services constitute 70.7% while farmers had no access to extension services constitute 20.7%. Results on visitations by extension workers indicates that, 70.7% of farmers have visits from extension officers while only 20.7% of farmers said they have never been visited by extension personnel. This analysis shows the extent to which the extension workers are assisting the farmers in order to improve on agriculture in this region.

### 3.2 Socio-economic constraints to sunflower production among emerging sunflower farmers

From the results of the regression analysis in Table 1, the factors which did not yield statistically significant coefficients **we**re; age of farmer, household sizes of farmers, number of dependents by farmer, land tenure, farming a full time job, total cost of planting seeds and seeds dressing, cost of machinery per year, distance of market from production area, total marketing expenditure for sunflower per year, access to credit and whether access to credit is a constraint to sunflower production. The results show that the gender of farmers affects sunflower output positively and significant (**p**=0.076) as most of the sunflower producers were male. This may be attributed to the fact that, males have more time to spend on their farms than females who are mostly engage in household activities and spend limited time on their fields. Males contribute directly in terms of labour and supervise workers and have strong bargaining power than women when it comes to loan negotiations and buying of inputs. A positive and significant (p=0.057) relationship between the number of planting times and output per ton. It must be mentioned that sunflower is an annual crop that is produce once a year however, number of planting times refers to the differences in time as the farmers do their planting. Total storage costs is positively significant (p=0.001). According to the results in Table 1, an increase in output per hectare will result in 0.345 increases in the cost of storage all other factors remaining constant. Positive relationship exists between output per hectare and cost of storage, which implies as output increases, cost of storage also increases. Similar positive results were found by Odulaja, and Kiros, (1996.

The results of the regression in Table 4.8 indicates that current price per ton of sunflower is statistically significant at (P = 0.000), signaling an increase of 0.968 in output per hectare when price per ton increases by one unit. Hence the relationship between current price per tone and output per hectare is positive. Total income per year is negative and statistically significant (P=0.003), signaling an increase in output per hectare. The results also shows that, a unit change in output per hectare will result in -0.649 reductions in farmer's income. This is attributed to the fact that despite the unprecedented increase in price per ton of sunflower oilseeds, inputs prices such as fuel cost, machinery cost, storage costs have been rising at faster rate than the increase in price per ton of sunflower. Extension contact has positive and significant (p=0.088) effects on output of sunflower per hectare. Improvements in extension

contacts and services will results in a 0.202 increase in sunflower output per hectare. Thirtle *et al* (1998) tested induced innovation hypothesis based on data from South Africa commercial farms. The result was positive with respect to farm size, research and extension including policies variables which are similar to the result obtain from the study. The results of the regression analysis is significant at (p=0.041) indicating a negative relationship between market accessibility and output per hectare. Reduction in market access will result to -1.145 in sunflower output. The reason is that the existing market systems do not allow farmers to explore outside of the systems there by leading to unfair transactions between the farmers and buyers. A positive and highly significant (p=0.006) relationship between output per hectare and land size under sunflower production. The result shows that an increase in the number of hectares cultivated will result to 0.343 increases in output per hectare all the other factors remain constant. The main reason is that, as the farm size of the farmer increases, the amount of grants receives also increases.

Table 1: Regression estimates of effects of significant variables on sunflower output per hectare.

| | Beta | t | p |
|---|---|---|---|
| (Constant) | | -.450 | .654 |
| Farmers' gender | .164 | 1.807 | .076** |
| Employed | .333 | 3.267 | .002*** |
| Planting times per year | .190 | 1.944 | .057** |
| Storage costs per year | .345 | 3.404 | .001*** |
| Price per ton of sunflower | .968 | 4.528 | .000*** |
| Income per year | -.649 | -3.064 | .003*** |
| Access to extension services | .202 | 1.737 | .088** |
| Access to the market | -1.145 | -2.088 | .041*** |
| Land size cultivated | .343 | 2.833 | .006*** |
| Adjusted $R^2$ | | 0.610 | |
| F-Statistic | | 6.131 | |
| Durbin-Watson | | 1.661 | |
| Significant. | | 0.000[a] | |

## 4. Conclusion

The study has shown that socio-economic factors affect sunflower production in the Bojanala Region of the North-West province of South Africa. Van Zyl (1995) concluded that large scale mechanization farms are generally inefficient with respect to food security when compared to small-scale family type's farm models. There are real economies of scale in larger farms but they are mostly false because they are as a result of policies that favor larger farms over smaller ones. However, it could be said that sunflower producers would generally be capable of making rational decisions if their numerous constraints are removed through effective and efficient government policies and farmer support programmes. It was evident from the results that socio-economic factors such inadequate finance, high interest rate, machinery costs, land tenure, changes in climatic, technical practices/tillage systems including lack of insurance affects sunflower producer addressing the initial research question and objective of the study. Further analysis using linear regression on selling price per ton, total income from sales of sunflower per year, access to market, Land size under sunflower production, total storage cost per year and access to market positively or negatively impacts on sunflower output thereby addresses the following hypothesis; selling price per ton affects sunflower output., total income from sales of sunflower per year affects sunflower output, access to market affects sunflower output.

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# Adult Learning and the related requirements

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**Abstract:** Adults learn most effectively when they have an inner motivation to develop a new skill or gain new knowledge. They resist learning material if it is forced on them, or if the only reason given is that the material will, in some vague way, be "good for them to know." Adults need to know why they are being asked to learn something; and they definitely will want to know what the benefits will be before they begin learning. This means the best motivators for adult learners are explicit interest and self benefit. If they can be shown that the program will benefit them pragmatically and practically, they will learn better, and the benefits will be much longer lasting. Typical motivations include a desire for better handling of personal money matters, say in retirement, wanting a new or first job, promotion, job enrichment, a need to reinforce old skills in say, handling credit or learn new ones, a need to adapt to community changes such as on-line banking and so on. Remember the tone of the program should be motivating. Your program should employ methodologies so that your trainers establish a friendly, open atmosphere that shows the participants they will help them learn rather than present as 'experts' imparting knowledge.
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**Keywords:** adult education, Adult Learning

## Introduction:

Types of content and educational resources in various parts of adult curriculum materials motivational book, course materials, supplementary materials, track materials (continued) participatory form and materials. Incentives aimed at providing content that audiences are produced primarily to attract different groups of adults interested in design, so that their participation in learning programs are encouraged. Motivational training materials for learners and have great importance even in support of successful applications over learners, planners and executors for educational programs is important.

Material often set different types of materials and educational content in books and pamphlets, books, training guides, trainers, equipment auxiliary audio, visual and material are included such that during actual teaching sessions, are used in the transmission and content but also to achieve the goals of making education programs are important.

Additional material for the next stage of learning often means to be expected when developing your learning skills Learners to increase awareness and enjoyment of reading and studying to operate.

To impr ve the quality of life, learning materials should reinforce the skills they acquired previous. This material should have access to information and provide new technology should also have to make learning more fun. Additional materials should provide opportunities for literacy skills to read and to strengthen their cognitive awareness.

Track materials (continued) which increased literacy skills and knowledge gained is also effective in enriching learning environment for learners are important. Participatory materials to ensure the participation of learners in the learning process and codification are included out of class activities, dialogue, role playing, etc., adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

## Concept of adult education:

Several definitions of adult education has been done Community

- Adult Education is a⎤in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age⎤ limits as formal and informal, voluntary and at different levels of time, place

- Adult Education is a process in which people who⎤and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after⎤ school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

**Adult characteristics:**

To understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

**Operating speed:**

Slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

**Consciousness:**

No stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

**Health:**

What is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

**Background of knowledge - skills and beliefs of adults:**

Adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

**Principles of Adult Learning**

**1) PURPOSE**

The Financial Literacy Foundation has prepared this document to provide education materials developers with information on the key principles of adult learning. It is a short summary of a very broad area of research and advice, prepared with the input of Adult Leaning Australia, the national peak body representing organisations and individuals in the adult learning field.

**2) NEEDS, WANTS, CONCERNS AND ABILITIES OF YOUR LEARNERS**

Assess the needs, wants, concerns and current abilities of the target learners. Each target group will have their own special needs and probably expect different outcomes from undertaking your training program. Common themes you can prepare for are:

**Why are you here?** - no-one readily admits to not knowing something fundamental that may impact on their life chances. Therefore program material, particularly that designed for adult learners should always treat aspects of why learners are in the training sensitively. Describe the outcomes expected from the training in positive, enhancing terms and not as redressing a weakness or failure on the part of the learner. For example, “Undertaking this program will improve (rather than redress a failing) the way you manage your money”.

**Tell me more** - learners may well enter programs like this with poor past experiences of money matters or at least some trepidation about handling personal finances in the future. Recognise this in the program introduction but individual learners should never be required to expose any of their negative experiences in a group. It might seem a good ‘ice-breaker’ to ask a new group of learners to share what they expect from the program but resist going too far when asking learners to talk about past problems they may have had with finances. Firstly, they may be uncomfortable doing this in a group and secondly you could start the program in a sea of negative views about financial matters generally. A successful program introduction will focus on where the learners will go rather than dwell too much on where they may have been.

**What do you know?** - Gauge the likely capabilities of your target groups. Overestimating their current skills in dealing with money could mean the program

misses fundamental principles and understandings. Underestimating existing knowledge is also not good as plodding through basic material most already are familiar with will bore participants and the full program content will not be assimilated.

**What will I be able to do?** – above all these target groups will want to be hands on and demonstrate to themselves and their peers that that can do something they could not before the training; and do it well. Let them know right at the beginning that they will be able to do things that will be of great benefit to them, not just know more.

**Build on small successes** – if a target group of learners has had limited positives in their life or work experiences its important to provide small and regular 'success' points in the program. Simply exposing the content and assuming everyone is assimilating it, putting it all together holistically and building up their skills is not enough. The beginning of the program should be designed so that a discrete piece of learning that the learners can use right away builds their confidence to move on. The program should be a series of steps where the learners confirm their progress and reinforce one new skill by relating it to another they can already confidently apply.

**Testing!** – many adults and people not regularly engaged in learning fear testing. Many may have had bad experiences of assessment in school and view the practice among peers as stressful. Make sure they understand that what they are in is a life skills program and no-one can 'fail' as such. In fact each can support others in things they do well that fellow learners may need help with so it's a cooperative not competitive environment that they are learning in. Build in some teamed exercises and assessments to avoid people feeling isolated in their learning and fearful of failure in front of the group.

**Special needs**. You need to consider learners with special needs and those who have English as their second language. Reasonable adjustment should be made depending on each individual learner's particular needs and abilities. Your program material should include advice to the trainer on how to determine the need to make adjustments which, depending on a learner's abilities may include:

- providing interpreters for people who are deaf;
- ensuring access, for example by conducting training and assessment in facilities which have ramps for people using wheelchairs and adjustable desks for people with physical disabilities;
- allowing for access of personal assistants or note takers;
- allowing additional time for assessments;
- allowing oral instead of written responses to questions;
- adaptive technology such as screen readers, speech synthesisers, computer software or hardware; and,
- assistance with managing stress and anxiety.

**3) HOW DO ADULTS LEARN?**

Your program needs to account for:

• Motivation of the learner;
• Reinforcement of the skills and knowledge being developed;
• Retention of key learning; and,
• Transference of what is learnt to new situations.

**Motivation** - Adults learn most effectively when they have an inner motivation to develop a new skill or gain new knowledge. They resist learning material if it is forced on them, or if the only reason given is that the material will, in some vague way, be "good for them to know." Adults need to know why they are being asked to learn something; and they definitely will want to know what the benefits will be before they begin learning. This means the best motivators for adult learners are explicit interest and self benefit. If they can be shown that the program will benefit them pragmatically and practically, they will learn better, and the benefits will be much longer lasting. Typical motivations include a desire for better handling of personal money matters, say in retirement, wanting a new or first job, promotion, job enrichment, a need to reinforce old skills in say, handling credit or learn new ones, a need to adapt to community changes such as on-line banking and so on. Remember the tone of the program should be motivating. Your program should employ methodologies so that your trainers establish a friendly, open atmosphere that shows the participants they will help them learn rather than present as 'experts' imparting knowledge. No-one engages well with a trainer/teacher who is just 'showing off' what they know. Financial services have a plethora of jargon and complicated ideas that can put many lay people off. Exposing this sort of terminology and explaining it in simple terms – or deciding whether some of it needs exposure at all – is paramount to keeping your learner's trust and interest.

**Appropriate level of difficulty.** The degree of difficulty of your financial literacy program should be set high enough to expose all the essential elements of the topic and challenge learners to succeed, but not so high that they become frustrated by information overload. Too much financial industry terminology strung together can be a complete turn off for people who may already struggle with the

fundamentals – is it really a necessary part of the skills they need?

So start with financial information and techniques that relate directly to the learner's own personal needs and wants. Personal budgeting is always useful and less complicated than say, comparing mortgage options. Don't make what could be a lesser used skill so important in the program it de-motivates the learners and loses their interest.

Motivational reward does not necessarily have to be in the monetary sphere; it can be simply a demonstration of social or workplace benefits to be realised from new financial management skills. Older participants could perhaps learn how to help their children with financial decisions. People could be shown how to utilise better financial planning in a club or society they belong to. Its about improving whole of life experiences not just direct monetary reward. The overall thrust of the program should be motivating and, like all good teaching and learning programs, course material should ensure other key adult learning elements are covered.

**Reinforcement.** As we know reinforcement is a very necessary part of any teaching/learning process. Through it, trainers encourage correct modes of behaviour and performance and discourage bad habits. Your program should use both reinforcement techniques throughout. Positive reinforcement is normally used when participants learn new skills. As implied, positive reinforcement is "good" and reinforces "good" (or positive) behaviour. Negative reinforcement is useful in trying to change bad habits or inappropriate modes of behaviour. The intention is extinction -- that is, the trainer uses negative reinforcement until the "bad" behaviour disappears or the learner understands why past practice is not beneficial to them. Examples could be ensuring participants always compare different rates of interest available to them before signing up for any new debt (a positive reinforcement) and not considering credit purchases that leave them with no income safety net for unforeseen circumstances (negative reinforcement).

**Retention.** Learners must retain what the program delivers to them in order to benefit from the learning. In order for participants to retain the information taught, they must see a meaning or purpose for that information. They must also understand and be able to interpret and apply the information in their own real life contexts. Understanding includes their ability to assign the correct degree of importance to the material and its application in the future. The amount of retention is always directly affected by the degree of original learning. In other words if the learners did not learn the material well initially, they will not retain it well either. Retention by the participants is directly affected by their amount of practice during the learning. After the students demonstrate they can apply new financial skills, they should be urged to practice in their own time and for their own personal needs to retain and maintain the desired performance.

**Transference.** Transfer of learning is the result of training and is simply the ability to use the information taught in your program but in new settings and contexts. As with reinforcement, both types of transfer: positive and negative should be used in the program approach. Positive transference, like positive reinforcement, occurs when the learner uses the skill learnt in your program. It is very important for any learner's orientation to the new skills they develop that they can practice in their own situations. Using knowledge from financial literacy training to work out the best way to use (or not use) credit in their lives is an important tool that many participants could use immediately. Participants can check how much credit debt they have, what interest they are paying and what alternatives there may be. Negative transference, again like negative reinforcement, occurs when the learners applying the skill do not do what they are told not to do. This also results in a positive (desired) outcome. This means it's important to find out what the participants in your program have been using their new skills for. Check to see if they are applying the techniques properly or whether they have misunderstood a key aspect of the program. Once wrong information is absorbed and used again and again it simply becomes another bad habit that could make financial decision-making worse instead of better.

Transference is most likely to occur in the following situations:

• **Association**: participants can associate the new information with something that they already know. What skills have the learners already mastered that they can bring to bear on better financial planning for example? Perhaps they have a hobby where it is necessary to access information from written materials or the Internet and the same skills could be used to obtain and analyse better financial data to use in their budgeting.

• **Similarity**: the information is similar to material that participants already know; that is, it revisits a logical framework or pattern. Using calendars or electronic planners to plan future holidays, work shifts etc can be transferred to setting up a long-term budget planner for financial payments and income.

• **Critical attribute element**: the information learned contains elements that are extremely beneficial (critical) in personal life or in the workplace. Try to

reinforce the importance of aspects of the financial literacy program to the learner's own goals, whether these are in their home life, getting a job or improving their prospects in work they already have. People can even start their own small business ventures if they have the financial skills to work out the costs and benefits first.

**4) DELIVERY STRATEGIES**

Finally in developing your program consider that adults have different personal and social lives than young people in formal schooling or college. Unlike children and teenagers, adults have many responsibilities that they must balance against the demands of learning. Because of these responsibilities, adults may have barriers against participating in learning. These barriers could include lack of time, money, confidence, or interest, lack of information about opportunities to learn, scheduling problems, "red tape," and problems with child care and transportation. Try to consider these factors when scheduling the program. If it is to be delivered to people in a workplace it should fit around their work times and not require them to come back hours later well after they have completed a hard day's work. Week-ends might seem like good free time to learn but many adult learners are conditioned to week-ends being for family pursuits and are likely to be reluctant to give up hours away from this for financial training. Try to identify groups of learners for each program that can support each other in transport to where the program is delivered, assistance in minding young children and common interests outside of the formal learning. Groups seeking employment or those soon to retire are obvious examples of participants who will have similar interests and motivations and can help each other to access the training and learn collaboratively to use the new skills.

**5) ENGAGEMENT OF THE LEARNER**

Good program strategies encourage real learning, where the learner increasingly:

• takes responsibility and ownership of their learning;
• engages in experiential learning;
• partakes in cooperative learning; and,
• engages in reflective learning.

By requiring or encouraging your learners to take a more directive and active role in the program as it is delivered you are encouraging them to engage in the critical processes of:

• making meaning out of the new financial management knowledge they have;
• distilling principles from the program, which will aid their transference of financial skills to new contexts; and,
• practising their financial planning skills and mastering processes to improve their money management.

In your financial literacy program learner directed activities can also encourage greater levels of motivation. The learning is more purposeful, because they have a sense of ownership over what they achieve and identify themselves as the key beneficiaries of the outcomes. An abstract exercise in developing a savings plan for an imaginary person or family may appear to introduce the right principles but it may not resonate with the individuals you are training. Think of your target group. What are their savings goals?

What aspects of their income are available to saving and how can they work this out?

What form of saving is best for them in terms of achievable targets, regular contributions and limited risk?

Teachers and trainers often develop example exercises based on imaginary situations because, frankly, they appear to put everyone on the same testing level and it is easier to assess because there are a common set of 'right' answers. This is not the way to make financial literacy learning work for the target groups. They should be encouraged to work on individual situations entirely relevant to them. This may mean more effort on the part of the trainer in assisting with the work each person is doing and assessing outcomes but the result will be practical exercises that keep the learners involved and motivated.

**6) ASSESSING PROGRESS AND OUTCOMES**

Good assessment is a collaborative process involving the assessor, learners and others, where appropriate. Your assessment process should be transparent and allow for ongoing feedback from and to the learners. Remember these adult learners want to improve their skills in managing money and are not necessarily interested in formal recognition or being ranked against their peers in the group. Where possible, presenters should emphasise from the start that no-one is going to 'fail' the program. Even where students are seeking formal certification of their achievement, presenters can advise that there is no competition between the learners in the group or between an individual and the topic material – it's all achievable and everyone can make it work for them. Make sure they understand that they will all leave with better financial skills than they have at the beginning. If someone in the group is somehow 'better' or 'faster' at understanding superannuation than others that is their good fortune but makes no difference to the benefits everyone in the group gains from knowledge and skills in handling this important

financial tool. Everyone will improve their life chances through participating in the program and outside of training for formal certification, assessment is to demonstrate this to them and no-one else.

If you want further Information on collaboration in the design of assessment materials and the role of learners in the assessment process this can be found in:

• Guide One – Training Package Assessment Materials Kit and Guide Five – Candidate's Kit in the Training Package Assessment Guides; and,

• Learning Circles Resource Manual for Facilitators and Learners (developed by Adult Learning Australia).

**Conclusion:**

In traditional programs that the principles of psychology and curriculum planning, less attention is the form of content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided Affect the selection of pictures and images related to the concepts and content produced by including them.

Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. Duties are placed on the learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

1- - Preparation for adults to learn how much he depends on previous learning. Knowledge that has accumulated because of an ability to absorb new information more person is. Past educational experience features a diverse group of adult learners, the starting point of any activity on the diversity training is emphasized.

2- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable

3- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

4- To maximize learning, information must be provided an organized manner. Entries can be simple or complex can be arranged around related concepts are organized. Starting point for organizing content knowledge for adults and adults is linked to past experiences

5- Learning, especially regarding skills development, will be added frequently.

6 - Duties and meaningful content than meaningless subjects are learned more easily and are later forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

7- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

8- Environmental factors affect the learning. Tangible things such as noise, crowded places, temperature, light and ... Learning process can be prevented. Other factors such as stress, ridicule, pressure, fatigue and low health can also reduce learning.

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# Assessing of Ways to Strengthen Adult Education

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**Abstract:** Adult learners have a different approach to learning. By the time you reach adulthood, you're most likely responsible for your own success and you're perfectly capable of making your own decisions once you have the information you need. Adults learn best when learning is focused on them, not the teacher. This is called andragogy, the process of helping adults learn. Types of content and educational resources in various parts of adult curriculum materials motivational book, course materials, supplementary materials, track materials (continued) participatory form and materials. Incentives aimed at providing content that audiences are produced primarily to attract different groups of adults interested in design, so that their participation in learning programs are encouraged. Motivational training materials for learners and have great importance even in support of successful applications over learners, planners and executors for educational programs is important.
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**Keywords:** Adult Education; learn; course

## Introduction:

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal.

Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy" refers to the knowledge, skills, and competencies of individuals. The federal Adult Education and Family Literacy Act (Title II of the Workforce Investment Act)1 defines literacy as "an individual's ability to read, write, speak in English, compute and solve problems, at levels of proficiency necessary to function on the job, in the family of the individual, and in society." Literacy is often defined in terms of specific domains such as "basic academic skills," "workplace skills," "life skills," "parenting skills," or skills necessary to exercise one's rights and responsibilities for citizenship. Different dimensions of literacy are often categorized by terms that cluster several dimensions of literacy important for different clients. Examples include workplace literacy (combining both basic academic skills and workplace skills), and family literacy (combining basic academic skills and other skills essential for successful parenting).
2. "Education attainment" usually refers to the numbers of years of schooling completed or the level of credential (e.g., high school diploma or associate degree) an individual has obtained. Despite concerns about the meaning of credentials, there is a strong correlation between educational attainment and literacy.
3. "Literacy initiatives" often are defined in terms of the needs of a particular target group. These may be parents of young children, youth who have dropped out of high school without earning a high school diploma, welfare recipients, persons with limited English-speaking ability, incarcerated adults, or adults in the workforce.
4. Other literacy initiatives are defined in terms of a particular educational service, strategy, or means to address a target population's literacy problems. "Adult basic education" and "family literacy" are examples. These initiatives are often defined in terms of a particular configuration of services for the target population (e.g., assessment and information and counseling services).
5. The term "lifelong learning" is often associated with "literacy." Lifelong learning is a means to the goal of maintaining necessary levels of literacy throughout one's lifetime. The goal of lifelong learning has implications for both individual adult's learning behavior as well as education policy and the design of the education system.

Goal six of the National Education Goals illustrates a broadly stated goal that incorporates expectations about both adult literacy and the kinds of policies and services that should be in place to improve literacy. Goal six, "Adult Literacy and Lifelong Learning," states that, "By the year 2000, every adult will be literate and possess the knowledge

and skills necessary to compete in a global economy and exercise the rights and responsibilities of citizenship." The objectives related to this goal touch on several of the common elements of definitions listed above, for example:

- Different dimensions of literacy (e.g., academic and workplace skills),
- The level of education attainment (e.g., increasing the number of persons who complete postsecondary degrees),
- The needs of target groups (e.g., parents, minorities, or part-time learners),
- The need to increase the availability of particular educational services, strategies or means (e.g., accessibility of libraries to part-time learners or opportunities for parental involvement), and
- The importance of lifelong learning, both in the learning behavior of individuals and in the educational system's responsiveness to the needs of adult learners.

**Characteristics of adult education:**

**Flexibility in time:**

In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

**Flexibility in the location:**

One of the aspects of flexible space is that individuals can, regardless of their residence to the study and advancing their knowledge and skills pay. For example, adults in remote villages should like people who live in the city use of educational programs. After flexibility in other places is that the issue of specificity of location is not considered primarily educational.

**Flexibility in age:**

Educational opportunities for certain age should not use it for all regardless of their age, is possible. In fact, educational programs must use people of different ages to prepare.

**Flexibility in admission:**

No adult should not only be deprived of education because of the necessary conditions for admission in the class does. Of course this is not such a person without academic records to participate in university classes is accepted, Adoption order is that the adults in educational programs at different levels, according to the possibility of using the opportunity that is provided must be based on the experience and knowledge and their knowledge is.

**To combine education and job responsibilities:**

Adults should be able to work during that time engaged in training classes take them. In other words, their presence in the class should be considered part of their work. This means that low-literate or illiterate working people who are allowed to work an hour of your daily spending surpassed participation in educational programs.

**Ways to Strengthen Adult Education**

**1- Create a culture that supports adult study**

1. Communicate that learning is intrinsic to faith development. Lift up ongoing study, including adult education, as an essential function of any Christian community.
2. Reinforce the expectation of study participation from the pulpit and with new members.
3. Make Bible study a part of other church activities such as committee meetings and mission activities.
4. Use scripture meaningfully in worship. Don't assume your worshippers know the context of the passages read. Use sermons as an opportunity to teach the Bible.

**2- Offer a variety of formats, schedules, and approaches**

5. Experiment with a variety of times -- Sunday morning classes, weeknight groups, retreats, oneday events, and breakfast-hour or noon-time classes – depending on lifestyles in your congregation.
6. Consider scheduling some classes or small groups in homes or other community locations. Christian education doesn't happen only in church buildings.
7. Start new studies and groups often. Despite their best intentions, ongoing groups have a tendency to become cliquish. Newcomers are far more likely to feel comfortable joining something new.
8. Have as your goal a Bible study program that exposes church members to the entire biblical witness over time.
9. Recognize different learning styles among individuals and age groups. Older folks tend to be most comfortable with traditional classroom structures. Boomers are inclined to question authority and enjoy discussion. Younger persons are more accustomed to media and technology and prefer a fast-paced, informal style.
10. Make use of a variety of different approaches, including lectionary-based studies, topical studies, character studies, etc.

11. Incorporate different learning strategies, such as role playing, dramatization, guided meditation, even memorization.
12. Churches too small for a large number of groups can vary their approach by rotating different studies and curricula with groups.
13. Don't teach "about" the Bible in a way that doesn't allow people to encounter the texts for themselves. Encourage individual reading or make it part of the group's time together.
14. Encourage active, discussion-based learning. Break into small conversation groups frequently.
15. Allow for diversity in perspectives.
16. Encourage the use of a variety of different biblical translations. Those less experienced in Bible study may find it helpful to read from a paraphrase.

**3- Meet people where they are**

17. Acknowledge biblical illiteracy among many adult church-goers – even the well-educated – and strive for methods that straddle this paradox.
18. Recognize that some beginners will be turned off by "homework." Use videos, in-class readings, dramatizations, or audio tapes as alternative ways of getting everyone "on the same page" and ready for discussion, all the while encouraging the habit of daily scripture reading.
19. Provide short-term classes for those who won't commit to a long-term study or ongoing class, but make these short-term learning experiences "stepping stones" toward greater involvement.
20. Conduct "taster" classes for those who want to try out the experience before they commit to it. Select topics that will appeal to those new to Bible study.
21. Break an ongoing class into shorter, defined segments, each with a clearly identified focus. With each new segment, take the opportunity to publicize the topic and invite newcomers.
22. Teach stewardship of time to counteract "busyness." Just as with financial stewardship, persons need to be encouraged to make Christian education a priority. Encourage "first fruits" commitments of time.
23. Be clear about expectations with regard to attendance, participation, and preparation.

**4- Promote participation effectively**

24. Link group study topics to sermon series and encourage participation from the pulpit.
25. Emphasize study during Lent. Select a topic or curriculum for church-wide study during this period and encourage all to take part. Tie the topic into preaching and worship.
26. Lift up study leaders and participants. Celebrate every time a new group starts or completes a study program. Use the newsletter, a photo board, or a dedication service in worship.
27. Ask class members to write a newsletter article or testify about the significance of their learning experiences.
28. Remember that personal invitations are usually the most effective way of getting someone involved in any activity.
29. Capitalize on the current popularity of book clubs and films by creating opportunities for those who enjoy these activities. Check out "Reel Time" from Cokesbury.

**5- Foster strong leadership**

30. Recruit leaders as the first step toward forming groups. Groups will often form around a gifted leader.
31. Stress the group leader's role as facilitator, rather than teacher. Setting up one person as "the expert" creates a poor group dynamic and discourages new people from stepping into leadership. Thinking of group leaders as facilitators allows Scripture and the Holy Spirit to do the teaching.
32. Expect your pastor to model the importance of ongoing adult education by leading and participating in study, but don't reinforce the notion that only the ordained can lead study groups.
33. Take advantage of the leader training opportunities provided in conjunction with many popular study curricula.
34. Provide orientation and ongoing support for group leaders.
35. Train leaders in group process so they can keep their groups on track, being sensitive to the need to keep more outspoken participants in check and draw out the more reserved using phrases like, "Let's hear from some of the others," or "You look like you have something to say."
36. Emphasize the importance of leader preparation, especially mapping out discussion questions in advance.
37. Encourage team leadership. Experienced leaders should invite a newer person to pair with them in leading groups to develop the less experienced leader.
38. Rotate the leadership responsibility within a group so that all participants get experience leading sessions.
39. Know that Sunday School classes and small groups are one of the best places to develop lay leaders and lay relationships that strengthen the church.

**6- Use resources effectively**

40. Stay abreast of new resources, including those available from other denominations or traditions and the secular press.
41. Don't be afraid to introduce ideas and resources from a variety of theological perspectives. Trust the discernment abilities of individuals and the group.
42. Use workbook-style studies creatively. Nothing is more boring than a lesson read straight out of a leader's manual. Find ways to make pre-packaged lesson plans come alive.
43. Use videos to bring expert perspectives to bear and to get everyone "on the same page" for discussion. But avoid class sessions that are no more than viewing a video, or participants will soon wonder why they shouldn't stay home and watch their own TV.
44. Create a resource center with reference materials, maps, and other items to support your leaders and participants.
45. Don't allow your church library to become a museum. Update the collection. Offer books and resources linked to sermon topics and congregational study themes.
46. Consider a book sales kiosk and stock it with things you'd like your congregants to be reading. Many busy people would rather buy a book than worry about due dates and library fines.

**7- Stress spiritual formation**

47. Remember, the goal is formation, not information. Every class should be deliberate in helping members accept God's grace, grow in faith, deepen their relationship to the Christian community, and answer Christ's call to discipleship.
48. Include prayer as part of every study session and encourage group members to pray for one another daily.
49. Encourage a covenantal relationship within study groups.
50. Nurture a sense of Christian community and connectedness within groups. A Sunday School class or small group can be a "home" for individuals within a larger church.

**Conclusion:**

As indicated earlier, a strength of adult education is the dedication of the many teachers often serving under difficult conditions, without adequate support, and often with compensation and benefits less than teachers in the public schools. Testimony before the task force characterized the work of adult educators as "missionary" work. Recognizing the seriousness of the adult literacy issue in Kentucky, it should be a major concern that the Commonwealth does not have a comprehensive approach to the professional preparation, development, and support of adult educators.

The challenge for Kentucky will be to move from a system that still depends on teachers with limited training in working with adults, to one in which professional competence in working with adults is a basic requirement. Any strategy to make this transition must involve both professional development and support for the teachers now in the field as well as a new system for a new generation of adult educators.

Beyond the issues relating directly to DAEL(Department of Adult Education and Literacy), the task force heard a number of concerns about the Commonwealth's overall approach to adult literacy.

- Lack of coherent statewide leadership and coordination among multiple complementary initiatives aimed at the same problem.
- Lack of continuity in state leadership. Cited in particular was the difficulty sustaining a high level commitment to the issue long enough to make a difference because of changes in priorities of the state's political leaders. A high level of turnover in the leadership of the Department of Adult Education and Literacy has also contributed to the instability.
- Tendency to think of adult education as a separate categorical program rather than a strategy that cuts across the mission and responsibility of multiple Commonwealth programs and initiatives (e.g., early childhood education, welfare reform, economic development, and corrections).
- Multiple uncoordinated categorical federal initiatives that tend to drive (and fragment) policy for an overall state effort that is largely funded by Kentucky.
- A tendency to commingle and confuse different functions. The most important distinction is between functions focused on the needs of clients (adult learners, employers, communities, regions, and the Commonwealth as a whole) and functions associated with the operations and performance of providers. It is important that each of these functions receive attention, yet the tendency is for one (e.g., overseeing a network of providers) to drive out attention to overall system strategy.
- Inadequate coordination of services to meet the needs of individual adults, communities, employers, and regions is hindered by:

- Vertical financing and regulatory relationships between separate federal and state programs and local providers and administrative units. These vertical

relationships can hinder the horizontal coordination of services for individual adult learners, communities, and employers.
- Turf wars among providers, local politics, and long-standing conflicts among neighboring counties.

- Inadequate links with and leverage of other public and private initiatives and investments to reach the target population. Major sources of help include employers, postsecondary education, and workforce development.
- Lack of a state financing policy and strategy for provider performance incentives and collaboration, and tax and other employer incentives for leverage of non-state resources.
- Lack of programmatic and administrative flexibility to meet the rapidly changing needs of adult learners, employers, regional economies, and communities.

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# Methicillin Resistant *Staphylococcus aureus* - Post surgical Infections in Egyptian Hospital

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**Abstract:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of bacteria that is resistant to certain antibiotics including methicillin, oxacillin, penicillin and amoxicillin. Our study investigated the reason of Methicillin Resistant *Staphylococcus aureus* (MRSA) infection in an Egyptian hospital in which multiple drug resistant *S. aureus* was isolated from pus, sputum and blood of infected cases. Our objective was to detect the *mec-A* gene using PCR analysis to confirm that the multiple drug resistant *S. aureus* is MRSA as well to find the drug of choice to be used for competing such infections and to find a safe method for competing MRSA using probiotics. The antibacterial effect of probiotic strains isolated from different animals was tested against MRSA isolates. The results obtained from molecular analysis identified the *mec-A* gene in six out of seven tested samples with a great success with an incidence 85.71%. Moreover, the results revealed that cefobid as well as claforan are the drugs of choice for competing MRSA. *B.subtilus* followed by *L. acidophilus* isolated from colostrum of mare showed great capability of hindrance of MRSA, then *L. palantarum*, *Bifidobacterium* and finally *L.acidophilus* isolated from goat colostrums, *L. acidophilus* isolated from buffalo-cow milk on the contrary showed no activity against MRSA. Our study identified *mec-A* gene from MRSA strains was confirmed to be the main cause of MRSA outbreak in infected hospital patients subjected to stressful conditions due to severe skin infections.
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**Keywords:** MRSA, PCR, mec-*A* gene, Antibiotic sensitivity, Human; Probiotic.

## 1.Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of bacteria that is resistant to certain antibiotics including methicillin, oxacillin, penicillin and amoxicillin (Wichelhaus *et al*. 1997). *S. aureus* remains one of the most intensively investigated bacterial species in human and animal pathogen, it can cause a variety of nosocomial and community-acquired infections ranging from minor skin abscesses to serious potentially life-threatening diseases such as bone and soft tissue intra- surgical infections, sepsis and invasive endocarditis (Chambers, 2001; Enright *et al.* 2002). In terms of resistance, *S. aureus* infections cause an increasing problem. Methicillin-resistant *S. aureus* (MRSA) have spread worldwide, with infection rates >40% in Japan and Southern Europe ( Bozdogan *et al.* 2003 and Chang *et al.* 2003) and 20%-25% in the United States (Singh *et al.* 2006).

Conventional methods (culture, biochemical tests and antimicrobial susceptibility) are limited and time consuming. In particular, PCR appears to be a rapid, sensitive, and specific assay for *mec-A* gene (Geha *et al.* 1994). Murakami *et al.* (1991) determined the resistance to methicillin by the *mec-A* gene, which allows a bacterium to be resistant to antibiotics such as methicillin, and other penicillin-like antibiotic, because it does not allow the ring like structure of penicillin-like antibiotics to attack the enzymes that help forming the cell wall of the bacterium, and hence the bacteria is allowed to replicate normally.

Alternative medicine treatments have been practiced for thousands of years around the world, because the effectiveness of currently available antibiotics is decreased due to increasing the number of resistant strains causing infections including MRSA. Lactic acid bacteria (LAB) are famous as friendly bacteria for human health due to their production of bacteriocins (Settanni and Corsetti, 2008).

The aim of the present work is isolation and biochemical characterization of MRSA as well as detection of *mec-A gene* using PCR. MRSA sensitivity was checked against different antibiotics as well as different probiotic strains isolated from different sources.

## 2. Materials and Methods:

### Human clinical sampling:

An investigation was carried out in a hospital showing spread of multiple drug resistant *S. aureus* (18 strains) isolated from different samples including 13 pus (10 males and 3 females) as well as 4 sputum (3 males and 1 female) and 1 blood sample from male as shown in table (1).

### Bacteriological sampling and monitoring bacterial profile:

Bacterial swabs were collected under aseptic conditions, cultivation of samples, isolation and purification of the isolates were carried out using different media which were purchased from (Oxoid); swabs were inoculated into a tube containing 10 ml tryptic soy broth. The broth was incubated at 37ºC for 24 hrs then streaked from the enriched broth onto nutrient, mannitol, blood and MacConkey agar plates. Identification of isolates includes morphological examination by Gram's stain (Cruickshank *et al.* 1975). Biochemical identification was carried out according to Collee *et al.* (1996); CDC (2005) including catalase, oxidase, coagulase, gelatin liquefaction, acetone production and sugar fermentation test including; glucose, maltose, lactose, sucrose, sucrose and mannitol with the production of acid without gas. Mannitol fermentation may be used for provisional identification of human *S.aureus*.

***S.aureus* identification and characterization**:

*Staphylococcus* isolates were streaked onto mannitol salt agar with 2 µg/ml oxacillin and incubated aerobically at 35°C for 48 hrs. Colonies identified as *S. aureus* were diagnosed according to Bottone *et al.* (1984); CDC, (2005). Confirmation of strains was carried out using Staphytect plus dry spot (Oxoid) as latex identification for *S. aureus*.

**Antibiotic sensitivity test:**

*In vitro* sensitivity of *S.aureus* strains (18) were done against 15 different Antibiotics was carried out using Agar diffusion antibiotic sensitivity test was carried out for all isolated strains during the outbreak according to Beaney *et al.,* 1970. Interpretation was carried out according to NCCLS, (2002), Antibiotic discs were obtained from Oxoid including B-lactams [penicillin-G (10 units), amoxicillin/clavulinic acid (20/10 µg/ml), cefotaxime (30 µg/ml)] , macrolides [erythromycin (15 µg/ml)], aminoglcosides [gentamicin (10 µg/ml)], fluoroquinolones [ciprofloxacin (5 µg/ml), ofloxacin (5 µg/ml)] cefadroxil (30 µg/ml), cefobid (75mcg), tetracycline (30 µg/ml), tobramycin (10 µg/ml), sulpha/ trimetho (23.75+1.25 µg/ml), amikacin (30 µg/ml) , amoxy/fluclox (25 µg/ml) and claforan (30mcg).The percentages of sensitive, intermediate and resistant are shown in Tables (2).

**Polymerase Chain Reaction**

**DNA extraction from culture samples**

DNA from cultured bacteria was extracted Biospin Bacteria Genomic DNA Extraction Kit, Bioflux. Seven multiple drug resistant *S. aureus* were selected for investigating the presence of *mec-A* gene to prove whether the tested strains are MRSA or not. Single colonies of isolates were cultured in Luria-Bertani medium and incubated for 16 h at 37ºC. An aliquot (4 ml) of overnight culture ($10^9$ CFU) was pelleted by centrifugation (13.000 rpm for 4 min). Bacterial pellet was resuspended in 100µl of Elution buffer and extraction was carried out according to instruction by Bioflux Company using (Biospin Bacteria Genomic DNA Extraction Kit).

**PCR amplification:**

On the basis of the DNA sequences of the *mec-A* gene, the following oligonucleotides were used in PCR amplification: primers M1 (TGG CTA TCG TGT CAC AAT CG) and M2 (CTG GAA CTT GTT GAG CAG AG), which amplified a 310-bp fragment of the *mec-A* gene (Vannuffel *et al.* 1995). The amplification was carried out using Pyrostart Fast PCR Master Mix (Fermentas Company). Molecular and conventional tests were performed in different laboratories and the results were compared.

***In vitro* antimicrobial activity of probiotic bacteria against MRSA using well diffusion assay (Sgouras *et al.*, 2004):**

*mec-A* gene positive MRSA strains were tested for their sensitivity toward different probiotic strains as follow; Bifidobacterium, *L.palantarum, L. acidophilus* (buffaloe –cow milk, cow milk, mare colostrum as well as goat colostrum), *B. suibtlus*. Strains were plated onto Mueller Hinton agar plates. The *In vitro* antibacterial activity of the tested probiotic strains using agar well diffusion test was carried out as follow; Muller Hinton agar plates were prepared and wells were drilled out using Pasture pipettes, the plates were inoculated with MRSA strains prepared in conc. equivalent with 0.5 MacFerland tube and streaked onto the agar plates using sterile swabs, and then 50 µl aliquots of cell free cultures supernatant in fresh DeMan Rogaso Sharpe (M.R.S.) broth of the probiotic strains were suspended in the agar wells. Plates were incubated at 37˚C for 24hrs under aerobic conditions and the diameters of inhibition zones around wells were measured in mm using a ruler. The experiment was carried out in duplicate and the mean of the zone of inhibition was estimated as follows; ++ showed zone of inhibition ≥ 8 mm, + showed zone ≤ 7mm and – indicate complete absence of inhibition.

## 3. Results

Traditional analysis of 18 samples collected from post-surgical side infected patients including; 14 males and 4 females (13 pus, 1 blood and 4 sputum) Table (2) were carried out using bacterial isolation and biochemical identification. Results revealed the presence of Gram positive, non-spore forming cocci, arranged in form of grapes or in irregular clusters. The colonies are circular, smooth and glistening. On blood agar, they are beta-hemolytic. Colonies are golden yellow. Biochemically; they were catalase, coagulase positive and mannitol fermenter which proved to be *S. aureus*. Antibiotic sensitivity test was

carried out using fifteen antibiotic disks (Oxoid). *In vitro* antibiotic sensitivity test against eighteen *S. aureus* isolated strains, showed resistance against cefotaxime, cefadroxil, sulph/trimetho and amoxy/fluclox with an incidence equal 94.40%. Resistance against pencillin–G, erythromycin, gentamycin, tetracycline and amickacin was shown with an incidence 89.00% and resistance aganist amoxicillin/clavulinic acid, ciprofloxacin and ofloxacin with an incidence 83.30%. Drugs that were capable of hindrance of such strains were cefobid and claforan with an incidence of 83.30%, thus they were used successfully for treatment of infected cases as shown in Table (2, 3). Molecular analysis was carried out for detection of *mec-A* gene which was responsible for multiple drug resistance of the tested strains to confirm that tested strains were MRSA. Results revealed that the isolated strains from pus samples of patients subjected to stressful conditions due to post operative infections were MRSA; Molecular analysis identified the *mec-A* gene in six out of seven samples with an incidence equal 85.71% as shown in Photo (1). On investigating the use of probiotics for competing MRSA as a safe method for hindrance of multiple drug resistant strains, results revealed that *B.subtilus* isolated from mare fecal swab followed by *L. acidophilus* isolated from mare colostrum showed great capability of hindrance of MRSA, followed by *L. palantarum* then *Bifidobacterium* and finally *L.acidophilus* isolated from goat colostrums. On the contrary, *L. acidophilus* isolated from buffalo-cow milk showed no activity against MRSA as shown in Photos (2, 3) and Table (4).

**Table (1): Diagnosis of Cases showing multiple drug resistant *S. aureus* and site of sample collection.**

| Sample type | Case Diagnosis | Male | Female | Total |
|---|---|---|---|---|
| **Pus** | **Burn** | 0 | 1 | 1 |
| | **Boil** | 2 | 1 | 3 |
| | **SSI** | 4 | 1 | 5 |
| | **Bone Fracture** | 2 | 0 | 2 |
| | **Plastic flap** | 1 | 0 | 1 |
| | **Osteomyelitis** | 1 | 0 | 1 |
| **Sputum** | **Cancer Larynx** | 1 | 0 | 1 |
| | **Chest infection** | 2 | 0 | 2 |
| | **Stroke** | 0 | 1 | 1 |
| **Blood** | **Head trauma** | 1 | 0 | 1 |
| | **Total** | 14 | 4 | 18 |

**Table (2): Antibiotic sensitivity test against *S. aureus* (18 strains)**

| Antimicrobial agent | Conc. of disk | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % |
| **B-lactams** | | | | | | | |
| Penicillin-G | 10 units | 0 | 0.00 | 2 | 11.00 | 16 | 89.00 |
| Amoxicillin/clavulinic acid | 20/10 µg/ml | 0 | 0.00 | 3 | 16.70 | 15 | 83.30 |
| Cefotaxime | 30 µg/ml | 0 | 0.00 | 1 | 5.60 | 17 | 94.40 |
| **Macrolides** | | | | | | | |
| Erythromycin | 15 µg/ml | 0 | 0.00 | 2 | 11.00 | 16 | 89.00 |
| **Aminoglcosides** | | | | | | | |
| Gentamicin | 10 µg/ml | 0 | 0.00 | 2 | 11.00 | 16 | 89.00 |
| **Fluoroquinolones** | | | | | | | |
| Ciprofloxacin | 5 µg/ml | 0 | 0.00 | 3 | 16.70 | 15 | 83.30 |
| Ofloxacin | 5 µg/ml | 0 | 0.00 | 3 | 16.70 | 15 | 83.30 |
| **Cefadroxil** | 30 µg/ml | 0 | 0.00 | 1 | 5.60 | 17 | 94.40 |
| **Cefobid** | 75mcg | 15 | 83.30 | 3 | 16.70 | 0 | 0.00 |
| **Tetracycline** | 30 µg/ml | 0 | 0.00 | 2 | 11.00 | 16 | 89.00 |
| **Tobramycin** | 10 µg/ml | 0 | 0.00 | 4 | 22.00 | 14 | 78.00 |
| **Sulpha/ trimetho** | 23.75+1.25 µg/ml | 0 | 0.00 | 1 | 5.60 | 17 | 94.40 |
| **Amikacin** | 30 µg/ml | 0 | 0.00 | 2 | 11.00 | 16 | 89.00 |
| **Amoxy/fluclox** | 25 µg/ml | 0 | 0.00 | 1 | 5.60 | 17 | 94.40 |
| **Claforan** | 30mcg | 15 | 83.30 | 3 | 16.70 | 0 | 0.00 |

**Table (3): *S. aureus* strains isolated from pus samples tested for *mec-A* gene presence and antibiotics used for cases treatment.**

| PCR (pus) Sample | Age (years) | Sex | Diagnosis | Antibiotic used for treatment |
|---|---|---|---|---|
| **1** | 30 | male | Bone fracture | Claforan and Cefobid |
| **2** | 8 | male | Bone fracture | Claforan and Cefobid |
| **3** | 30 | male | Plastic flap | Claforan and Amikane |
| **4** | 44 | male | SSI | Claforan and Cefobid |
| **5** | 22 | male | SSI | Claforan and Cefobid |
| **6** | 10 | male | SSI | Claforan and Cefobid |
| **7** | 41 | female | SSI | Claforan and Cefobid |

**SSI=Surgical side infection**

**Table (4): The antimicrobial activity of the tested probiotic strains against MRSA.**

| Probiotic strains | Source | Tested MRSA strains | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| ***B.subtilus*** | fecal swab of mare | ++ | ++ | + | + | + | ++ | ++ |
| ***L. acidophilus*** | buffaloe-cow milk | - | - | - | - | - | - | - |
| ***Bifidobacterium*** | cow milk | + | + | + | - | - | + | + |
| ***L. acidophilus*** | goat colostrums | + | - | - | - | - | - | - |
| ***L. acidophilus*** | mare colostrum | ++ | ++ | + | + | + | + | + |
| ***L. palantarum*** | cow milk | ++ | ++ | + | - | + | + | + |

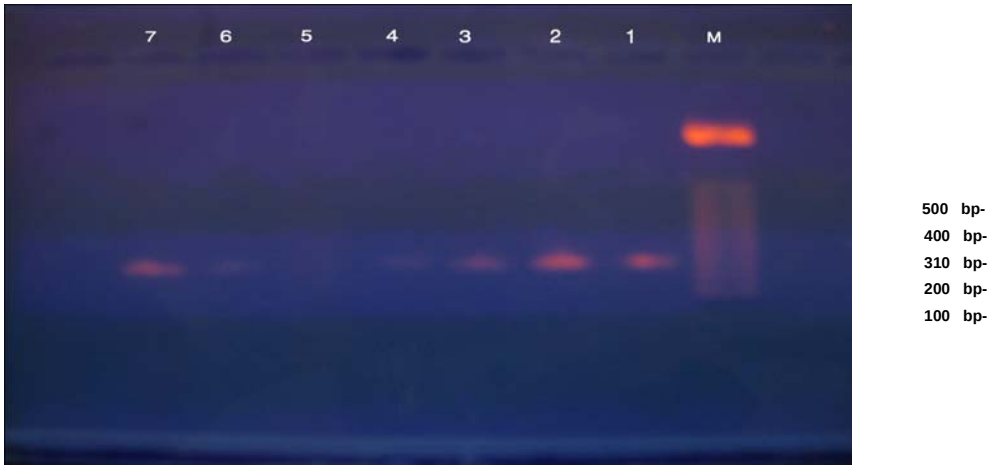


**Photo (1)** Gel electrophoresis of DNA fragments showing 310 bp amplified fragment of *mec-A* gene among the examined *S.aureus* isolated from hospitalized patients. Lane M represents DNA ladder. Lanes 1 to 7 represent *S. aureus* isolated strains as follow; (1) Pus from bone fracture of male, (2) Pus from bone fracture of male, (3) Pus from plastic flap of male, (4) Pus from surgical side infection of male, (5) Pus from surgical side infection of male (6) Pus from surgical side infection of male, (7) Pus from surgical side infection of female

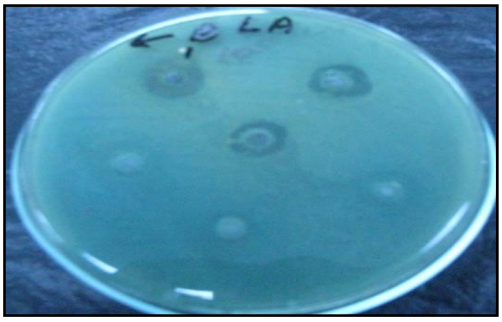

**Photo (2)** MRSA strain (1) isolated from pus of human, show variable sensitivity toward *B.subtilus* isolated from the fecal swab of mare, followed by *L. acidophilus* isolated from colostrum of goat, *Bifidobacterium* isolated from cow milk sample, *L. acidophilus* isolated from Buffalo-cow milk then *L. acidophilus* isolated from colostrum of mare, *L. palantarum* isolated from cow milk, in sequence with the arrow.

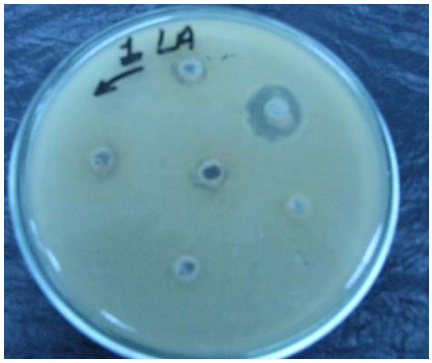


**Photo (3)** MRSA strain (2) isolated from pus of human, show variable sensitivity toward *B.subtilus* isolated from the fecal swab of mare, followed by *L. acidophilus* isolated from colostrum of goat, *Bifidobacterium* isolated from cow milk sample, *L. acidophilus* isolated from Buffalo-cow milk then *L. acidophilus* isolated from colostrum of mare, *L. palantarum* isolated from cow milk, in sequence with the arrow**.**

**4. Discussion**

The present study was carried out to investigate the cause of development of multiple drugs resistant strains as side infection following surgical operations in an Egyptian hospital. Results showed that the outbreak was due to infection with multiple drug resistant *S. aureus* (MRSA). This study proved that MRSA is an opportunistic pathogen which is abused of being a critical pathogen responsible for a great morbidity especially among immunosuppressed cases. MRSA outbreaks in hospitals suggested that this organism cause an emerging problem in patients subjected to multiple stressful conditions. Results agree with Hudson (1994) and Cookson (1998) who proved that the treatment of *S. aureus* infections may be complicated by multiple antibiotic resistances and specific virulence factors, causing temporary or long-lasting carriage. The nasal carriage of MRSA is a main risk factor for community-acquired infections and in hospital settings (nosocomial sepsis). Also, results agree with Hiramatsu *et al.* (2002) who found that infection rate in carriers of *S. aureus* is higher than in non-carriers, and it has been well documented that humans are usually infected with their own nasal isolate. Results also agree with Gomez-Lucía *et al.* (1989); Kloos and Bannerman (1999) who mentioned that *S. aureus* is an opportunistic pathogen which can cause diseases ranging from superficial soft-tissue infections to life-threatening bacteraemia and toxic shock syndrome. These findings agree with Quinn *et al.* (2002) who abuse MRSA of being a critical pathogen responsible for a great morbidity and mortality especially among immunosuppressed cases.

Antibiotic sensitivity test against eighteen isolated strains showed resistant against cefotaxime, cefadroxil, sulph/trimetho and amoxy/fluclox with an incidence equal 94.40%. Resistance against pencillin –G, erythromycin, gentamycin, tetracycline and amickacin was shown with an incidence 89.00%. Amoxicillin/clavulinic acid, ciprofloxacin and ofloxacin were resistant with an incidence 83.30%, on the other hand, the tested strains were sensitive against cefobid and claforan with an incidence of 83.30%. These results agree with Quinn *et al.* (2002) who proved that MRSA either produce potent toxins or resist a wide range of antibiotics. Results agree with Karska-Wysocki *et al.* (2010) who showed that MRSA is a multidrug-resistant microorganism and the principal nosocomial pathogen worldwide. Tiwari *et al.* (2009) compared the performances of four phenotypic tests used to detect methicillin resistant *S. aureus* (MRSA) with the *mec-A* gene polymerase chain reaction. Two hundred thirty-seven *S. aureus* isolates were isolated from different patients visiting Hospital and subjected to cefoxitin and oxacillin disc diffusion tests, oxacillin minimum inhibitory concentration (MIC) test, and oxacillin screen agar test. The authers stated that cefoxitin disc diffusion test can be considered as the best method for routine detection of MRSA when molecular techniques are not available.

Molecular analysis performed in the present study was necessary to assess the feasibility of the PCR approach for the identification of *S. aureus* multiple drug resistant strain. Our study revealed that PCR analysis verified that 85.71% of the tested strains carried *mec-A* gene at 310 bp fragment. On the basis of these results, the PCR strategy could give rapid and reliable information to clinicians not only for the identification of pathogenic bacteria but also for therapeutic management. Results agree with Montanari *et al.* (1990) who explained the absence of

*mec-A* gene in the 25 MRSA isolates by overproduction of penicillinase. Also, Oshima *et al.* (1993) amplified *mec- A* gene by PCR and reported that the gene was positive in all MRSA strains. They reported that identification of MRSA by drug susceptibility tests alone presented a serious problem because numbers of clinical *S. aureus* isolates are border line resistant to methicillin. Hence quick, accurate and sensitive method of PCR based amplification for the detection of the *mec-A* gene is necessary. They added that detection of *mec-A* gene by PCR is extremely important for appropriate treatment of MRSA. Our results agree with Vannuffel *et al.* (1995) who indicated that MRSA has become a major nosocomial pathogen not only in tertiary care hospitals but also in chronic care facilities. Also results agree with Anderson and Weese (2006) who found that conventional identification of MRSA requires between 24-48 hours after sampling and recommended rapid and sensitive method of identification as PCR for detection of *mec-A* gene which codes for the drug resistant penicillin-binding protein 2a(PBP2a) or 2(PBP2). Klotz *et al.* (2005) reported an increase in the frequency of MRSA as an important causative agent of nosocomial infections worldwide, in spite of optimal hygienic conditions high number (24.1%) was isolated from human stool samples.

The present study focused on studying the effect of different probiotic strains and tested their efficiency on hindrance of the growth of MRSA, Results revealed that *B. subtilus* isolated from fecal swab of mare followed by *L. acidophilus* isolated from colostrum of mare showed great capability of hindrance of MRSA, then *L. palantarum*, *Bifidobacterium* isolated from milk and finally *L.acidophilus* isolated from goat colostrum. On the contrary *L. acidophilus* isolated from Buffalo-cow milk showed no activity against MRSA. Results agree with Karska-Wysocki *et al.* (2010) who tested the antibacterial activity of lactic acid bacteria against MRSA from ten human clinical isolate using *L. acidophilus* CL1285® and *L. casei* LBC80R as pure cultures. They demonstrate that the direct interaction of lactic acid bacteria and MRSA in a mixture led to the elimination of 99% of the MRSA cells after 24 h of their incubation at 37 °C.

## 5. Conclusion and Recommendations

Misuse of antibiotics must be controlled as it might be the main cause of outbreaks due to their immunosuppressive effect on infected cases. Rapid diagnosis should be carried accurately including screening of unusual causes of multiple drug resistant strains in hospitalized patients. Researchers recommended that hospitals should initiate surveillance programs for MRSA infections using quantitative PCR, particularly in post surgical operations to clarify the role of MRSA in drug resistant outbreaks. Research group advice the use of probiotics as *B.suibtlus* or *L.acidophilus* as feed additives in human subjected to stressful conditions for hindrance of opportunistic microorganisms during stressful conditions.

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# Factors Influencing the Adoption of Nanocides in Controlling the Fire Blight among Apple Producers in Iran

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**Abstract:** Apple producers in the Province of East Azarbaijan were surveyed in order to explore their perception about factors influencing the adoption of nanocides in controlling Fire Blight among apple producers in Iran. As the factor analysis showed, the factors were categorized into four groups, namely marketing, social, regulatory and economic, ordered by the magnitude of their impact.
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**Keywords:** Apple producers, Nanotechnology, East Azarbaijan Province, Adoption, Nanocide, Fire Blight

## 1. Introduction

The emerging field of nanotechnology has the potential to bring about changes as big as the European Industrial revolution in the late 18th and early 19th century. A hundred and fifty years ago, the mechanization of industry, the introduction of steam power and improved transportation systems brought huge technological, socioeconomic and cultural changes. Today, nanotechnology is forecast to underpin "the next industrial revolution", leading to far-reaching changes in social, economic and ecological relations (Miller and Senjan, 2006).

As well as developing improved systems for monitoring environmental conditions and delivering nutrients or pesticides as appropriate, nanotechnology can improve our understanding of the biology of different crops and thus potentially enhance yields or nutritional values. In addition, it can offer routes to added value crops or environmental remediation.

In regard to application of Nanocides in encapsulation of pesticides, researchers pointed out the impetus for formulating pesticides on the nano-scale is the changed behavior of the reformulated product: the strength of the active ingredient can be maximized and biological activity is longer-lasting (ETC, 2004).

Nanocide is one of the most effective antibiotics made in human history. It destroys more than 650 species of bacteria, fungi, and viruses. Nanocide is produced by using natural materials and new technology. It has lack of microbial resistance in long use and maintains the sterilized environment for a long time. Nanocide also has an optimum performance in various ph and does not have any side effect.

The adoption of any new technology and innovations has not been an easy task and it is not spontaneous, the technology has to be taught and learned –adopted to existing experience and integrated into production. As is often the case with technological-innovation potential and expectations can outpace reality (Gelb and Bonati, 2005).

Several parameters have been identified as influencing the adoption behavior of farmers and social scientists investigating farmers who adopt the biotechnology showing the demographic variables, technology characteristics, information source, knowledge, awareness, attitude and group influence affect adoption behavior (Oladele, 2005).

Successful adoption of any new technology in developing countries will depend on the availability of technologies appropriate for local agricultural conditions, and policies that enhance the ability of poor farmers to obtain these technologies (Ameden et al., 2005).

A major issue that will affect successful applications of new technology such as bio and nanotechnologies to agriculture is the regulatory climatic governing the release of new products. Developing societies will need to develop and implement regulatory measures to manage any environmental, economic, health and social risks associated with genetic engineering (Ozor, 2008).

The results of the study by Spielman and others (2006) suggest that the regulatory environment governing the introduction of new technologies is slowing the forward movement of research into later stages of product development.

Nanotechnology can play an important role in improving the quality and quantity of agricultural products. Therefore, it is necessary to remove the

impediments faced by farmers and provide basic information to enable the spread of nanotechnology. This would enable nanotechnology to be part of a comprehensive development strategy for agricultural sector.

Developing countries such as Iran have adopted their own nanotechnology programs with a specific focus on agricultural applications. The Iranian Agricultural ministry is supporting a consortium of 35 laboratories working on a project to expand the use of nanotechnology in agro sector. The ministry is also planning to hold training programs to develop specialized human resources in the field (Joseph and Morrison, 2006).

In the year 2001, the Iran presidential technology cooperation office initiated a smart move in the field of nanotechnology. Through these efforts, nanotechnology gained national priority in the country and in 2003 the Iranian Nanotechnology Initiative was set up with the aim of pursuing the development of nanotechnology in Iran.

The attitudes and interests of stakeholders involved in national public debates on the risks and benefits of agricultural technology are having a significant influence on public opinion as well as public policy outcomes in developed and developing countries (Aerni, 2005).

Evidence shows that even small efforts to informing farmers and increasing their knowledge about the new technologies can have big results. However, the promise has yet to be realized due to the lack of information and access to this technology among rural communities. Therefore, it is necessary to remove the impediments faced by rural population and provide basic information in rural areas to enable the spread of new technologies.

Fire Blight is one of the major diseases in granular fruit trees in the world and Iran. Even if the disease spreads in limited areas, it will cause serious damage to trees. Therefore, precise control program to contain the disease and its distribution area should be implemented.

The disease in the provinces of East Azarbaijan has caused serious damages to the apple gardens. Ministry of Agriculture in this province has started a program to recommend the diffusion and adoption of nanocides in controlling Fire Blight in the apple trees among gardeners. The research question for this study is: what are the perceptions of gardeners about factors influencing the adoption of nanocides in controlling Fire Blight among apple producers in Iran?

## 2. Material and Methods

A series of in-depth interviews were conducted with some senior experts in the nanotechnology to examine the validity of questionnaire. A questionnaire was developed based on these interviews and relevant literature. The questionnaire included both open-ended and fixed-choice questions. The open-ended questions were used to gather information not covered by the fixed-choice questions and to encourage participants to provide feedback. The total population for this study was 61 apple producers in the East Azarbaijan Province.

Measuring respondents' attitudes towards factors influencing the adoption of nanocides has been achieved largely though structured questionnaire surveys. The final questionnaire was divided into several sections. The first section was designed to gather information about personal characteristics of respondents. The second section was designed to measure the attitudes of respondents about factors influencing the adoption of nanocides in controlling fire blight disease. Four factors were presented in a 5-point Likert format. The variables and their measurement scale are presented in Table 1.

Content and face validity were established by a panel of experts consisting of faculty members at Islamic Azad University, Science and Research Branch and some specialists in the nanotechnology. Minor wording and structuring of the instrument were made based on the recommendation of the panel of experts.

A pilot study was conducted to determine the reliability of the questionnaire for the study. Computed Cronbach's Alpha score was 89.0%, which indicated that the questionnaire was highly reliable. The data collected by interviewing the respondents and analyzed by using ordinal factor analysis technique.

**Table 1:** Variables and their measurement scale

| Variables | Measurement Scale |
|---|---|
| Regulatory Factors | Five- point Likert |
| Economic Factors | Five- point Likert |
| Social Factors | Five- point Likert |
| Marketing Factors | Five- point Likert |

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## 3. Results

The results of descriptive statistics indicated that majority of respondents were male. The educational level of all respondents was under high school diploma.

The classification of the factors into four latent variables was displayed in table 2. The variables were classified in economic, social, marketing and regulatory factors. The basic idea of factor analysis is to find a set of latent variables that contain the same information. The classic factor

analysis assumes that the both observed and the latent variables are continuous variables.

KMO and Bartlet test were used to show the extent variables have correlation and dependence to each other. In factorial analysis when KMO is less than 0.5, data are not suitable for factorial analysis and when KMO is between 0.5-0.7, data are suitable for factorial analysis. KMO amount and meaningful level of Bartlet test indicated in Table 3, that shows are very suitable fir factorial analysis.

The results show that these factors contributed about 56 percent of variance in the perception of respondents about factors influencing the adoption of nanocides in controlling fire blight disease. Table 2 represents components of each factor, as well as, portion of each factor from the total common variance. As one may observe, about 56% of total common variance is explained by these four factors, where the majority of it has been explained by the marketing factors (17.50%).

**Table 2**. Classification of factors that influence the adoption of nanocides in controlling fire blight disease by Using Factor Analysis

| Category | Variance by Factor |
|---|---|
| Marketing | 17.50 |
| Regulatory | 12.19 |
| Economic | 10.24 |
| Social | 16.11 |
| Total | 56.04 |

**Table 3:** KMO amount and meaningful level of Bartlet test

| Factorial Analysis | KMO | Bartlet Test Sig |
|---|---|---|
| Factors | 0.664 | 8201.205 0.00 |

**4. Discussions**

A wide range of economic, social, marketing and regulatory factors influences the adoption of nanocides by apple producers. Wheeler (2005) citing Rogers and Pannell pointed the factors which influence the adoption of new innovations by farmers. She mentioned factors such as perception about risk and profitability; uncertainty and certainty about adoption; amount of required information and attitude about risk and uncertainty.

The findings are in accordance with the result of study by Hosseini and Alikarami (2009) that factors such as social and regulatory factors in adopting the new technology. Innovation is not only based on the technology's agronomic suitability to specific environments.

Like any other new technology, public confidence, trust and acceptance are likely to be the key factors determining the success or failure of nanotechnology applications for the agriculture sector. It is well known that uncertainties and lack of knowledge of potential effects and impacts of new technologies, or the lack of a clear communication of risks and benefits can raise concern amongst public (Chaudhry et al., 2008).

A regulatory process should ensure the democratic control of and public participation in decision making on nanotechnology and other new technologies. It is recommend the initiation of a wide range of participatory processes to enable direct input from the general public into new technology assessment and determination of priorities and principles for public policy, R&D and legislation (Johnston, et al., 2007).

It is becoming increasingly clear that nanotechnology requires a holistic and tightly integrated regulatory framework for dealing with the range of health, ecological, economic, and socio-political issues that this technology raises (Johnston, et al., 2007).

As in the case of any new technology, the gains from modern technology are accompanied with certain negative effects and concerns. The nature and extent of the positive and negative impacts will depend on the choice of technique, place and mode of application of technique, ultimate use of the product, concerned policies and regulatory measures including risk assessment and management ability and finally on the need, priority, aspiration and capacity of individual countries (Ameden et al., 2005).

However, the application of nanotechnology by farmers in Iran faces challenges and obstacles. There is no single appropriate way to introduce and promote nanotechnology in the developing countries: constraints and opportunities vary from country to country and therefore require location-specific approaches.

There is need for more training and education to change the attitude of farmers and enhance their confidence about the role of nanotechnology in agriculture. It is also important to develop policies that benefit small-scale farmers and attend their technological needs.

Based upon the results of this study, it is apparent that there is still need to further research about the role of nanotechnology in agriculture sector in Iran. In this regard, strengthening the linkage between research and extension institutions and increasing the role of farmers in developing appropriate technology would accelerate the adoption by the farmers.

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# Blood Ras-Association Domain Family 1 A Gene Methylation Status In Some Liver Diseases

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**ABSTRACT: BACKGROUND:** Hepatocellular carcinoma (HCC) is one of the most common human malignancies and its impact on mortality is significant and well documented. Biomarkers have been developed for early HCC detection, with serum α-fetoprotein (S.AFP) being the most widely used clinically, but with relatively low diagnostic sensitivity. Therefore new biomarkers are needed for early HCC detection to improve overall-survival rates. **METHODS:** Blood RASSF1A promoter methylation was evaluated using methylation specific PCR in patients with chronic liver diseases together with its potential use as a biomarker for detecting HCC in comparison to or in association with S.AFP. **RESULTS:** Blood RASSF1A promoter methylation was detected in 70% of HCC patients on top of hepatitis C virus- associated liver cirrhosis, 28.5% of hepatitis C virus-associated liver patients and 16.6% of bilharzial liver fibrosis patients. However none of the healthy control subjects showed blood RASSF1A promoter methylation. The sensitivity, specificity, PPV and NPP of blood RASSF1A promoter methylation for HCC diagnosis were 70%, 83.3%, 73.7% and 80.6% respectively. On the other hand the sensitivity, specificity, PPV and NPP of S.AFP with a cut off value of 33.6 for HCC diagnosis were 85%, 80%, 88.9% and 90.7% respectively. Moreover it was found that the combined use of RASSF1A promoter methylation status and S.AFP is better than S.AFP use alone in HCC prediction. **CONCLUSION:** RASSF1A promoter methylation plays an important role in the process of human hepatocarcinogenesis and is related to hepatic inflammation due to bilharziasis and viral hepatitis. Moreover it can be considered as an important biomarker for the diagnosis of HCC when combined with S.AFP.

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**Key words:** RASSF1A promoter methylation, AFP, HCC, Bilharziasis, Liver cirrhosis, blood.

## 1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common human malignancies and is a leading cause of cancer-related death worldwide[(1)]. In Africa, liver cancer has been ranked as the fourth common cancer, and most of liver cancers are HCC[(2)]. HCC incidence has doubled in Egypt in the past 10 years[(3)].Most HCC patients in African and Asian populations exhibit chronic hepatitis or cirrhosis caused by persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV)[(4)]. Of all the HCC cases, it is estimated that 66% are attributable to HBV and 42% are attributable to HCV, assuming that the relative risk of disease in both carriers is 20. However, in North Africa, infection with HBV is less common than in other regions. In Egypt, the prevalence of HCV infection is one of the highest in the world. HBV was found at high rates in Egypt, but after increase in HCV prevalence the rates of HBV declined[(2)]. In addition, HBV rates have declined after the introduction of the vaccine in 1992[(3)]. By contrast, the relative importance of the etiologic effect of schistosomiasis on liver cancer is still inconclusive. Schistosomiasis per se may cause the persistence of viremia due to reduced immunity or could play a minor role in the pathogenesis of HCC as a result of the copper sulfate sprayed in canals for snail control[(2)]. Therefore, schistosomiasis can be treated as a covariate in multivariable analysis for HCC.

Despite numerous advances in the treatment of HCC during the last decade, the 5-year survival rate remains <40% and late presentation remains an important obstacle to successful treatment. In fact, many HCC patients have already developed locally advanced disease or distant metastasis by the time of presentation. In this regard, biomarkers have been developed for early HCC detection, with serum α-fetoprotein (S.AFP) being the most widely used clinically[(5)]. However, its use in the early detection of HCC is limited, especially because about one-third of patients with HCC have normal levels of S.AFP. Serum AFP is a marker that has low sensitivity and high specificity[(6)]. Therefore, new biomarkers for early HCC detection are needed to improve overall-survival rates.

HCC might be related to genetic or epigenetic alterations. Hypermethylation of tumor suppressor genes is frequently observed in HCC[(7,8)]. Such

epigenetic changes are potential markers for detecting and monitoring HCC. Recently, methods for the detection of circulating hypermethylated DNA sequences were developed[9].

RASSF1A (Ras association domain family 1 isoform A) is a recently discovered tumor suppressor gene located within 3 P21.3 locus. RASSF1A protein modulates multiple apoptotic and cell cycle checkpoint pathways which are commonly deregulated in cancer. It is mostly inactivated by transcriptional silencing of the gene by inappropriate promoter methylation in many cancers including HCC [10].

Our study was designed to evaluate the biochemical changes of blood RASSF1A promoter methylation in patients with chronic liver diseases including bilharziasis, HCV- associated liver cirrhosis as well as HCC on top of HCV- associated liver cirrhosis and its potential use as a marker for detecting HCC in comparison to or in association with S.AFP.

## 2. SUBJECTS AND METHODS

Our study was conducted on 56 subjects selected from internal medicine department, Benha University Hospitals. They were categorized into 1- *Control group*; consisted of 10 healthy volunteers 2- *Bilharzial liver fibrosis group*; consisted of 12 bilharzial liver fibrosis patients, free of HCV or HBV infection. *3- Liver cirrhosis group*; consisted of 14 HCV- associated liver cirrhosis patients, free from bilharziasis. 4- *HCC group*; consisted of 20 HCC patients on top of HCV- associated liver cirrhosis and free from bilharziasis. HCC was diagnosed by S.AFP, abdominal ultrasound & spiral CT with or without liver biopsy. Patients with hepatic metastasis due to other malignancies or with HBV infection were excluded from the study. Approval of the ethical committee was obtained and written informed consents were taken from all subjects of the study.

**Sample collection and preparation:**

Venous blood sample (10 ml) was withdrawn from patients and control. The blood samples were divided into two parts:

- The 1st part was left to clot, centrifuged and the sera were separated for measurement of S.AFP levels by enzyme linked fluorescent assay using VIDAS AFP kits supplied by bioMerieux sa,France, Bilharzial antibody titre by indirect haemagglutination using schistosomiasis fumouze kits supplied by Fumouze Diagnostics, France and HCV antibody test and hepatitis b surface antigen by immunochromatographic analysis using ACON® HCVone step test and ACON® HBsAg one step test respectively supplied by ACON Laboratories Inc..
- The 2nd part: was applied into EDTA containing vacutainer and stored at – 80°C for later detection of methylation status of RASSFIA gene by methylation specific PCR (MSP) technique.

**A- DNA extraction:**

100 µl of eluted DNA was extracted from 200 µl of anticoagulated blood using the "Axy prep blood genomic DNA mini prep kit" supplied by Axygen biosciences.

**B- Bisulfite treatment (DNA modification):**

Bisulfite modification of genomic DNA would convert unmethylated cytosine residues into uracil residues. Conversely, methylated cytosine residues would remain unmodified. Thus, methylated and unmethylated DNA sequences would be distinguishable by using sequence-specific PCR primers[11]. Bisulfite modification was conducted using the EZ DNA methylation-Gold™ kit. 10 µl of eluted modified DNA was obtained from 20µl of DNA sample. The eluted modified DNA was stored at-70°C as modified DNA is fragile like RNA for subsequent MSP**.**

**C-Polymerase chain reaction (PCR):**

Bisulfite-modified DNA was amplified using primers specific for the methylated sequence 5′-GTGTTAACGCGTTGCGTATC-3′ and 5′-AACCCCG CGAACTAAAAACGA-3′ together with primers specific for the unmethylated sequence 5′-TTTGGTTG GAGTGTGTTAATGTG-3′ and 5′-CAAACCCCACA AACTAAAAACAA-3′ [11].

Amplification of the methylated and the unmethylated sequences was done using the GeneAmp DNA Amplification Kit and AmpliTaq Gold polymerase (Applied Biosystems, Perkin-Elmer, Foster City, CA). The optimized thermal profile included initial denaturation at 95°C for 12 min., followed by 45 cycles of 95°C for 45 sec., 54°C for 45 sec., 72°C for 1 min., and a final extension at 72°C for 10 min..

**D- Detection of amplified PCR product by Agarose gel electrophoresis:**

The amplification products were analyzed by agarose gel electrophoresis in 2٪ agarose gel, and ethidium bromide staining and photographed by Polaroid camera under UV light.

**STATISTICAL ANALYSIS**

The collected data were computed and statistically analyzed using SPSS version 17 software. Suitable statistical techniques were calculated as mean, ±SD. ANOVA & Z tests were used as tests of significance. ROC curve was used to predict cutoff values of S.AFP with the optimum sensitivity and specificity of S.AFP & RASSF1A promoter

methylation for diagnosis of different liver diseases. In addition, logistic regression analysis was done to predict the equation that determines the probability of being HCC by S.AFP and RASSF1A promoter methylation.

Logit(Y) = $b_0 + b_1x_1 + b_2x_2 + \ldots\ldots$

(Probability >0.5 indicates that the test can predict the disease and the maximum of probability is being one).

Moreover spearman correlation coefficient was estimated to correlate the S.AFP and DNA methylation. P values less than 0.05 were considered significant.

## 3. RESULTS

The 56 subjects included in the study were 32 males and 24 females .The *control group* consisted of 4 males and 6 females with age ranging from 22 - 48 years and mean value of 35 ± 9.5, while the *bilharzial liver fibrosis group* consisted of 8 males and 4 females, with age ranging from 23 – 50 years and mean value of 32 ± 11.8. Moreover *the liver cirrhosis group* consisted of 7 males and 7 females, with age ranging from 47 – 80 years and mean value of 56.07 ± 9.2. In addition, *HCC group* consisted of 13 males and 7 females with age ranging from 40- 75 years and mean value of 59.35 ± 9.01.

S.AFP level was measured in the different study groups and it was found to be significantly elevated in the HCC group compared to the other study groups. Cut off values were determined for S.AFP in the different study groups (table 1) to determine the percentage of positive and negative S.AFP (Table 2). Also the percentage of positive and negative RASSF1A promoter methylation in the different study groups was determined (Table 2). Moreover our study showed that there was a positive significant correlation between S.AFP level and DNA methylation (r = 0.49, P<0.001).

**Table (1): The mean, ± SD and cutoff values of S.AFP in the different study groups**

| Parameter / Group | S.AFP (ng/ml) | | | | | |
|---|---|---|---|---|---|---|
| | **Mean ± SD** | ANOVA test | P value | Post hoc (Bonferroni test) | P value | **Cut off value** |
| **Control** | 3.45 ± 1.21 | 22.6 | < 0.001 | Control#HCC | < 0.001 | 4.55 |
| **Bilharzial liver fibrosis** | 5.30 ± 1.86 | | | Fibrosis#HCC | < 0.001 | 6.4 |
| **Liver cirrhosis** | 31.42 ± 12.21 | | | Cirrhosis#HCC | < 0.001 | 16.5 |
| **HCC** | 118.56 ± 67.30 | | | | | 33.5 |

**Table (2): The percentage of positive and negative S.AFP and RASSF1A promoter methylation in the different study groups.**

| Parameter / Group | S.AFP | | | | | | | RASSF1A promoter methylation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Negative** | | **Positive** | | **Z1 P** | **Z2 P** | **Z3 P** | **Negative** | | **Positive** | | **Z1 P** | **Z2 P** | **Z3 P** |
| | n | % | n | % | | | | n | % | n | % | | | |
| **Control (n=10)** | 8 | 80 | 2 | 20 | | | | 10 | 100 | 0 | 0 | | | |
| **Bilharzial liver fibrosis (n=6)** | 8 | 66.7 | 4 | 33.3 | 0.68 >0.05 | 3.77 <0.001 | | 10 | 83.3 | 2 | 16.7 | 1.36 >0.05 | 2.9 <0.001 | |
| **Liver cirrhosis (n=14)** | 1 | 7.1 | 13 | 92.9 | 3.6 <0.001 | 0.7 >0.05 | 3.2 <0.001 | 10 | 71.4 | 4 | 28.6 | 1.89 <0.05 | 2.4 <0.01 | 0.72 >0.05 |
| **HCC (n=20)** | 3 | 5 | 17 | 95 | 3.5 <0.001 | | | 6 | 30 | 14 | 70 | 3.6 <0.001 | | |

n: number of cases Z1 versus control Z2 versus HCC Z3 versus bilharzial liver fibrosis

**Table (3): Cut off values, sensitivity, specificity and predictive values of S.AFP and RASSF1A promoter methylation in the diagnosis of different liver diseases.**

| Group | Test | Cut off value | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) | P value | 95% confidence interval |
|---|---|---|---|---|---|---|---|---|---|
| **Control (n=10)** | **•S.AFP**<br>**• RASSF1A Methylation** | ≤ 4.55<br>– | 80<br>100 | 95<br>52.5 | 80<br>34.5 | 95<br>100 | 96<br>73.8 | < 0.001<br><0.05 | 0-1<br>0.6-0.88 |
| **Bilharzial liver fibrosis (n=12)** | **•S.AFP**<br>**• RASSF1A Methylation** | 6.4<br>– | 66.7<br>83.3 | 70.5<br>59.1 | 23.5<br>21.7 | 93.9<br>96.3 | 78<br>62.1 | <0.05<br>>0.05 | 0.66-0.91<br>0.4-0.84 |
| **Liver cirrhosis (n=14)** | **•S.AFP**<br>**• RASSF1A Methylation** | 16.5<br>– | 92.9<br>28.6 | 52.8<br>58.3 | 43.3<br>76 | 95<br>67.7 | 52.8<br>43.5 | >0.05<br>>0.05 | 0.37-0.69<br>0.26-0.61 |
| **HCC (n=20)** | **•S.AFP**<br>**• RASSF1A Methylation** | 33.5<br>– | 85<br>70 | 80<br>83.3 | 73.9<br>73.7 | 88.9<br>80.6 | 90.7<br>76.7 | < 0.001<br>< 0.01 | 0.79-1<br>0.62-0.91 |

According to the results of our study (table 3), bilharzial liver fibrosis is diagnosed with S.AFP level >6.4-16.5. In addition, liver cirrhosis is diagnosed with S.AFP level > 16.5- 33.5, while HCC is diagnosed with S.AFP level above 33.5. The sensitivity and specificity of S.AFP level as a diagnostic test for these diseases are shown in table (3). Also the sensitivity and specificity of RASSF1A promoter methylation as a diagnostic test for these diseases are shown in the same table.

According to the results of the logistic regression analysis:

●Logit (HCC) = -2.08 + 3.1 (S.AFP positive). So When S.AFP alone is positive the probability of the case to be HCC = 0.58.

●Logit (HCC) = -3.06 + 3.08 (S.AFP positive) + 2.4 (RASSF1A promoter methylation positive). So when both S.AFP and RASSF1A promoter methylation are positive, the probability of the case to be HCC = 0.82.

Also, according to the odds ratio calculated; it was found that individual with positive S.AFP is 21.7 times more likely to have HCC. Moreover, the individual with positive RASSF1A promoter methylation is 11.1 times more likely to have HCC (Table 4). The PCR products on the agarose gel are shown in figure (1).

**Table (4): Odds ratios calculated for positive S.AFP and RASSF1A promoter methylation tests.**

| Test | Odds ratio | P value | 95% confidence interval |
|---|---|---|---|
| Positive S.AFP | 21.7 | 0.001 | 3.7-127.4 |
| Positive RASSF1A methylation | 11.1 | 0.007 | 1.9-64.3 |

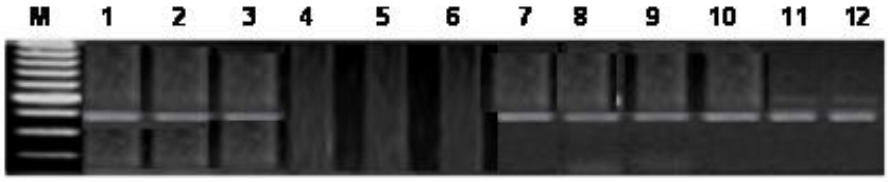


**Figure (1): verification of the PCR products on agarose gel:** Lane M shows PCR marker (100bp), lanes 1, 2, 3, 7, 8, 9, 10, 11 and 12 show bands of RASSF1A promoter methylation of **300 bp** length while lanes 4, 5 and 6 are negative for RASSF1A. Promoter methylation.

## 4. DISCUSSION

Hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing of these genes and genomic instability. CpG hypermethylation acts as an alternative and/or complementary mechanism to gene mutations causing gene inactivation, and it is now recognized as an important mechanism in carcinogenesis. Although the mechanisms responsible for CpG island hypermethylation in cancer are poorly understood, it has been hypothesized that epigenetic silencing depends on activation of a number of proteins known as DNA methyltransferases that possess de novo methylation activity[12]. A growing number of genes have been reported to undergo CpG island hypermethylation in HCCs, which indicates the potential role of CpG island hypermethylation in hepatocarcinogenesis[13].

RASSF1A hypermethylation has been detected frequently in the tissues of different cancers including HCC[14]. Tissue RASSF1A promoter methylation has been documented in 85%[15], 100%[16], 93%[7] and 66.7%[13] of HCC. Moreover, it was detected slightly less frequently in the hepatitic / cirrhotic tissue adjacent to HCC, ranging from 70%[15] to 82.75%[16].On the other hand, absence of RASSF1A promoter methylation was detected in the non neoplastic hepatitic / cirrhotic tissue far from tumors[13,15], hepatitic tissue with or without cirrhosis in patients with absent HCC [13] and normal liver tissue in patients without HCC[15,16]. This indicates that RASSF1A promoter methylation occurs as an early event in hepatitis and cirrhosis before the development of HCC and is closely linked to hepatitis and cirrhosis in hepatocellular carcinoma patients. This suggests that it plays an important role in the human hepatocarcinogenesis. In addition, RASSF1A promoter methylation has been documented in different body fluids of different cancers including the serum in HCC which indicated its value for the early-stage diagnosis of tumors[14]. All of these data indicates that RASSF1A promoter methylation may play a role as a potential marker for cancer risk assessment and early detection of human HCC as like any ideal biomarker, it appears early in the course of disease and is detectable in biological samples that can be obtained noninvasively. This is in spite of the unclear exact mechanism of how the tumor DNA enters systemic circulation.

This study had evaluated RASSF1A promoter methylation in the blood of three types of chronic liver diseases including bilharziasis, HCV associated liver cirrhosis as well as HCC on top of HCV associated liver cirrhosis. Methylation was evaluated using MSP technique. MSP is the most widely used technique for studying the methylation of CpG islands of genes and is one of the most effective choices for investigating the methylation profile of these regions[17].

According to the results of our study, there was a significant elevation in the percentage of positive blood RASSF1A promoter methylation in the HCC group (70%) in comparison to the healthy control group (0%), bilharzial liver fibrosis group (16.6%) and the liver cirrhosis group (28.5%). RASSF1A promoter methylation was also significantly higher in the liver cirrhosis group compared to the healthy control group and non significantly higher compared to the bilharzial liver fibrosis group. Furthermore, RASSF1A promoter methylation was non-significantly higher in bilharzial liver fibrosis than in the control group.

RASSF1A promoter methylation was detected in the serum of 31%[14] and plasma of 44.1%[18] of HCC patients on top of HBV- associated liver cirrhosis compared to 79.3%[14] and 91.2%[18] in the corresponding cancerous tissue of the same patients using MSP. Moreover, serum RASSF1A hypermethylation was reported in 70% of HCV associated HCC and 80% of HBV associated HCC cases using MSP[19]. On the other hand, No RASSF1A methylation was detected in the serum[14] or plasma[18] or peripheral blood mononuclear cells[20] of 10 healthy blood donors in each study. However RASSF1A promoter methylation was reported in three out of 35 control subjects, but two or three of these subjects(as the authors did not clarify) had either hepatitis B virus or/and HCV infections; one subject had a history of smoking and alcohol drinking. They explained the hypermethylation in serum DNA from controls was perhaps due to hepatitis virus infection and chemical carcinogen exposure and that another possibility was that some normal controls have cryptogenic hepatic cirrhosis[19].

Hypermethylated RASSF1A sequences were detected in the sera of 93% of HCC patients mostly on top of HBV infection(89%), 58% of HBV carriers, and 8% of the healthy volunteers using real-time PCR after digestion with a methylation-sensitive restriction enzyme. The sensitivity of this technique is higher than MSP [5]. This explains the higher RASSF1A methylation frequency in HCC patients (93%) to approach that of tissues detected by other studies[7,15,16,18]. It also explains the higher RASSF1A methylation frequency in HBV carriers and the presence of RASSF1A methylation in 8% of the healthy volunteers. These results are higher than our study and the previous studies using MSP[14,18,19] or using bisulfite sequencing and PCR-RFLP[20]. The lower sensitivity of MSP or bisulfite sequencing and PCR-RFLP is due to substantial degradation of DNA (up to 96%) caused by the bisulfite conversion step. However with using real-time PCR after digestion with

a methylation-sensitive restriction enzyme, there is specific degradation of unmethylated sequence by the methylation-sensitive restriction enzyme, in contrast to the nondiscriminatory degradation of both methylated and unmethylated DNA with bisulfite conversion[21].

The absence of RASSF1A methylation in the peripheral blood mononuclear cells[20] indicates that the source of RASSF1A methylation in the blood of the HCC, liver cirrhosis and bilharzial liver fibrosis groups using MSP in our study is not from the peripheral blood mononuclear cells but from diffusion from the liver tissue into the blood.

The development and progression of HCC is a multistep process whereby the normal hepatocytes undergo inflammation, fibrosis by the hepatitis virus or other stimuli, followed by liver cirrhosis, which then progresses to HCC or dysplastic nodule and subsequent HCC[13].

RASSF1A promoter methylation in our HCV- associated liver cirrhosis and in HCC on top of HCV- associated liver cirrhosis can be explained by increased DNA methyltransferase mRNA expression that have been observed to occur early in the HCC tissues and in liver tissues showing hepatitis [22,23] . Persistent inflammatory stimulation caused by chronic hepatitis and cirrhosis results in aberrant hypermethylation that with its continuation finally lead to HCC, as it has been reported that inflammatory proliferative diseases such as ulcerative colitis[24], Barrett's esophagitis[25], and Epstein-Barr virus-associated gastritis[26] are strongly related to aberrant hypermethylation of various CpG islands. Also several studies reported frequent silencing of multiple genes by CPG island methylation, including RASSF1A and other genes in hepatitis B virus associated HCC which accumulate during the pathogenesis of human HCC[20]. It was reported that there were no associations between the methylation status of *RASSF1A* and other tumour suppressor genes and the type of hepatitis virus[13]. Moreover, the status of promoter methylation of RASSF1A and other tumour suppressor genes were significantly correlated with the viral infections in the background liver parenchyma[27]. In addition, HBV- or HCV-positive HCCs showed more frequent hypermethylation of CpG islands than virus-negative ones. Furthermore, there is possibility that the regeneration process that is characteristic of chronic liver disease may be associated with aberrant methylation that may involve tumour suppressor genes[20].

It is possible that the detection of blood RASSF1A promoter methylation in the HCV associated -liver cirrhosis cases in our study is indicator that these cases are going to develop HCC and that this methylation is a step towards HCC development. This is evidenced by the presence of RASSF1A promoter methylation in the hepatitic / cirrhotic tissue adjacent to HCC[15,16] and its absence in the hepatitic / cirrhotic tissue far from the tumour or in patients without HCC[13]. So, it is recommended to follow up these cases more frequently to find out if they are going to develop HCC and the time of occurrence of HCC in comparison to the liver cirrhosis cases without RASSF1A promoter methylation. Also, it is recommended to quantitate those cases of HCV associated -liver cirrhosis with RASSF1A methylation to follow up these cases by quantitative measurement of RASSF1A methylation to find out if they exceed the cut off value for HCC reported by Allen and associates [5] . This is to ensure the usefulness of the use of RASSF1A promoter methylation as a predictor of the future development of HCC in cases of HCV associated liver cirrhosis and thus its usefulness as a potential molecular biomarker for cancer risk assessment in the precancerous lesions.

The results of our study showed that the PPV of RASSF1A methylation in cases of HCC was 73.7% and in cases of liver cirrhosis was 76%. This supports the use of RASSF1A methylation as a powerful marker in the diagnosis of both liver cirrhosis and HCC and thus differentiating these diseases from other liver diseases. . However the PPV of RASSF1A methylation in the diagnosis of bilharzial liver fibrosis is low indicating that that it is a weak biomarker for its diagnosis.

As regards schistosomal liver fibrosis, it was reported that the liver injury produced by schistosomal egg-induced inflammatory response is mild or limited compared to the necro-inflammatory reaction produced by the HCV. Also, the liver mesenchymal cells (myofibroblasts) involved in fibrogenesis were increased in both schistosomal periportal fibrosis and HCV-induced cirrhosis than in normal liver, but higher in HCV-induced cirrhosis[28]. This indicates that the regenerative process is stronger in response to the more inflammatory reaction produced by HCV infection. So the stronger inflammatory reaction and thus the higher regenerative process in response to HCV infection may explain the higher RASSF1A promoter methylation in HCV- associated liver cirrhosis group and HCC group than in schistosomal liver fibrosis group. Also the limited inflammatory response to schistosoma eggs may explain the non significant mild increase of RASSF1A methylation in the schistosomal liver fibrosis group in comparison to the control group.

To our knowledge, there are no previous studies conducted to assess RASSF1A promoter methylation in schistosomal liver patients. However, it was found that mice infested with schistosoma mansoni have promutagenic methylation damage in liver, but

not in kidney, spleen or bladder[29]. Also remnants of schistosomal eggs were found in the severe granulomatous reaction present in a well-differentiated hepatocellular carcinoma that had developed in a chimpanzee devoid of hepatitis B or C markers[30]. On the other hand, it was stated that in bladder cancers, schistosoma-associated tumors had more genes methylated than non-Schistosoma tumors and they suggest that schistosomal involvement in bladder cancers associates with a greater degree of epigenetic changes in the urothelium [31]. However, infection with schistosoma mansoni is not classified as being carcinogenic to humans, while infection with schistosoma haematobium is carcinogenic to humans, but schistosoma mansoni may still be linked to hepatocellular carcinoma through potentiating the effects of hepatitis B virus and hepatitis C virus on the liver[32]. Moreover a study supported the rapid progressive course of HCV infection in the presence of bilharzial infection in Egypt leading to rapid development and aggressive course of cirrhosis and higher incidence of HCC[33].

The presence of RASSF1A methylation in 16.6% of bilharzial liver fibrosis, which is classified as non carcinogenic to humans may contradict the possibility that the detection of blood RASSF1A promoter methylation in the HCV associated liver cirrhosis cases in our study is indicator that these cases are going to develop HCC..However, we suggest that continuous limited inflammation and regeneration in the bilharzial liver fibrosis without viral hepatitis may result in limited RASSF1A methylation unable to conduct the development of HCC. Moreover, when schistosoma mansoni infection is combined with HCV infection that causes further methylation, HCC may develop. Therefore quantification of blood and tissue RASSF1A methylation in bilharzial liver fibrosis with and without HCV infection is recommended.

As regards S.AFP, our results showed that its level was significantly higher in the HCC group compared to the other groups. Also, the percentage of positive S.AFP according to our cut off values was significantly higher in the HCC group (95%) compared to the control (20%) and bilharzial liver fibrosis (33.3%) groups and in the liver cirrhosis group (92.9%) compared to the control group and bilharzial liver fibrosis groups.

AFP is a fetal-specific glycoprotein, synthesized primarily by the embryonic liver, by cells of the vitelline sac and by the fetal intestinal tract in the first trimester of pregnancy. The expression of AFP is repressed within a few weeks after birth. Pathologically, patients with chronic liver disease, particularly those associated with a high degree of hepatocyte regeneration, can express AFP in the absence of cancer. Also, S.AFP is elevated in hepatocarcinogenesis, embryonic carcinomas and in gastric and lung cancer. S.AFP is not elevated in all patients with HCC. Up to 42% of patients with HCC present with S.AFP levels within normal values[34].

The results of our study showed that the PPV of S.AFP (with a cut off value 33.5 ng/ml) in cases of HCC was 73.9%. This supports the use of S.AFP as a powerful marker in the diagnosis of HCC. However the PPV of S.AFP in the diagnosis of bilharzial liver fibrosis and liver cirrhosis are low indicating that it is weak biomarker for diagnosis of these diseases. The high sensitivity, specificity and NPV of S.AFP (with a cut off value 33.5 ng/ml ) in our study which were 85%, 80% and 88.9% respectively, indicates the importance of S.AFP at this cut off value in the diagnosis of HCC.

In accordance to our study, a study revealed the diagnostic importance of S.AFP in differentiating HCC from other liver diseases as they reported that the PPV of S.AFP in HCC was high (60%) [35].

A group of scientists analyzed five studies detecting the sensitivity and specificity of S.AFP for detecting HCC in patients with HCV. They reported that by using the most commonly reported cutoff value of a positive test result for HCC (AFP level > 20 ng/ml), the ranges of test characteristics were as follows; sensitivity, 41% to 65%; specificity, 80% to 94%; positive likelihood ratios, 3.1 to 6.8; and negative likelihood ratios, 0.4 to 0.6. Four of the 5 studies reported sensitivity and specificity for S.AFP cutoff value higher than 200 ng/ml, a value that is frequently reported to be specific for the diagnosis of hepatocellular carcinoma. The range of specificities for hepatocellular carcinoma was very high at this cutoff value (99% to 100%), but the sensitivity was very low (20% to 45%) [36].

However, a study reported that the best discriminating AFP value between HCC and chronic liver disease was 16 ng/ml. Using S.AFP level of 20 ng/ml (the upper normal range) as the cut-off yielded equivalent sensitivity (60.0% *vs.* 62.4%) and specificity (90.6% *vs.* 89.4%). The PPV and NPV were 84.6% and 69.7%, respectively. In non-infected patients with either HCV or HBV the PPV was 100% and the NPV ranged from 59.0 to 73.0% [37].

Also, another study revealed that the best discriminant cut-off value of S.AFP between liver cirrhosis and HCC on top of liver cirrhosis was 30 ng/ml which approaches that of our study. The etiology of liver cirrhosis and HCC in this study was mostly HCV (more than 70%), and less frequently non viral cause, HBV or combined HCV and HBV respectively. At this cut-off value 65% sensitivity, 89% specificity, 74% PPV and 79% NPV were determined. This cut-off

value was more useful in detecting non-viral HCC, because PPV to diagnose non viral HCC was significantly (94%) higher than in viral HCC (70%). In the non-viral diseases PPV reached 100% for S.AFP levels of 100 ng/ml, while in the viral diseases PPV was 100% when S.AFP was greater than 400 ng/ml. There were no significant differences in specificity, sensitivity or NPV between viral and non-viral liver diseases[38].

Our study had revealed a positive significant correlation between the serum concentrations of AFP and RASSF1A methylation status. This disagrees with another study that reported no statistically significant correlation of plasma methylation with S. AFP levels[18]. However a significant association between methylation status of tissue RASSF1A, and S.AFP level was detected before [39].

The results of our study showed that combined use of RASSF1A promoter methylation and S.AFP is better than the use of S.AFP alone in the prediction of HCC. This result agrees with a study which indicated the usefulness of combined measurement of S. AFP and RASSF1A levels in HCC diagnosis than the measurement of S.AFP alone. It reported that the diagnostic sensitivity and specificity were 77% and 89%, respectively, for combined AFP and RASSF1A levels analysis compared with 65% and 87%, respectively, for AFP measurement alone[5].

In conclusion, RASSF1A methylation plays an important role in the process of human hepatocarcinogenesis and is an important biomarker for the diagnosis of HCC when combined with S.AFP.

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# Different aspects of adult learning principles

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**Abstract:** Learners must retain what the program delivers to them in order to benefit from the learning. In order for participants to retain the information taught, they must see a meaning or purpose for that information. They must also understand and be able to interpret and apply the information in their own real life contexts. Understanding includes their ability to assign the correct degree of importance to the material and its application in the future. The amount of retention is always directly affected by the degree of original learning. In other words if the learners did not learn the material well initially, they will not retain it well either. Retention by the participants is directly affected by their amount of practice during the learning. After the students demonstrate they can apply new financial skills, they should be urged to practice in their own time and for their own personal needs to retain and maintain the desired performance.
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**Introduction:**

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal.

Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. Other literacy initiatives are defined in terms of a particular educational service, strategy, or means to address a target population's literacy problems. "Adult basic education" and "family literacy" are examples. These initiatives are often defined in terms of a particular configuration of services for the target population (e.g., assessment and information and counseling services).
2. The term "lifelong learning" is often associated with "literacy." Lifelong learning is a means to the goal of maintaining necessary levels of literacy throughout one's lifetime. The goal of lifelong learning has implications for both individual adult's learning behavior as well as education policy and the design of the education system.

Goal six of the National Education Goals illustrates a broadly stated goal that incorporates expectations about both adult literacy and the kinds of policies and services that should be in place to improve literacy. Goal six, "Adult Literacy and Lifelong Learning," states that, "By the year 2000, every adult will be literate and possess the knowledge and skills necessary to compete in a global economy and exercise the rights and responsibilities of citizenship." The objectives related to this goal touch on several of the common elements of definitions listed above, for example:

- Different dimensions of literacy (e.g., academic and workplace skills),
- The level of education attainment (e.g., increasing the number of persons who complete postsecondary degrees),
- The needs of target groups (e.g., parents, minorities, or part-time learners),
- The need to increase the availability of particular educational services, strategies or means (e.g., accessibility of libraries to part-time learners or opportunities for parental involvement), and
- The importance of lifelong learning, both in the learning behavior of individuals and in the educational system's responsiveness to the needs of adult learners.

Adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

Several definitions of adult education has been done Community

- Adult Education is a⎫in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age⎫ limits as formal and informal, voluntary and at different levels of time, place
- Adult Education is a process in which people who⎫and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after⎫ school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

**Characteristics of adult education:**

**flexibility in time:**

In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

**Flexibility in the location:**

One of the aspects of flexible space is that individuals can, regardless of their residence to the study and advancing their knowledge and skills pay. For example, adults in remote villages should like people who live in the city use of educational programs. After flexibility in other places is that the issue of specificity of location is not considered primarily educational.

**Flexibility in age:**

Educational opportunities for certain age should not use it for all regardless of their age, is possible. In fact, educational programs must use people of different ages to prepare.

**Flexibility in admission:**

No adult should not only be deprived of education because of the necessary conditions for admission in the class does. Of course this is not such a person without academic records to participate in university classes is accepted, Adoption order is that the adults in educational programs at different levels, according to the possibility of using the opportunity that is provided must be based on the experience and knowledge and their knowledge is.

**To combine education and job responsibilities:**

Adults should be able to work during that time engaged in training classes take them. In other words, their presence in the class should be considered part of their work. This means that low-literate or illiterate working people who are allowed to work an hour of your daily spending surpassed participation in educational programs.

**Principles of Adult Learning**

**1) PURPOSE**

The Financial Literacy Foundation has prepared this document to provide education materials developers with information on the key principles of adult learning. It is a short summary of a very broad area of research and advice, prepared with the input of Adult Leaning Australia, the national peak body representing organisations and individuals in the adult learning field.

**2) NEEDS, WANTS, CONCERNS AND ABILITIES OF YOUR LEARNERS**

Assess the needs, wants, concerns and current abilities of the target learners. Each target group will have their own special needs and probably expect different outcomes from undertaking your training program. Common themes you can prepare for are:

**Why are you here?** - no-one readily admits to not knowing something fundamental that may impact on their life chances. Therefore program material, particularly that designed for adult learners should always treat aspects of why learners are in the training sensitively. Describe the outcomes expected from the training in positive, enhancing terms and not as redressing a weakness or failure on the part of the learner. For example, “Undertaking this program will improve (rather than redress a failing) the way you manage your money”.

**Tell me more** - learners may well enter programs like this with poor past experiences of money matters or at least some trepidation about handling personal finances in the future. Recognise this in the program introduction but individual learners should never be required to expose any of their negative experiences in a group. It might seem a good ‘ice-breaker’ to ask a new group of learners to share what they expect from the program but resist going too far when asking learners to talk about past problems they may have had with finances. Firstly, they may be uncomfortable doing this in a group and secondly you could start the program in a sea of negative

views about financial matters generally. A successful program introduction will focus on where the learners will go rather than dwell too much on where they may have been.

**What do you know? -** Gauge the likely capabilities of your target groups. Overestimating their current skills in dealing with money could mean the program misses fundamental principles and understandings. Underestimating existing knowledge is also not good as plodding through basic material most already are familiar with will bore participants and the full program content will not be assimilated.

**What will I be able to do?** – above all these target groups will want to be hands on and demonstrate to themselves and their peers that that can do something they could not before the training; and do it well. Let them know right at the beginning that they will be able to do things that will be of great benefit to them, not just know more.

**Build on small successes** – if a target group of learners has had limited positives in their life or work experiences its important to provide small and regular 'success' points in the program. Simply exposing the content and assuming everyone is assimilating it, putting it all together holistically and building up their skills is not enough. The beginning of the program should be designed so that a discrete piece of learning that the learners can use right away builds their confidence to move on. The program should be a series of steps where the learners confirm their progress and reinforce one new skill by relating it to another they can already confidently apply.

**Testing!** – many adults and people not regularly engaged in learning fear testing. Many may have had bad experiences of assessment in school and view the practice among peers as stressful. Make sure they understand that what they are in is a life skills program and no-one can 'fail' as such. In fact each can support others in things they do well that fellow learners may need help with so it's a cooperative not competitive environment that they are learning in. Build in some teamed exercises and assessments to avoid people feeling isolated in their learning and fearful of failure in front of the group.

**Special needs**. You need to consider learners with special needs and those who have English as their second language. Reasonable adjustment should be made depending on each individual learner's particular needs and abilities. Your program material should include advice to the trainer on how to determine the need to make adjustments which, depending on a learner's abilities may include:

- providing interpreters for people who are deaf;
- ensuring access, for example by conducting training and assessment in facilities which have ramps for people using wheelchairs and adjustable desks for people with physical disabilities;
- allowing for access of personal assistants or note takers;
- allowing additional time for assessments;
- allowing oral instead of written responses to questions;
- adaptive technology such as screen readers, speech synthesisers, computer software or hardware; and,
- assistance with managing stress and anxiety.

**3) HOW DO ADULTS LEARN?**

Your program needs to account for:

• Motivation of the learner;
• Reinforcement of the skills and knowledge being developed;
• Retention of key learning; and,
• Transference of what is learnt to new situations.

**Motivation -** Adults learn most effectively when they have an inner motivation to develop a new skill or gain new knowledge. They resist learning material if it is forced on them, or if the only reason given is that the material will, in some vague way, be "good for them to know." Adults need to know why they are being asked to learn something; and they definitely will want to know what the benefits will be before they begin learning. This means the best motivators for adult learners are explicit interest and self benefit. If they can be shown that the program will benefit them pragmatically and practically, they will learn better, and the benefits will be much longer lasting. Typical motivations include a desire for better handling of personal money matters, say in retirement, wanting a new or first job, promotion, job enrichment, a need to reinforce old skills in say, handling credit or learn new ones, a need to adapt to community changes such as on-line banking and so on. Remember the tone of the program should be motivating. Your program should employ methodologies so that your trainers establish a friendly, open atmosphere that shows the participants they will help them learn rather than present as 'experts' imparting knowledge. No-one engages well with a trainer/teacher who is just 'showing off' what they know. Financial services have a plethora of jargon and complicated ideas that can put many lay people off. Exposing this sort of terminology and explaining it in simple terms – or deciding whether some of it needs exposure at all – is paramount to keeping your learner's trust and interest.

**Appropriate level of difficulty.** The degree of difficulty of your financial literacy program should be set high enough to expose all the essential

elements of the topic and challenge learners to succeed, but not so high that they become frustrated by information overload. Too much financial industry terminology strung together can be a complete turn off for people who may already struggle with the fundamentals – is it really a necessary part of the skills they need?

So start with financial information and techniques that relate directly to the learner's own personal needs and wants. Personal budgeting is always useful and less complicated than say, comparing mortgage options. Don't make what could be a lesser used skill so important in the program it de-motivates the learners and loses their interest.

Motivational reward does not necessarily have to be in the monetary sphere; it can be simply a demonstration of social or workplace benefits to be realised from new financial management skills. Older participants could perhaps learn how to help their children with financial decisions. People could be shown how to utilise better financial planning in a club or society they belong to. Its about improving whole of life experiences not just direct monetary reward. The overall thrust of the program should be motivating and, like all good teaching and learning programs, course material should ensure other key adult learning elements are covered.

**Reinforcement.** As we know reinforcement is a very necessary part of any teaching/learning process. Through it, trainers encourage correct modes of behaviour and performance and discourage bad habits. Your program should use both reinforcement techniques throughout. Positive reinforcement is normally used when participants learn new skills. As implied, positive reinforcement is "good" and reinforces "good" (or positive) behaviour. Negative reinforcement is useful in trying to change bad habits or inappropriate modes of behaviour. The intention is extinction -- that is, the trainer uses negative reinforcement until the "bad" behaviour disappears or the learner understands why past practice is not beneficial to them. Examples could be ensuring participants always compare different rates of interest available to them before signing up for any new debt (a positive reinforcement) and not considering credit purchases that leave them with no income safety net for unforeseen circumstances (negative reinforcement).

**Retention.** Learners must retain what the program delivers to them in order to benefit from the learning. In order for participants to retain the information taught, they must see a meaning or purpose for that information. They must also understand and be able to interpret and apply the information in their own real life contexts. Understanding includes their ability to assign the correct degree of importance to the material and its application in the future. The amount of retention is always directly affected by the degree of original learning. In other words if the learners did not learn the material well initially, they will not retain it well either. Retention by the participants is directly affected by their amount of practice during the learning. After the students demonstrate they can apply new financial skills, they should be urged to practice in their own time and for their own personal needs to retain and maintain the desired performance.

**Transference.** Transfer of learning is the result of training and is simply the ability to use the information taught in your program but in new settings and contexts. As with reinforcement, both types of transfer: positive and negative should be used in the program approach. Positive transference, like positive reinforcement, occurs when the learner uses the skill learnt in your program. It is very important for any learner's orientation to the new skills they develop that they can practice in their own situations. Using knowledge from financial literacy training to work out the best way to use (or not use) credit in their lives is an important tool that many participants could use immediately. Participants can check how much credit debt they have, what interest they are paying and what alternatives there may be. Negative transference, again like negative reinforcement, occurs when the learners applying the skill do not do what they are told not to do. This also results in a positive (desired) outcome. This means it's important to find out what the participants in your program have been using their new skills for. Check to see if they are applying the techniques properly or whether they have misunderstood a key aspect of the program. Once wrong information is absorbed and used again and again it simply becomes another bad habit that could make financial decision-making worse instead of better.

Transference is most likely to occur in the following situations:

• **Association**: participants can associate the new information with something that they already know. What skills have the learners already mastered that they can bring to bear on better financial planning for example? Perhaps they have a hobby where it is necessary to access information from written materials or the Internet and the same skills could be used to obtain and analyse better financial data to use in their budgeting.

• **Similarity**: the information is similar to material that participants already know; that is, it revisits a logical framework or pattern. Using calendars or electronic planners to plan future holidays, work

shifts etc can be transferred to setting up a long-term budget planner for financial payments and income.

• **Critical attribute element**: the information learned contains elements that are extremely beneficial (critical) in personal life or in the workplace. Try to reinforce the importance of aspects of the financial literacy program to the learner's own goals, whether these are in their home life, getting a job or improving their prospects in work they already have. People can even start their own small business ventures if they have the financial skills to work out the costs and benefits first.

**4) DELIVERY STRATEGIES**

Finally in developing your program consider that adults have different personal and social lives than young people in formal schooling or college. Unlike children and teenagers, adults have many responsibilities that they must balance against the demands of learning. Because of these responsibilities, adults may have barriers against participating in learning. These barriers could include lack of time, money, confidence, or interest, lack of information about opportunities to learn, scheduling problems, "red tape," and problems with child care and transportation. Try to consider these factors when scheduling the program. If it is to be delivered to people in a workplace it should fit around their work times and not require them to come back hours later well after they have completed a hard day's work. Week-ends might seem like good free time to learn but many adult learners are conditioned to week-ends being for family pursuits and are likely to be reluctant to give up hours away from this for financial training. Try to identify groups of learners for each program that can support each other in transport to where the program is delivered, assistance in minding young children and common interests outside of the formal learning. Groups seeking employment or those soon to retire are obvious examples of participants who will have similar interests and motivations and can help each other to access the training and learn collaboratively to use the new skills.

**5) ENGAGEMENT OF THE LEARNER**

Good program strategies encourage real learning, where the learner increasingly:

• takes responsibility and ownership of their learning;
• engages in experiential learning;
• partakes in cooperative learning; and,
• engages in reflective learning.

By requiring or encouraging your learners to take a more directive and active role in the program as it is delivered you are encouraging them to engage in the critical processes of:

• making meaning out of the new financial management knowledge they have;
• distilling principles from the program, which will aid their transference of financial skills to new contexts; and,
• practising their financial planning skills and mastering processes to improve their money management.

In your financial literacy program learner directed activities can also encourage greater levels of motivation. The learning is more purposeful, because they have a sense of ownership over what they achieve and identify themselves as the key beneficiaries of the outcomes. An abstract exercise in developing a savings plan for an imaginary person or family may appear to introduce the right principles but it may not resonate with the individuals you are training. Think of your target group. What are their savings goals?

What aspects of their income are available to saving and how can they work this out?

What form of saving is best for them in terms of achievable targets, regular contributions and limited risk?

Teachers and trainers often develop example exercises based on imaginary situations because, frankly, they appear to put everyone on the same testing level and it is easier to assess because there are a common set of 'right' answers. This is not the way to make financial literacy learning work for the target groups. They should be encouraged to work on individual situations entirely relevant to them. This may mean more effort on the part of the trainer in assisting with the work each person is doing and assessing outcomes but the result will be practical exercises that keep the learners involved and motivated.

**6) ASSESSING PROGRESS AND OUTCOMES**

Good assessment is a collaborative process involving the assessor, learners and others, where appropriate. Your assessment process should be transparent and allow for ongoing feedback from and to the learners. Remember these adult learners want to improve their skills in managing money and are not necessarily interested in formal recognition or being ranked against their peers in the group. Where possible, presenters should emphasise from the start that no-one is going to 'fail' the program. Even where students are seeking formal certification of their achievement, presenters can advise that there is no competition between the learners in the group or between an individual and the topic material – it's all achievable and everyone can make it work for them. Make sure they understand that they will all leave with better financial skills than they have at the

beginning. If someone in the group is somehow 'better' or 'faster' at understanding superannuation than others that is their good fortune but makes no difference to the benefits everyone in the group gains from knowledge and skills in handling this important financial tool. Everyone will improve their life chances through participating in program and outside of training for formal certification, assessment is to demonstrate this to them and no-one else.

If you want further Information on collaboration in the design of assessment materials and the role of learners in the assessment process this can be found in:

• Guide One – Training Package Assessment Materials Kit and Guide Five – Candidate's Kit in the Training Package Assessment Guides; and,

• Learning Circles Resource Manual for Facilitators and Learners (developed by Adult Learning Australia).

**Conclusion:**

Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. Duties are placed on the learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning. The task force's policy recommendations are guided by these principles:

- Shift from top-down implementation of a federal or state program to leading a statewide public campaign that depends fundamentally on a bottom-up commitment of communities, employers, and educational institutions. The campaign must engage all aspects of Kentucky life—all dimensions of state and local government, all education levels, the state's business and civic leaders, voluntary organizations, and all others whose work affects—or is affected by—the problem of adult illiteracy.
- The future of Kentucky depends on narrowing the disparities among counties by improving the adult literacy of the population in all regions of the state.
- Shift from an emphasis on providers to the needs of clients. Measure performance and progress in terms of impact on the quality of life and economic well being of:
  - Individuals
  - Communities
  - Regions
  - The Commonwealth as a whole.
- Shift from an emphasis on programs and pilots to a focus on systemic impact on adult literacy in all counties of the Commonwealth.
- Focus on all adults who are in need of significant improvement in their knowledge and skills to be full participants in Kentucky's workforce and society, to develop and maintain healthy families, and to continue their education and training as necessary throughout their lifetimes.
- recognize multiple dimensions of the issue and, consequently, the importance and efficacy of multiple, separate but coordinated strategies aimed at the needs of different target populations, including, but not limited to:

- Parents of young children.
- Adults in the workforce, including those with secondary education credentials, for basic literacy and workplace skills and for retraining and upgrading of knowledge, skills, and competencies.
- Youth from 16 to 18 who drop out of school or are not well served by traditional secondary programs.
- Adults with significant learning disabilities that limit their ability to take advantage of further education and training.
- Adults with limited English language literacy.
- Incarcerated adults.
- Adults whose access to further education is severely restricted by geography, transportation, technology, and other economic and social barriers.
- All Kentucky adults who will need lifelong learning opportunities from basic literacy through postsecondary education to succeed in the changing society and economy.

- Emphasize both continuity and development of basic human and physical assets to provide services as well as performance in serving client, community, and Commonwealth needs and priorities.
- Recognize the current and traditional roles and strengths of public schools, postsecondary

institutions, employers, and other providers; as a corollary, avoid assigning to key players responsibilities that are inconsistent with their strengths.

- Recognize, support, and build upon the work of existing providers, if they meet performance expectations.
- Reward those who have performed and continue to perform effectively.

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# Strengthen Adult Education: Methods and Procedures

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**Abstract:** Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. duties are placed on the learner, a resource for developing knowledge, skills and insights he considered. Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his. another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will. to ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning
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**Keywords:** Adult Education; learn

## Introduction:

Adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge. Several definitions of adult education has been done Community

- Adult Education is a in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age limits as formal and informal, voluntary and at different levels of time, place
- Adult Education is a process in which people who and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

in developed countries, adult education is a form of informal education for people above 24 years is presented. In fact, a means of expanding knowledge, skills and abilities of adults. In these countries, adult education helps adults to variable conditions of political, social, economic and cultural adjustment, and pay to fix their shortcomings.

In developing countries and backward because the problems in primary education, lack of resources and facilities, poverty, social existence, economic and cultural concept of adult education is different. In such countries the concept of adult education, literacy education is.

Concept of adult education in revolutionary countries, is a combination of these two concepts. Changes in these countries due to social, political and cultural revolution, resulting from, literacy and continuing education necessary to find because of the revolution, there is cultural poverty on the other hand the implementation of development plans and the need for skilled personnel are expert. General adult education system based on economic conditions - social and cultural community is different and each specific goals will follow. General objectives of adult education and literacy in two categories is divided into professional education.

## Adult characteristics:

to understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

**Operating speed:**
slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

**Consciousness:**
no stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

**Health:**
what is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

**Background of knowledge - skills and beliefs of adults:**
adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

**Characteristics of adult education:**

**flexibility in time:**
In the past, usually one of the obstacles in the way of learning and development of adult education was being inflexible and time courses were programs. But now most countries have to consider that the speed limit of time and learning ability and facilities must be adults. Flexibility in time means that not only should the time classes and programs for adults is appropriate, but necessary facilities should be provided for independent study.

**Flexibility in the location:**
One of the aspects of flexible space is that individuals can, regardless of their residence to the study and advancing their knowledge and skills pay. For example, adults in remote villages should like people who live in the city use of educational programs. After flexibility in other places is that the issue of specificity of location is not considered primarily educational.

**Flexibility in age:**
Educational opportunities for certain age should not use it for all regardless of their age, is possible. In fact, educational programs must use people of different ages to prepare.

**Flexibility in admission:**
No adult should not only be deprived of education because of the necessary conditions for admission in the class does. Of course this is not such a person without academic records to participate in university classes is accepted, Adoption order is that the adults in educational programs at different levels, according to the possibility of using the opportunity that is provided must be based on the experience and knowledge and their knowledge is.

**To combine education and job responsibilities:**
Adults should be able to work during that time engaged in training classes take them. In other words, their presence in the class should be considered part of their work. This means that low-literate or illiterate working people who are allowed to work an hour of your daily spending surpassed participation in educational programs.

**Ways to Strengthen Adult Education**

**1- Create a culture that supports adult study**

1. Communicate that learning is intrinsic to faith development. Lift up ongoing study, including adult education, as an essential function of any Christian community.
2. Reinforce the expectation of study participation from the pulpit and with new members.
3. Make Bible study a part of other church activities such as committee meetings and mission activities.
4. Use scripture meaningfully in worship. Don't assume your worshippers know the context of the passages read. Use sermons as an opportunity to teach the Bible.

**2- Offer a variety of formats, schedules, and approaches**

5. Experiment with a variety of times -- Sunday morning classes, weeknight groups, retreats, oneday events, and breakfast-hour or noon-time classes – depending on lifestyles in your congregation.
6. Consider scheduling some classes or small groups in homes or other community locations. Christian education doesn't happen only in church buildings.
7. Start new studies and groups often. Despite their best intentions, ongoing groups have a tendency to become cliquish. Newcomers are far more likely to feel comfortable joining something new.
8. Have as your goal a Bible study program that exposes church members to the entire biblical witness over time.
9. Recognize different learning styles among individuals and age groups. Older folks tend to be most comfortable with traditional classroom

structures. Boomers are inclined to question authority and enjoy discussion. Younger persons are more accustomed to media and technology and prefer a fast-paced, informal style.
10. Make use of a variety of different approaches, including lectionary-based studies, topical studies, character studies, etc.
11. Incorporate different learning strategies, such as role playing, dramatization, guided meditation, even memorization.
12. Churches too small for a large number of groups can vary their approach by rotating different studies and curricula with groups.
13. Don't teach "about" the Bible in a way that doesn't allow people to encounter the texts for themselves. Encourage individual reading or make it part of the group's time together.
14. Encourage active, discussion-based learning. Break into small conversation groups frequently.
15. Allow for diversity in perspectives.
16. Encourage the use of a variety of different biblical translations. Those less experienced in Bible study may find it helpful to read from a paraphrase.

**3- Meet people where they are**
17. Acknowledge biblical illiteracy among many adult church-goers – even the well-educated – and strive for methods that straddle this paradox.
18. Recognize that some beginners will be turned off by "homework." Use videos, in-class readings, dramatizations, or audio tapes as alternative ways of getting everyone "on the same page" and ready for discussion, all the while encouraging the habit of daily scripture reading.
19. Provide short-term classes for those who won't commit to a long-term study or ongoing class, but make these short-term learning experiences "stepping stones" toward greater involvement.
20. Conduct "taster" classes for those who want to try out the experience before they commit to it. Select topics that will appeal to those new to Bible study.
21. Break an ongoing class into shorter, defined segments, each with a clearly identified focus. With each new segment, take the opportunity to publicize the topic and invite newcomers.
22. Teach stewardship of time to counteract "busyness." Just as with financial stewardship, persons need to be encouraged to make Christian education a priority. Encourage "first fruits" commitments of time.
23. Be clear about expectations with regard to attendance, participation, and preparation.

**4- Promote participation effectively**
24. Link group study topics to sermon series and encourage participation from the pulpit.
25. Emphasize study during Lent. Select a topic or curriculum for church-wide study during this period and encourage all to take part. Tie the topic into preaching and worship.
26. Lift up study leaders and participants. Celebrate every time a new group starts or completes a study program. Use the newsletter, a photo board, or a dedication service in worship.
27. Ask class members to write a newsletter article or testify about the significance of their learning experiences.
28. Remember that personal invitations are usually the most effective way of getting someone involved in any activity.
29. Capitalize on the current popularity of book clubs and films by creating opportunities for those who enjoy these activities. Check out "Reel Time" from Cokesbury.

**5- Foster strong leadership**
30. Recruit leaders as the first step toward forming groups. Groups will often form around a gifted leader.
31. Stress the group leader's role as facilitator, rather than teacher. Setting up one person as "the expert" creates a poor group dynamic and discourages new people from stepping into leadership. Thinking of group leaders as facilitators allows Scripture and the Holy Spirit to do the teaching.
32. Expect your pastor to model the importance of ongoing adult education by leading and participating in study, but don't reinforce the notion that only the ordained can lead study groups.
33. Take advantage of the leader training opportunities provided in conjunction with many popular study curricula.
34. Provide orientation and ongoing support for group leaders.
35. Train leaders in group process so they can keep their groups on track, being sensitive to the need to keep more outspoken participants in check and draw out the more reserved using phrases like, "Let's hear from some of the others," or "You look like you have something to say."
36. Emphasize the importance of leader preparation, especially mapping out discussion questions in advance.
37. Encourage team leadership. Experienced leaders should invite a newer person to pair with them in leading groups to develop the less experienced leader.
38. Rotate the leadership responsibility within a group so that all participants get experience leading sessions.

39. Know that Sunday School classes and small groups are one of the best places to develop lay leaders and lay relationships that strengthen the church.

**6- Use resources effectively**

40. Stay abreast of new resources, including those available from other denominations or traditions and the secular press.
41. Don't be afraid to introduce ideas and resources from a variety of theological perspectives. Trust the discernment abilities of individuals and the group.
42. Use workbook-style studies creatively. Nothing is more boring than a lesson read straight out of a leader's manual. Find ways to make pre-packaged lesson plans come alive.
43. Use videos to bring expert perspectives to bear and to get everyone "on the same page" for discussion. But avoid class sessions that are no more than viewing a video, or participants will soon wonder why they shouldn't stay home and watch their own TV.
44. Create a resource center with reference materials, maps, and other items to support your leaders and participants.
45. Don't allow your church library to become a museum. Update the collection. Offer books and resources linked to sermon topics and congregational study themes.
46. Consider a book sales kiosk and stock it with things you'd like your congregants to be reading. Many busy people would rather buy a book than worry about due dates and library fines.

**7- Stress spiritual formation**

47. Remember, the goal is formation, not information. Every class should be deliberate in helping members accept God's grace, grow in faith, deepen their relationship to the Christian community, and answer Christ's call to discipleship.
48. Include prayer as part of every study session and encourage group members to pray for one another daily.
49. Encourage a covenantal relationship within study groups.
50. Nurture a sense of Christian community and connectedness within groups. A Sunday School class or small group can be a "home" for individuals within a larger church.

**Conclusion:**

Adult learners have a different approach to learning. By the time you reach adulthood, you're most likely responsible for your own success and you're perfectly capable of making your own decisions once you have the information you need.

Adults learn best when learning is focused on them, not the teacher. This is called andragogy, the process of helping adults learn.
Malcolm Knowles, a pioneer in the study of adult learning, observed that adults learn best when:

1. They understand why something is important to know or do.
2. They have the freedom to learn in their own way.
3. Learning is experiential
4. The time is right for them to learn.
5. The process is positive and encouraging.

Types of content and educational resources in various parts of adult curriculum materials motivational book, course materials, supplementary materials, track materials (continued) participatory form and materials. Incentives aimed at providing content that audiences are produced primarily to attract different groups of adults interested in design, so that their participation in learning programs are encouraged. Motivational training materials for learners and have great importance even in support of successful applications over learners, planners and executors for educational programs is important.
Material often set different types of materials and educational content in books and pamphlets, books, training guides, trainers, equipment auxiliary audio, visual and material are included such that during actual teaching sessions, are used in the transmission and content but also to achieve the goals of making education programs are important.
Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:
1- - Preparation for adults to learn how much he depends on previous learning. Knowledge that has accumulated because of an ability to absorb new information more person is. Past educational experience features a diverse group of adult learners, the starting point of any activity on the diversity training is emphasized.
2- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable
3- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

4- To maximize learning, information must be provided an organized manner. Entries can be simple or complex can be arranged around related concepts are organized. Starting point for organizing content knowledge for adults and adults is linked to past experiences

5- Learning, especially regarding skills development, will be added frequently.

6 - Duties and meaningful content than meaningless subjects are learned more easily and are later forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

7- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

8- Environmental factors affect the learning. Tangible things such as noise, crowded places, temperature, light and ... Learning process can be prevented. Other factors such as stress, ridicule, pressure, fatigue and low health can also reduce learning.

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4/19/2011

# Lens Protein Changes Associated With Cigarette Smoking

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**Abstract: Purpose**: Smoking is an independent risk factor that has dose-response effect. The goal of the present work is to study the biophysical and biological effects of smoking on the crystalline lens of the rabbits. **Materials and methods**: Twenty New Zealand albino rabbits used in this study were classified into five groups in which group I (n=4) served as control. The other groups were exposed to different durations of cigarette smoke (five cigarettes per day). Animals were decapitated after 2, 4, 6 and 8 weeks and soluble lens proteins were separated and the following measurements were carried out: estimation of total soluble protein, refractive index measurement, sodium dodocyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and determination of sodium, calcium and potassium concentrations. **Results**: The results showed that, exposure of the animals to cigarette smoke resulted in decrease of the protein concentration and potassium content that was accompanied by an increase in the refractive index of the soluble lens proteins and an increase in sodium and calcium content. In addition, there were changes in the molecular structure of soluble lens proteins demonstrated by SDS-PAGE. **Conclusion**: smoking causes morphological and functional changes to the lens that may lead to cataract.
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Key words: Ultrasound, Rabbits, Lens, Refractive index, Proteins, SDS

## 1. Introduction

Cigarette or tobacco smoking is a well-recognized major risk factor for a wide range of diseases, such as cardiovascular, respiratory, and malignant diseases[1]. Human[2,3,4] and animal studies[5,6,7] have demonstrated that maternal smoking has several detrimental and teratogenic impacts on pregnancy and its outcomes such as placental insufficiency, foetal growth retardation, low birth weight, foetal brain damage, and sudden infant death syndrome. Smoking also has adverse ocular effects. It has been shown to be a risk factor for many common and severe eye diseases, such as Graves' ophthalmopathy[8], age related macular degeneration [9], glaucoma [10], and cataract [11,12,13]. Many of these diseases lead to irreversible blindness. The epidemiological relationship between smoking and cataracts has been well studied by case-controlled [14,15], cross-sectional [16,18], and prospective studies [19-20]. There is a dose-response relationship between the cumulative amount of smoking and the risk of nuclear cataract developing [21]. A major teratogenic component of tobacco smoke responsible for adverse effects is nicotine. A typical smoker using 20 cigarettes a day will absorb about 0.3 mg/kg nicotine daily, resulting in peak plasma nicotine concentrations in the range of 10 to 50 ng/ml[22,23,24]. The present work deals with the biophysical and biological effects of smoking on the crystalline lens of the rabbits after different periods of exposure namely 2, 4, 6 and 8 weeks to provide support for the hypothesis that cigarette smoking increases the risk of cataract formations.

## 2. Materials and methods

Twenty New Zealand albino rabbits with an average body weight of 2.5±0.5 Kg were selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The research protocol was approved by the local ethical committee that applies the ARVO (THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY) statements for using animals in ophthalmic and vision research. The animals were classified into five groups in which group I (n=4) served as control. The other groups were exposed to cigarette smoke (five cigarettes per day) [25] by using eye speculum to insure that their eyes were exposed to smoke. Groups II, III, IV and V were exposed to smoke for 2, 4, 6 and 8 weeks, respectively. Animals were decapitated after different periods of exposure and eyes were enucleated. Then, the lenses were freed from the eye. The lenses without their capsules were weighed, homogenized separately in de-ionized water and centrifuged at 16,000 rpm to extract soluble lens proteins then stored at -20$^{o}$C for the following measurements.

**Estimation of total soluble lens proteins:**

Total proteins in the soluble part of the crystalline lens were determined by the method of Lowry et al.[26].

**Refractive index:**

The refractive index of native soluble lens protein was measured using Abb's-refractometer attached with temperature control unit type W Lauda (Germany).

**SDS polyacrylamide gel electrophoresis:**

Soluble lens proteins were separated according to their molecular weights by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli[27] using 5% stacking gel and 12% separating gel. The data represented graphically with an automatic scanner (model R-112, manufactured by Beckman).

**Sodium ($Na^+$), Potassium ($K^+$) and calcium ($Ca^{++}$) content:**

Sodium, potassium and calcium content in lens homogenate were recorded using atomic absorption spectroscopy in order to study the membrane permeability of the lens.

**Statistical analysis:**

Data were expressed as the mean ± SD. Comparison between groups was performed using analysis of variance (ANOVA), commercially available statistical software package (SPSS-11, for windows) was used where the significance level was set at $p<0.05$.

**3. Results:**

Fig (1) shows the total soluble lens proteins of control and exposed rabbits to cigarette smoking after 2, 4, 6 and 8 weeks. The protein concentration in control (group I) was 290.7±3.5 mg/g lens tissue wet weight. After 2 week of exposure to smoke the protein content was 288.1±3.9 that indicate no change in group II. But the rest of exposed animals' groups show a significant decrease in the soluble lens protein content to 280±4 (group III), 260.4±5.2 (group IV) and 255±4.3 (group V) after 4, 6 and 8 weeks, respectively.

Fig (2) shows the refractive index of soluble lens protein for control and after exposure to smoke for all periods. It is clear from the figure that the groups exposed to smoke were characterized by an increase in the refractive index relative to control, except group II.

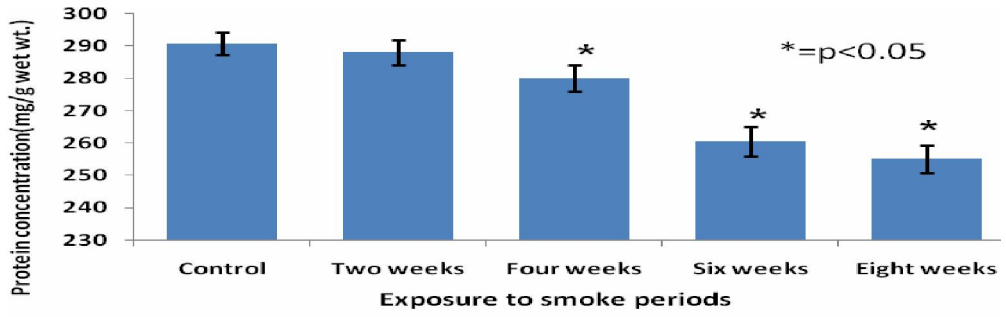



Fig 1. Protein concentration of rabbit lens for control and after exposure to smoking for different periods.

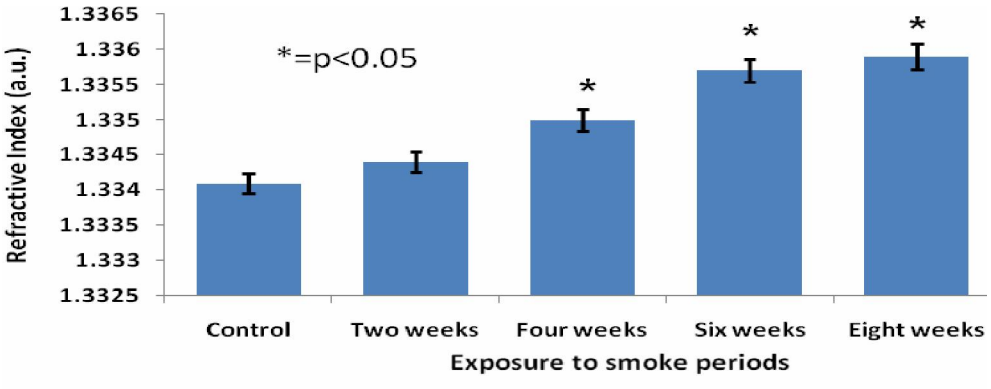
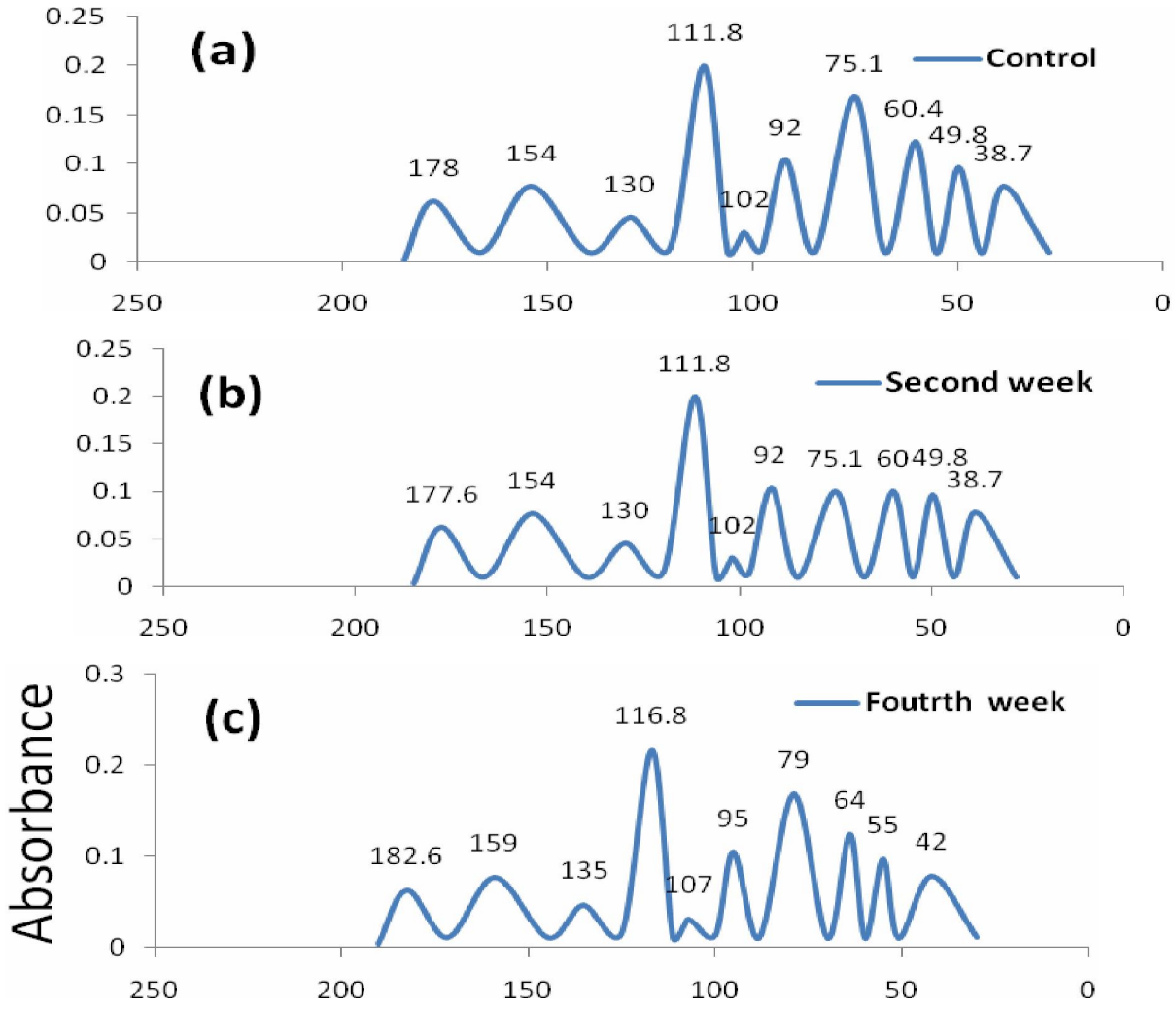



Fig 2. Refractive Index of rabbit lens protein for control and all groups exposed to smoking for different periods.

Panel (a) of Fig (3) shows the electrophoretic patterns of lens proteins for control rabbits, it was characterized by the presence of 10 peaks, which reflect the different soluble protein fractions with specific intensities and broadening that covered the molecular weight range 39 – 178 KDa. In panel (b) of fig (3), the patterns of lens proteins for group II which were exposed to cigarette smoke for 2 weeks revealed no change in the electrophoretic mobility and the intensity of all peaks proteins fractions. After 4 weeks of exposure to cigarette smoke (panel c of fig 3), the pattern revealed some shift to high molecular weight for all fractions and covered the molecular weight range 42-183 KDa. Also a decrease in the intensity of the 75 KDa peak is observed to be 0.1 compared to the control which is 0.17. Panels (d) and(e) of fig (3) shows the electrophoretic pattern of soluble lens proteins for animals exposed to cigarette smoke for 6 and 8 weeks, respectively. The two patterns revealed propagation of the same phenomenon; shift in all fractions to high molecular weight and covered the molecular weight range 40-185 KDa and 65-195 KDa after exposure to smoke for 6 and 8 weeks, respectively. Panel (d) characterized by decrease in the intensity of low fractions mobile groups. Also panel (e) (8 weeks exposure to smoke) is characterized by reduction of fractions to 9 peaks.

The cigarette smoke influence on the concentration of cations in the lens protein homogenates was illustrated in Figs 4-6. Sodium, $Ca^{++}$ and $K^{+}$ concentrations did not change after 2 weeks of exposure to cigarette smoke. Four, 6 and 8 weeks of exposure produced a remarkable increase of both $Na^{+}$ and $Ca^{++}$ concentrations in exposed rabbits (Fig 4, 5). On the contrary exposure to cigarette smoke caused a pronounced decrease of $K^{+}$ concentration with respect to control samples (Fig 6).



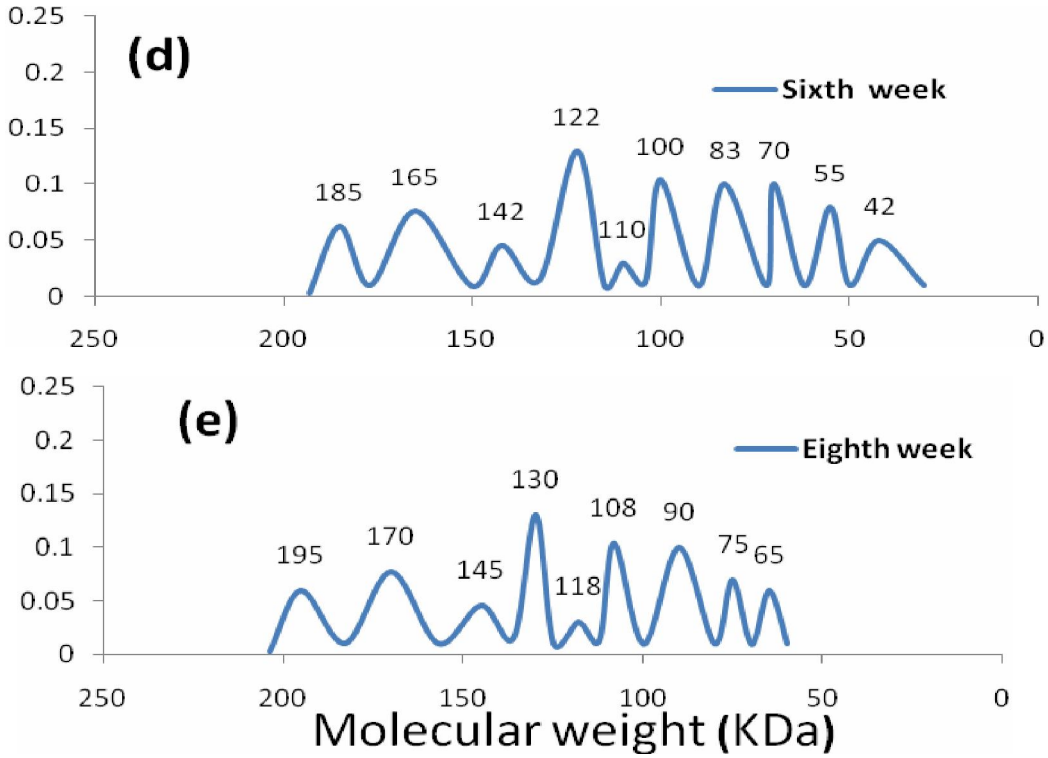



**Fig3. Electrophoretic pattern for (a) control animals, (b), (c),(d) and (e) animals exposed to cigarette smoking for 2,4,6 and 8 weeks, respectively**

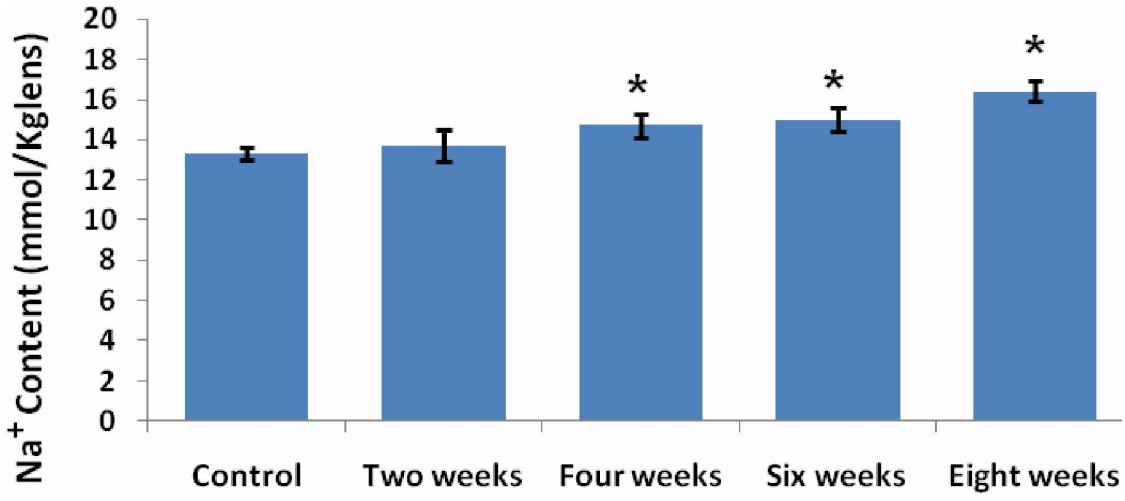



Fig 4. Sodiem concentration in lens homogenate of control and exposed to smoking for different periods.

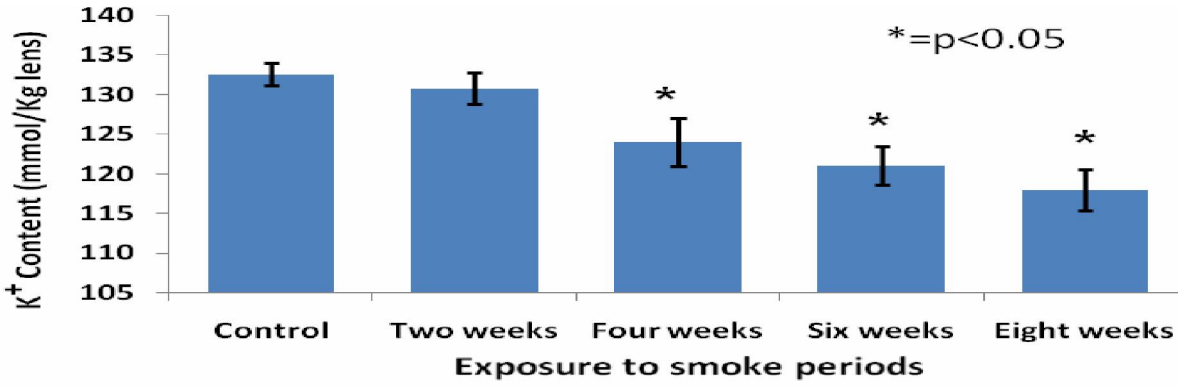



**Fig 6. Potassium concentration in lens homogenate of control and groups exposed to smoking for differemt periods.**

**4. Discussion**

Cigarette smoke contains numerous organic and metallic compounds emitted as gases and condensed tar particles, many of them being oxidants and prooxidants, capable of producing reactive oxygen species (ROS). These chemically ROS are known to be present or formed in cigarette smoke which may lead to modification of biological macromolecules [28]. Smoking causes morphological and functional changes to the lens due to its atherosclerotic and thrombotic effects on the ocular capillaries. Smoking also enhances the generation of free radicals and decreases the levels of antioxidants in the blood circulation, aqueous humour, and ocular tissue. Thus, the eyes are more at risk of having free-radical and oxidation attacks in smokers.

Total soluble lens proteins of the rabbits lens and refractive index were quantitatively changed after exposure to cigarette smoke more than 2 weeks. These changes may give an interpretation about the appearance of high molecular weight aggregates which could be attributed to the formation of new protein molecules that differ from the native protein of the control lens. Also these changes are supported by the electrophoretic studies in which shift of all fractions to high molecular weight due to either loss of surface charge or increase in the molecular weight.

The present study supports the hypothesis that damage to lens cell membrane affects ion exchange mechanisms with associated formation of cataract [29,30]. It has been demonstrated that in cataract formation there is a significant increase in lens cytosolic calcium and sodium concentrations, together with a decrease in cytosolic potassium levels [31] .The results obtained in our study suggest that increased levels of calcium and sodium and decreased levels of potassium are related to the oxidative damage of cigarette smoking relative to the period of smoking time. Duncan et al.[32] studied the physiological status of human eye lens membranes of different ages in the population. One likely mechanism of cataract formation is oxidation and precipitation of lens proteins. Smoking may increase the oxidative stress in the lens that increases lipid peroxidation and decreases plasma antioxidant levels. The relationship between smoking and cataracts has been well studied [14,16,19]. There is a dose-response relationship between the cumulative amount of smoking and the risk of nuclear cataract developing [21]. Heavy smokers are more at risk than other groups. The cigarette smoking contributes to the formation of cataracts in two ways. First, free radicals present in smoke assault the eye directly, potentially damaging lens proteins and fiber cell membrane in the lens. Second, smoking reduces the body's levels of antioxidants and certain enzymes which may help remove damaged protein from the lens.

In conclusion, Smoking -if continued- may lead to cataract, perpetuate further ocular damage and lead to permanent blindness. Cessation of smoking and avoidance of passive smoking is advised to minimize the harmful effects of smoking on the eyes.

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4/21/2011

# Key Factors in E- Banking: Concepts & Applications

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**ABSTRACT -** With the phenomenal growth of B2C e-commerce, most industries including banking and financial services sector have been influenced, in one way or another. Several studies suggest that customers have not adopted B2C e-commerce in the same degree primarily because of risk concerns and trust- related issues. This paper extends an area of information systems research into a marketing of financial services context by look in into the element of trust and risk in e- banking. A conceptual model of trust in e banking is proposed with two main antecedents that influence customer's trust: perceived security and perceived privacy. Trust is being defined as a function of degree of risk involved in the e- banking transaction, and the outcome of trust is proposed to be reduced perceived risk, leading to positive intentions towards adoption of e- banking.
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**Keywords:** Electronic Banking, Trust, perceived risk, satisfaction, Quality.

## Introduction

With the development of the internet, more knowledge is accessible to people anywhere at anytime. Facilitating communication, data transmission, and global interaction, the internet is a playing field unlike any other. Transcending the traditional barriers of time and space, the internet is redefining the world of banking. The internet has created new methods for carrying out a variety of financial transactions. With these developments, a new era of banking has emerged which has come to be known as “e-banking”. E-banking encompasses an array of financial transactions, once done through the tangible exchange of information, now are done electronically. While the benefits of such advancements have been welcomed, there also have been drawbacks. Issues such as security, fraud, and theft have deterred people from participating in the internet e-banking revolutions [1].

The extension of money and banking to the cyberspace is an inevitable development in the information age. Over the past few years, many financial institutions have launched e- retail banking over the internet. Given the requirements of matching marginal gains against marginal costs, evaluating the profitability of market development along specific dimensions and segments, and determining whether the new technology would be accepted, it is imperative that this decision is continually re-evaluated. Commercial banks face significant challenges on both the supply side and demand side, associated in particular with competition, product-service quality and differentiation, transaction security, cost efficiency, and demographic change [2].

Many banks have hired qualified teams of network administrators as a part of their IT departments to ensure the safety of both the customer and the institution that operate from and log into the banks network. Future compliance with these security measures also will lead to techniques such as biometrics and electronic fingerprint ability. Banks are also encouraged to focus on security from within by exploring scenarios of disgruntled employees or hackers from within the organization. The responsibility for safety and protection also lies with the customer [3]. The development of customer identification numbers, passwords, and other forms of customer identifications permitting users to into a banks web site and make secure transactions are the main emphasis behind consumer protection. Password protection is the one of the biggest problems facing customers. Creating passwords that are not easily recognizable prevents outside parties with malicious intent from computer hacking. Many banks now require passwords to be case sensitive, include a certain number of characters, and contain both numbers and letters. In addition, customers are recommended, and in some cases required, to change their password on a regular basis [4, 3].

Customers' trust on electronic banking transactions as compared with face to transactions have some unique dimensions, such as the extensive use of technology for transactions, the distant and impersonal nature of the online environment, and the implicit uncertainty of using an open technological infrastructure for transactions. The spatial and temporal separation of the bank branch and the customer, and that of the customer.

The financial advisor increases fears of opportunism arising from product and identify uncertainty. Customers trust in an internet environment thus, is very important as there is little guarantee that the online vendor will refrain from undesirables, unethical, opportunistic behavior, such as unfair pricing, presenting inaccurate information, distributing personal data and purchase activity without prior permission [4,5]. To further complicate the situation there is a concern about the reliability, of the underlying internet and related infrastructure the banks and financial service providers employ to interface with customer. Overall, these unique differences reduce customer perceptions of control over their online transactions, increasing their apprehension about adopting e-banking and providing unique challenges to banks and financial service providers to find ways in which to initiate and foster electronic relationships with their customers [6]. It is important to understand the factors that might influence consumer's intentions to engage in banking and financial services over internet. As discussed in the next section an important factor that is recognized as key for the continued growth of electronic banking is the concept of trust. Congruent with this, the aim of this paper is to explore the nature, drivers and consequences of customers trust on the banking and financial services over internet. such understanding of customers trust will provide the practitioners and researchers with a set of manageable, strategic levers to build such trust, which will promote greater acceptance of electronic banking and financial services [7,8].

## THEORETICAL PERSPECTIVE AND DEFINITION OF TRUST

Trust has long been considered as a catalyst in many buyer – seller transactions that can provide consumers with high expectations of satisfying exchange relationships [9]. Many researchers have argued that trust is essential for understanding interpersonal behavior and economic exchanges [10, 11] The notion of trust has been examined in various contexts over the years are related to bargaining [12], industrial buyer–seller relationships [13], distribution channels [14], partner co-operation in strategic alliances [15], 1998), and the use of market research [16] personality psychologists traditionally have viewed trust as an individual characteristic [17]. They have conceptualized trust as a belief, expectancy, or feeling deeply rooted in the personality and originating in the individuals early psychological development, also known as disposition to trust. However, this approach can only be taken into account but is an uncontrollable factor that cannot be influenced by the web merchant [18].

## RESEARCH ON TRUST IN E-BANKING

The particular case of electronic banking that lacks the physical presence of bank branch and a physical interaction between the bank personnel and the customer, render a unique environment, in which trust is of paramount importance. Retail banks can build mutually valuable relationships with customers through atrust–based collaboration process [19]. Howevre, the way in which trust may be gained and the impact it has on online banking outcomes are not yet well understood [20]. Trust in electronic banking is a new and emerging area of interest in the filed marketing of financial services research. Extant literature on trust related to online banking is scarce and focused on more general issues of e- commerce.

## BACKGROUND TO E-BANKING AND ITS SUCCESS FACTORS

Some researchers in the field of e-banking have been engaged in quantifying the current provision of electronic services by the banks from an innovation and marketing point of view [21]. Liao and cheung (2002), have explored the perception of customers about ebanking. King and liou (2004) and compared the e-channel with other channels. Some strategic issues such as outsourcing of e- banking initiatives have been discussed by Cantoni and Rossignoli (2000) or competitive advantage of e-banking by Griffiths and Finlay (2004), but the area of strategic organizational issues of e–banking has generally not been covered adequately by the current body of the literature. This research was aimed to help bridge this gap. This section summaries some of the research done in this area. We have divided these factors into three categories: strategic, operational and technical. This categorization will help to explain our findings in terms of the nature of success factors in e- banking adoption [22, 23].

## STRATEGIC FACTORS

The interactive nature of e-banking also creates an opportunity to gain a much deeper understanding of the customer during his/ her interaction with the bank can be analyzed using data mining techniques and this marketing decision capability may ultimately determine the success of the banks internet channel [4]. To succeed in the e-banking arena, companies need to transform their internal foundations to be effective because of the reasons mentioned above. The new type of business would consist of finely tuned integration of business, technology and processes [24]. Therefore one critical issue is re-

engineering of the business processes, which also includes technological processes.

**OPERATIONAL FACTORS**

The most common factor cited by many in literature, is good customer service [25] Legislation has increased customers rights while technology and competition have increased their choice of products and providers. The increasing amount of information on the internet and changes in social behaviours has reduced the loyalty factor considerably. These changes will result in the growth of users with sophisticated needs and new channels are required to serve most of these needs. Harden (2002) argues that e–channels erode a direct relationship with customers and stresses the need for personalization in customer communication. According to Jayawardhena and Foley (2000), banks must continually invent new products and services in light of changes brought by the internet and also make existing products more suitable for online delivery. Similarly, Riggins (2000) identified a number of critical success factors of internet banking in the context of the Australian banking industry. These include: developing the will to innovate rapidly, aggressively marketing the banks website address to generate first time visitors, online decision support tools for personal financial management, the creation of an online virtual community for financial services, and bundling of products/ services [26].

**TECHNICAL FACTORS**

Security, which may include protection of consumer's personal data and safe transactions to prevent misuse, is paramount for the growth of any sort of online trade, including e- banking. Security in this context includes secure transactions as well as secure front and back up systems [25, 27].

Franco and klein (2006) stress the importance of upgrading existing technological infrastructure (which may still largely depend on slow and fragmented legacy systems)to bring it up to the speed with the internet trade. Storey, Thompson, Bokma, and bradnum (2000) state that technology failures lead to loss of custom, often forever. Shortcomings in technological infrastructure are often the biggest hurdle in adoption of the e-banking channel and its integration with other channels [11].

**A MODEL OF E-TRUST FOR ELECTRONIC BANKING**

The literature on trust provides a useful basis for investigating consumer trust and its antecedents in the context of electronic commerce, but as pointed out by Mayer et al. (2005) many researchers confuse trust with its antecedents. This section aims to remove this confusion by proposing a simple yet parsimonious model of trust on electronic banking, with strong support from literature. While proposing their model of organizational trust Mayer et al. (2005) suggested that a parsimonious model with a manageable number of factors should provide a solid foundation for the empirical study of trust on another party. Based on the above discussion and the review of literature, a theoretical model for of trust in e-banking is proposed in Figure.1.

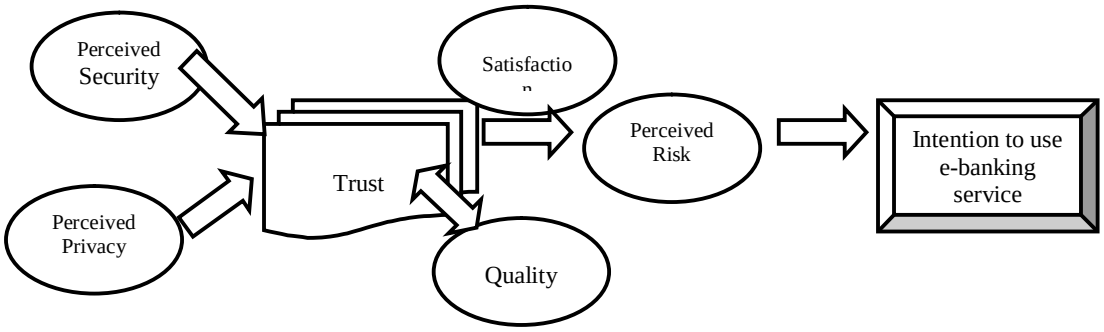


Fig 1. The proposed model of e-trust for e-banking [28]

**PERCEIVED SECURITY**

Security is being defended as a threat which creates circumstance, condition, or event with the potential to cause economic hardship to data or network resources in the form of destruction, disclosure, modification of data, denial of service, an/or fraud, waste, and abuse [22] under this definition, in context of electronic banking threats can be made either through network and data transaction attacks or through unauthorized access to

the account by means of false or defective authentication. Perceived security then is the customers perception of the degree of protection against these threats. Security has been widely recognized as one of the main obstacles to the adoption of electronic banking seems to remain one of the most significant barriers for adoption. The rapid developments in technology have made significant contributions to securing the internet for electronic business. However, the challenges remain in this area, and security remains a substantial issue for the development of electronic businesses, especially electronic banking. The need for security has already been recognized within the electronic banking community and a number of technologies have been developed to secure electronic transactions [29, 30].

**PERCEIVED PRIVACY**

Privacy has been identified to be a major, if not the most critical, impediment to e-commerce: In our view, the single, overwhelming barrier to rapid growth of e–commerce is a lack of consumer trust that consumer protection and privacy laws will apply in cyberspace. Consumers' worry, deservedly, that supposedly legitimate companies will take advantage of them by invading their privacy to capture information about them for marketing and other secondary purposes without their informed consent [2] Consumers in online environment in contrary to traditional retail environments, perceives little control over information privacy and this has a striking influence on their willingness to engage in exchange relationships with merchants. Due to the fall in cost data transmission and emerging technologies, it is now easier to collect personal information from customers and share it with third parties.

According to stone and stone (2000) customers are likely to have positive perceptions about privacy when: information is collected in the context of an existing relationship. (b) They perceive that they have the ability to control the future use of the information. (c) The information collected or used is relevant to the transaction, and (d) they believe that the information will be used to draw reliable and valid inferences about them [31].

**PERCEIVED TRUSTWORTHINESS**

People make important buying decisions based, in part, on their level in the product, salesperson, or the company. Similarly, electronic banking decision involves trust not simply on the transaction medium but also between the customer and the bank or financial service provider. Mayer and his colleagues have identified and validated three main element of trustworthiness: integrity (trustee honesty and promise keeping), benevolence (trustee caring and motivated to act in the trustor's interest and competence [1].

**QUALITY**

Quality can be defined as excellence. Dabholkar (2000) posited that within service contexts, the evidence supports an assertion that customers who view technology based service as easy-to-use, reliable, and enjoyable also perceive service quality in such technology–mediated service offerings (i.e.-service). Perceived service quality is believed to contribute to positive business outcomes such greater levels of customer satisfaction and, by extension, favorable marketing behaviors such as repurchase and positive word–of–mouth behaviors [32].

**SATISFACTION**

Satisfaction, on the other hand, is the consumer fulfillment response (Oliver, 2007). Szymanski and Hise (2000) argued for the impotence of e-satisfaction in technology–mediated relationships. The authors suggested that the conceptual domain of e-satisfaction appears similar to that understood from the general marketing literature. This assertion further supports our reliance on Oliver's (2007) constitutive definition for purposes of this research study. In addition, satisfaction judgments are generally believed to be superior to quality perceptions.

B2B, or business–to–business e- businesses, are companies that sell to one another online. B2C, or business–to- consumer e-businesses, are companies who sell to consumers via websites (Lerouge & Picard, 2000; Morrish, 2001) some writers argue that integrating both B2B and B2C capabilities may become essential to respond to customer demands and streamlining their supply chain management .C2B refers to trade between consumers and businesses, and is best exemplified by companies like Priceline. Com. C2C refers to trade between consumers and is best exemplified by companies like eBay.

The relationship between satisfaction and loyalty has also enjoyed a measure of attention in the recent literature. This research study envisions loyalty as super ordinate to satisfaction in that loyalty can capture long–term relationship elements that lie outside the domain of satisfaction in a business–to business (B2B) context (Barnes et al, 2000). This B2B perspective appears consistent with Heskett, Sasser, and Schlesinger (2007) and Hunter (2007) who asserted that three primary measurements of customer loyalty commonly known as the three R's included [33]:

1. Revenues and profits from retention of loyal customers.
2. Repeat sales
3. Referrals

From a practitioner perspective, Pastore (2001) suggested that customer loyalty and satisfaction will continue to play key roles as companies evaluate spending budgets based on a study by NFO prognostics (proprietary). In fact while the study suggested that satisfaction scores and reference ratings are generally strong, many professional service buyers are shopping around with each new IT project. Such shopping around behavior is an indicant of a weak marketing relationship. This finding strengthens the basic premise of this research study calling for relationship–marketing-based models specific to the e-banking industry. In summary, the weight of the evidence to date suggests that satisfaction should be subordinate to loyalty in the formation of customer behaviors .Assuming a base level of satisfaction, the research expects loyal customers to engage in activities that support and strengthen their relationship with the sponsoring e-banking company, as well as engage in positive word–of mouth activities within the professional Community.

**DISCUSSION AND CONCLUSIO**

Trust is been identified as key to e-commerce. If trust is vital, then building trust is even more crucial. This paper provides several preliminary insights into the role of perceived security, perceived privacy and the perceived trustworthiness attributes on the issue of trust in electronic banking. The paper also highlights the importance of using security and privacy as two distinct concepts, even though they are conceptually related. It has attempted to review the nature of customer's trust on ebanking and proposed a research model of customer trust on e-banking. The model presents the major relationship between customer trust and two major potential antecedents perceived security and perceived privacy. The trust model presented in this paper provides a coherent framework for further empirical research on the phenomenon of trust in e–banking [34].

This paper, tell the e-banking practitioners which trust antecedent to focus on in order to increase customer trust and thus increase the adoption rate of e- banking. Bearing in mind that we have proposed that trust antecedents are perceptual in nature, they can be influenced by appropriate advertising and marketing campaigns, visible privacy policies and the web site design of the bank. Finally, research into the trust model developed in this paper will help accelerate the adoption of e- banking by removing one of the major obstacles to its development, namely, lack of trust.

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# The role of indigenous knowledge toward improving agriculture

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**Abstract:** Experience shows that indigenous knowledge not only has no contradiction with formal knowledge but different indigenous knowledge features, put it as well complementary for formal knowledge. Indigenous knowledge is accessible, useful and cheap. Its perspective is holistic and its transmission is verbal. Knowledge is dynamic and time-tested, and while it has grown within local natural and social environment, so it is very sustainable with indigenous condition. Indigenous knowledge refers to both component and whole part of culture of each nation and this component and whole integration is so that stop to change traditional society of life without indigenous knowledge out of its cultural origin and therefore would lose it concept and effectiveness.
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**Keywords:** agriculture, indigenous knowledge

## Introduction:

In recent decades following issues had been recognized very essential : programming and performing development plans , indigenous knowledge at farming , pest control , ranching , veterinary , nutrition , medicine , watershed management , foresting , architecture , urban planning , social associations and decision making method as sustainable technology . At on hand, reason of this great evolution can be found due to wrong policy and at the other hand in undesirable environmental consequences of these policies.

studies have given new dimension to agriculture research. Now, in many countries the managers of agriculture resources are the people who are trained in western countries. So if the manager become familiar with the culture and environment roots of indigenous system of resource management, they won't do mistake. Indigenous agriculture is based on cooperation of farmer with nature.

Indigenous knowledge of each nation has enabled them to supply their needs from natural sources without reducing these sources. So, indigenous knowledge collection of world is valuable source of practices and time-tested tool that would be useful for sustainable development of all societies.

At third world countries, unconsidered triumph of world development policies has led to various social, economic, cultural and environmental issues (Agrawal, 2002).

Imbalance population growth, non-sustainable efficiency of natural sources and unequal distribution of resources, goods and services made involved societies in confusing issues and impasses. In these countries , inappropriate sampling of abroad countries and inordinate imports (e.g. heterogeneous and non-indigenous technology ) devastated independent collection of micro local systems , and instead has established heterogeneous and dependent system to global economy system , that obviously couldn't supply people's needs. Since , this development process is formed without considering social , cultural and environmental consequents so isn't continuing and human have to find strategies which can make development sustainable and humane(Popzan, 2002) .

Indigenous knowledge owners of world in current age (which known as information age) have valuable experiences from industry age and from inappropriate exploitation of their natural sources. These countries have learned that exporting produced goods is better than selling petroleum. enforcing indigenous productive system at villages and also encouraging youths and teens to learn indigenous knowledge at on hand , and preparing suitable research condition for applied-sciences scholars in order to identify better and increasing applied aptitude of indigenous knowledge at the other hand , is equal to protection and sustainable use of natural resources(Zare, H and Yaghoubi, 2003).

From Robert Chambers' view, power and wealth are at industry and at cities, and poverty and deprivation are part of villager's life. Power and wealth of cities of world has absorbed experts, sources and needed research facilities for producing and disseminating knowledge. Knowledge of these modern centers is considered scientific, advanced, and valid and enjoys premium technology. He labeled this group as "first" and in contrast "last" for deprived villagers. Because, preferences and values of these two groups are different.

Their knowledge and attitudes are also different. he believes that since "first" development remedies and their attitudes have led to fault , irregular and deprivation , so deprived villager's attitudes and knowledge should be considered serious in order to reach to improve conditions for this part of human society as they need and demand(Azkia, M and Imani, 2008) .

**Features of indigenous knowledge**

Some of these features are as follow:

Indigenous knowledge is holistic: indigenous knowledge is gained by sense and inspiration force and leads information unity. In spite of formal knowledge that is aural, visual and analytic.

Indigenous knowledge is verbal: writing and documenting indigenous knowledge would make it out of reach of villagers who can add to it, if it would not follow applied activities.

Indigenous knowledge is practical: it is possible to write about indigenous knowledge but it is impossible to educate and learn it through books and articles. Only way to learn it is close view and follow professor.

Indigenous knowledge isn't explanatory: it isn't possible to expect one master (e.g. mason, apothecary, farmer) to explain his method efficiency in a way that is apprehensible to us (literate people)

Indigenous knowledge is local: villager's knowledge has formed in itself environmental and climate framework. Effective indigenous knowledge at one geographical area isn't necessarily effective at other area (Nowroozi, A and Alagha, 2000).

Indigenous knowledge is general : while, formal knowledge emphasis is on saving time and removing ideas and also monopoly of knowledge at universities and research institutes , but indigenous knowledge is , receptive , incentive and needs to more people's participation at learning , developing and add to it. Furthermore, in verbal cultures, it is impossible to separate science from world and even include it to computer and book. Every human are important in indigenous knowledge.

Indigenous knowledge is deteriorating quickly: by every death of old indigenous people, great knowledge resources would be lost also, so every action toward gathering indigenous knowledge is necessary.

Learning by doing: repeating action in order to sustain and enforce indigenous knowledge through "learning by doing" is one of features of indigenous knowledge in real operation environment (Emadi and Abbasi, 2001)

Villager's knowledge and especially indigenous knowledge systems have various dimensions that is include linguistic knowledge, zoology, ecology, climate, agriculture, ranching and professional skills. Range and value of this knowledge hasn't been considered. Four aspects of various dimensions of rural knowledge were selected and were analyzed, In order to change attitudes and reformer's behavior of rural development. These dimensions are: agriculture operations, rural knowledge about nature, rural people's aptitudes and abilities and their experiences (Razavi, 2002).

In Chambers' opinion , indigenous knowledge or rural knowledge has various dimensions that he classified them to four parts in order to explain more and better about diversity of indigenous knowledge that are as follow : A: farming activity ; B: knowledge in relation to nature ; C : indigenous people's aptitude and ability ; D: indigenous people's test . indigenous people's knowledge originated from exact viewing of environment; since indigenous villagers have direct contact with phenomenon and also see all different processes at nature so have especial aptitude and ability compared to outside people . Maybe least known aspect of indigenous villager's knowledge is essence of tests that they do which maybe these tests are available to choose "bests" and some other for "minimizing risks" (Dewes, 1998).

**Sustainable agriculture**

Generally sustainable agriculture is every kind of production system which follows theses goals:

More complete mixing of natural processes such as food cycles, nitrogen fixation, and relation of pests and natural disasters with agriculture productions processes.

Decreasing use of that non-farming, outside and non-renewable inputs in order to reduce damage to environment or less damage to farmers and consumer's health.

More fair access to interests and productions opportunities and progress in order to access to forms of agriculture that is fairer, and also increasing self reliance between farmers and villagers (Chambers, 2000).

Using more potential biologic and genetic aptitude of plant and animal species.

Using more local knowledge including innovative approaches that scholars didn't understand it completely or farmers didn't accept it extensively.

Combined agriculture would prepare this opportunity for common systems to apply needed reforms without creating inclusive changes in it toward organic systems. Therefore, aforementioned systems are considered as medium between common intensive agriculture and organic agriculture methods.

Two principles have especial importance at sustainable agriculture that is:
at early 1980's , with the emergence of new concepts, renewable agriculture and sustainable agriculture evolved and indeed it was based on "ecological interplay affect" .now, this concept forms alter indigenous agriculture philosophy.
Sustainable agriculture presented from 1987 at global scale. In this principle, "agricultural interplay affects with society" is presented. Three issues are important about sustainability: first is enough income especially between poor people. Second is increasing access opportunity to food and its consumption. This means that more food should be prepared through increasing production and improving marketing. Third issue contains protecting and improving natural resources (Louise, 2000).

**Conclusion and discussion:**

At one research as a name of "analyzing position of indigenous knowledge at sustainable rural development" that was done by Buzarjomhore (2005) it was signified that although there are some differences between indigenous and formal knowledge, but they should not be compared, because they are complementary of each other and it is possible to gain successes by synthesizing them that is impossible lonely. Base on new paradigms of rural development in order to solve rural problems, we should first refer to indigenous solutions and if it was working, then we should reinforce it; if not we should test and use outside solutions. Findings of one research done by Emadi and Amiri (2004), as "Synthesizing indigenous knowledge and formal knowledge as necessity for accessing to sustainable rural development", has shown that dominated belief among educated groups toward natives and their knowledge is precondition of every interaction, synthesis and relation. Creating revolution in formal education systems in order to attending empirical knowledge area is considered as one of main necessity of this synthesis that is outcome of years of researches. Researchers attention to "exploiter's accumulated experimental and historical wisdom" is one of other necessities of this revolution by using cooperative, qualitative and filed methods. Also, applying mutual extension ways and creating revolution at communication system between governmental, education-extension centers and farmers and rural people so that they be interacting, was considered as precondition and necessities. At researches as "indigenous knowledge at development process" done by Karimi (2003) , findings show that indigenous knowledge is principal factor and main source at the field of research of sustainable development , decreasing poverty , enabling local men and attracting their participation at activities and rural development programs, developing and producing appropriate technology , self-reliance of rural societies and country.

So, effort and national commitment and multi-dimensional support is very critical for recording, valuing, extending and exchanging this rich source and also preparing mechanism and practical strategy for synthesizing this knowledge with new knowledge and agricultural development programs.

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# Aerobic Degradation of Synthetic-Based Drilling Mud Base Fluids by Gulf of Guinea Sediments under Natural Environmental Conditions

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**Abstract:** Synthetic-based fluids (SBF), which are composed mostly of linear alpha Olephins, Esters and Paraffins are used in drilling mud to lubricate the drill bit, control reservoir pressure and bring rock chips and cuttings to the surfaces which are subsequently released into the marine environment as a residue on the cuttings as they are discharged. Aerobic biodegradation is a major criterion for selecting synthetic –based fluids for drilling mud. In the present study, sediments were collected from four different locations in the Gulf of Guinea measuring from 100-500m depth and were used in indoor basin benthic chamber tests to measure degradation rates of 4 different Ester based synthetic fluids at room temperature over a 120 day test period. At each 30 day interval, residual organic carbons were measured by gas chromatograph while microbial populations were quantified with the most probable plate number method (MPN). At the end of the 120-day monitoring period, the following % degradation rates were recorded for the different ester based fluids used in the study; BR-EST (94%), CH-EST (91%), PFB-009 (94.8%), PFB-008 (93.8%). This result indicate that the Ester based fluids used in the experiment are readily biodegradable and the Gulf of Guinea sediments harbour considerable populations of indigenous hydrocarbon utilizing microorganisms that are capable of degrading the exogenous ester based synthetic fluids. This study addressed the fate of the synthetic ester base fluid portion of the drilling mud in Gulf of Guinea sediments by determining the potential of indigenous marine sediment microbes to degrade representative SBF under natural conditions.
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**Keywords:** Synthetic base fluids, Drilling mud, Cuttings, Biodegradable, Hydrocarbon utilizing microorganisms, Gulf of Guinea sediments.

## 1. Introduction

Three major types of cuttings can be defined depending on the drilling muds used to facilitate the boring process and also to carry the cuttings to the surface. They include:

i. Water based muds containing for example KCl/Polymers or glycol.
ii. ii. Pseudo-oil-based muds commonly comprising of olefins and Esters
iii. Oil based muds comprising either clean mineral oil or in early stages, diesel.

The oil based muds are considered to have the most deleterious effect on the local environment especially diesel, so their use has been gradually phased out in some countries (Kjeilen *et al*, 1996). Alternative types of drilling fluids have been developed as a consequence of increasingly strong environmental protection legislation. The alternative drilling fluids have been designed to have less negative impact on the environment i.e., they are more easily degradable and less toxic than oil based drilling fluids. These alternative drilling fluids are pseudo oil based comprising mainly of Olefins, Esters and Paraffins and they have been proved to be very important in difficult deepwater drilling operations (Deborah and Alan, 2006). They also combine the technical advantage of oil base fluids and the low toxicity of water base fluids and are used mainly to lubricate the drill bit, control reservoir pressure and bring rock chips or cuttings to the surface.

Ester based fluids are well known for their high biodegradation potential but they are susceptible to calcium and acidic gas combination as well as thermal limitations (West *et al*, 2009), despite that, Ester based fluids deliver outstanding performance even under extreme bore hole and formation conditions (Tapavicza, 2005). Ester quality (EQ) stands for a new generation of drilling fluids, they are based on vegetable esters derived from natural raw materials like palm kernel oil. The overall benefits of Ester based drilling fluids include; faster drilling, reduced drilling costs, superior lubricity, excellent hole cleaning, protection of drilling formations and proven track record on performance (Tapavicza, 2005). Esters strongly protect the geological formations, preventing the swelling of the reactive clay and shale formations. The polar Ester groups and

the balanced vegetable C-chain are the main factors conferring these properties. The biodegradable Ester based muds are better alternatives to oil based muds because oil based muds are not environment friendly and often involve recovering and transportation of drill cuttings to onshore locations for treatment and disposal which is very costly. In contrast, vegetable ester drill cuttings can be safely discharged into the ocean without harming the ecosystem if they meet the local regulatory requirement, In Nigeria, the discharge limit is less than 50ppm of oil (DPR, 1991). When they are discharged, the cuttings and adherent synthetic base fluids settle to the sea floor and their concentration in the sea floor environment may decrease with time due to re-suspension, bed transport, bioturbation and biodegradation but biodegradation is expected to be the most significant mechanism of synthetic based fluid removal and subsequent environmental recovery (Deborah and Alan, 2006).

Aerobic biodegradation is a key criterion for selecting a base fluid for bioremediation though there may be other important factors such as availability of the base fluids, drilling environment and the operator's policy and local legislation. Numerous studies on petroleum degradation in marine environment and soil demonstrate that organic ingredients in oily cuttings are biodegradable under aerobic conditions (Prince, 1993, Kjeilen, 1997, Deborah and Alan, 2006) but in the floor of some sediments for instance the Gulf of Mexico, the average oxygen concentration is 6.8mg/L (0.21nm) and the oxygen only diffuses a few centimetres into the sediment, an indication that oxygen availability can be limiting in deep offshore sediments (Deborah and Alan, 2006). A wide variety of aerobic hydrocarbon degrading microorganisms have been isolated from the Gulf of Guinea sediments namely; *Flavobacterium* sp., *Micrococcus* sp., *Alkaligenes* sp., *Corynebacterium* sp., *Aspergillus niger, Aspergillus fumigatus and Penicillium* sp (Okoro, $2010_a$), in a related development, an investigation carried out by Okoro ($2010_b$) revealed that aerobic microorganisms are very active in the Gulf of Guinea sediment up to a depth of 2-5cm with total heterotrophic bacterial counts of $3.20 \times 10^6$ cfu/g and $2.20 \times 10^4$ cfu/g respectively. Other aerobic microorganisms implicated in hydrocarbon degradation in marine sediments by other researchers include; *Pseudomonas* sp. (Tagger *et al*, 1990), *Flavobacterium* sp. (Okpokwasili *et al*, 1984), and *Vibrio* sp. (West *et al*, 1984).

In the present study, the biodegradation potential of 4-Ester based drilling fluids (BR-EST, CH-EST, PFB-009 and PFE-008) was tested under natural aerobic conditions using Gulf of Guinea sediments. The main objective of the study therefore is to determine the fate of these drilling fluids in the Gulf of Guinea sediments over time under natural aerobic environmental conditions.

## 2. Material and Methods:

### Experimental Design:

The experimental test set up consists of a series of 4 easily assessable rectangular shaped glass indoor basins called benthic chambers measuring approximately 18x30 inches (about 18 inches deep). Each of the glass containers was filled with the wet sediment collected from Escravos river (Located within the Gulf of Guinea) up to 12 inches depth followed by the introduction of 100mls of each of the Ester based fluids to the respective containers. The sediment/fluid mixture was mixed thoroughly by manual means using a metallic mixer. The experimental set up was allowed to settle for about 6hrs before the collection of the first sediment sample at day 0. The experiment was monitored for a period of 120 days and at each 30-day interval, sediment samples were collected and analysed for residual organic carbon and hydrocarbon utilizing bacteria. The entire set up was similar to the simulated sea bed experiment conducted by OGP (2003). The 4 sediment samples were labelled as follows; 1.SE-BR-EST, 2. SE-CH-EST, 3. SE-PFB-009, and 4. SE-PFB-008 depending on the type of ester based fluid added to the sediment.

### Description of the Synthetic-based fluids (SBF) used for the study.

The SBF samples which were collected from the Nigerian Department of Petroleum Resources (DPR) were coded and have the following descriptions.

1. BR-EST (BAROID ESTER)
2. CH-EST (CHEVRON ESTER)
3. PFB-009 (Mixture of Ester and Olefin)
4. PFE-008 (Ester of Aliphatic acid).

### Microbiological and Physicochemical Analysis of the Sediment samples

### Enumeration of Total Heterotrophic Bacterial and Fungal Counts.

Heterotrophic bacteria and Fungi were enumerated by adopting the standard plate count technique using spread plate method. Appropriate dilutions of samples were plated out on nutrient agar plates for bacteria and potato dextrose agar (PDA) plates for Fungi. The plates for bacteria were made in

duplicates and incubated aerobically at $29^0$C for 24hrs while that of Fungi were incubated aerobically for 3-4 days. 2µg/L of chloramphenicol was added to PDA plates to inhibit bacterial growth as described in Eaton *et al*, 1995.

**Enumeration of hydrocarbon carbon utilizing bacteria**

Hydrocarbon utilizing bacterial counts were obtained by plating out at low dilutions $10^{-1} - 10^{-3}$ of samples on mineral salt medium of Mills *et al* (1978). The composition of the medium in ( g/L) is as follows NaCl ( 10 ), $MgSO_4.7H_20$ ( 0.42), KCl (0.29), $KH_2PO_4$ (0.83), $Na_2HPO_4$ (1.25), $NaNO_3$ (0.42), Agar bacteriological (15), distilled water (1000 ml), and pH (7.2 ). The medium was autoclaved at 1.1 kg/cm$^2$ for 15 mins. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light ) and held in the lid of the petri dishes. The dishes were wrapped round with a masking tape so as to increase the vapor pressure within the Petri dishes while the plates were incubated at $29^0$C for 6 days after which the growth of hydrocarbon degrading bacteria were observed and counted. For fungal plates, 0.1g of Penicillin was added to 250ml mineral salt medium to inhibit bacterial growth.

**pH, Temperature measurement and Salinity**

The pH of the sediment was measured with a portable water proof pH meter (Jenway, 3150, USA),Temperature was measured using portable thermometer (Hanana , H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described in *(*Eaton *et al, 1995).*

**Estimation of Background Nutrient Concentration of the sediment**

Interstitial water samples were withdrawn with a simple apparatus as described in McKee *et al,* 1988. The collected interstitial water was filtered and inorganic nutrients such as Phosphorus and Potassium were analysed with ICP (Inductively coupled argon plasma emission spectrometer) as described in Eaton *et al,* 1995). Ammonium-Nitrogen was analysed with auto analyser as described in Eaton *et al,* 1995.

**Detection of heavy metals:**

Heavy metals were detected using the Atomic absorption Spectrophotometer (Perkin Elmer 5100PC, England) after sample preparation and digestion as previously described (Eaton *et al,1995).*

**Moisture content:**

The moisture content of the sediment was measured by simple gravimetric analysis. 10grams of the sample containing water was dried in the oven at a temperature of $200^o$C after which, the sample was measured again and the difference in weight is the moisture content as previously described *(*Eaton *et al,1995)*

**Solvent extraction of Residual Oil**

One gram of the sample was introduced into a separating funnel containing 50mls of Methylene chloride, this was followed by vigorous shaking for 10mins and filtration using Watman no.1 filter paper as previously described *(*Eaton *et al, 1995)* and the filtrate was collected in a clean conical flask.

**Gas Chromatography of Oils**

Degraded organic carbon were analyzed by gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter ( 900 series ) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1micron film thickness were used. A temperature program of 50-$305^0$C increasing at $3.5^0$C per minute for 27.15min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was $325^0$C while the injection port temperature was $305^0$C. 1 ml of the residual organic carbon extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of 0.2 µl was injected into the GC.

**Identification Microorganisms capable of utilizing SBF**

The growth and morphology of bacterial isolates in minimal salts medium and on nutrient
Agar plates were noted with regards to the following characteristics; Form, Pigmentation,
Texture, Colour and Elevation. Fungal cultures were stained with Methylene blue and observed under a microscope (x40) and each fungal culture was identified based on its morphological characteristics. Bacterial cultures were stained using grams staining procedure and proper identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

**3. Results.**

**Microbiological and Physicochemical properties of Gulf of Guinea sediments**

The total heterotrophic bacterial counts of the four sediments investigated ranged between 1.20-3.10 x $10^6$ cfu/g while the hydrocarbon utilizing bacterial counts ranged from 0.011-0.080 x $10^6$ cfu/g. Heterotrophic fungal and yeast counts in the sediments ranged between 0.0034 – 0.018 x $10^6$ cfu/g while the hydrocarbon utilizing fungal and yeast counts ranged from 0.00016 – 0.00042 x $10^6$ cfu/g.

The total organic carbon (TOC) in all the sediments tested were less than 10ppm suggesting that the sediment is pristine and have not undergone any significant pollution in the past. The levels of Nitrogen, Potassium and Phosphorus in all the sediments tested indicate that the sediments have sufficient nutrient that can sustain microbial growth and proliferation. The detailed results of the microbiological and physicochemical properties of the Gulf of Guinea sediments are shown on table 1.

**Aerobic degradation of Ester based drilling fluids in the sediment**

Aerobic microorganisms comprising of Bacteria and Fungi considerably degraded the Ester based fluids in the sediment within the 120 day experimental period. At day 60, all the ester based fluids tested achieved over 60 % degradation in the sediment and by the end of the 120 day period, almost all the residual ester present in the sediment (over 90%) were degraded by the microorganisms as shown in Figure 1.

**Changes in the population dynamics of microorganisms with the capability to utilize the ester based fluids as the sole carbon source during biodegradation studies**

Changes in the population dynamics of microorganisms with the capability to utilize the ester based fluids as their sole carbon sources in the sediment were monitored during the 120 day experimental period and the results showed that the population densities of the microorganisms increased progressively from day 0 to day 60 which was the peak population density. The microbial population however showed a gradual decline after day 60 and this can be attributed to the considerable drop in the concentration of the ester based fluids as biodegradation progressed. The results are shown on table 2.

**Microorganisms isolated from Gulf of Guinea Sediments with the capability to utilize the SBF.**

A wide variety of microorganisms with the capability to utilize the synthetic based fluids were isolated from the Gulf of Guinea sediments during the 120 day period the experiment lasted. At day 0, various bacterial and fungal species were isolated from the various sediments tested and the predominant microbial flora comprised of *Pseudomonas* sp., *Micrococcus* sp., *Alkaligenes* sp., *Corynebacterium* sp., *Actinomycetes* sp., *Enterobacter* sp., *Acinetobacter sp., Aspergillus niger, Penicillium* sp., *Penicillium crysogenum and Candida* sp. but at the end of the experiment at day 120, the predominant microbial flora in the sediment declined to *Pseudomonas* sp., *Alkaligenes* sp., *Micrococcus* sp., *Achromobacter* sp., *Aspergillus niger, Penicillium sp. and Penicillium crysogenum.* The percentage of total heterotrophs in the sediment with the capability to utilize SBF ranged from 0.92-3.30%, the detailed results are shown on table 3.

**Table 1. Microbiological and Physicochemical properties of Gulf of Guinea sediments**

| | | SE-BR-EST | SE-CH-EST | SE-PFB-009 | SE-PFE-008 |
|---|---|---|---|---|---|
| 1 | Total Heterotrophic Bacterial Counts (Cfu/g x $10^6$) | 1.20 | 2.60 | 1.40 | 3.10 |
| 2 | Hydrocarbon utilizing bacterial counts (Cfu/g x $10^6$) | 0.011 | 0.086 | 0.023 | 0.041 |
| 3 | Total Heterotrophic Fungi/Yeast Counts. (Cfu/g x $10^6$) | 0.004 | 0.016 | 0.0034 | 0.018 |
| 4 | Hydrocarbon Utilizing Fungi/Yeasts. (Cfu/g x $10^6$) | 0.00016 | 0.00042 | 0.00016 | 0.00022 |
| 5 | Total Organic Carbon (TOC) (ppm) | 6.50 | 7.60 | 9.50 | 8.40 |
| 6 | pH | 6.70 | 6.80 | 6.70 | 6.90 |
| 7 | Temperature ($^0$C) | 23 | 24 | 24 | 23 |
| 8 | Salinity (mg/g) | 5280 | 5360 | 5540 | 5920 |
| 9 | Moisture content (%) | 56 | 58 | 60 | 58 |
| 10 | Phosphorus (mg/g) | 106 | 128 | 98 | 130 |
| 11 | Potassium (mg/g) | 98 | 83 | 76 | 82 |
| 12 | Ammonia-N (mg/g) | 3.20 | 3.11 | 2.80 | 3.0 |
| 13 | Heavy Metals detected | Pb(0.032), Cr (0.10) | Cd(0.05), Pb(0.018) | Fe(0.022), Zn (0.04) | Fe (0.031), Cd (0.006), Zn (0.03) |

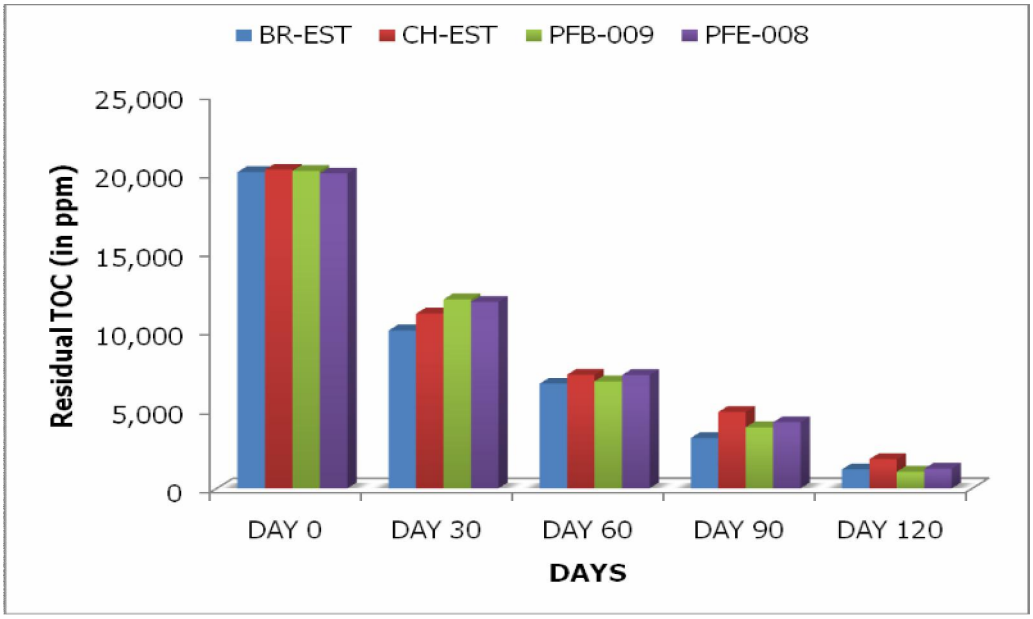


**Figure 1. Degradation of Ester based drilling fluid in the sediment by aerobic microorganisms.**

**4. Discussion:**

The degradation of surrogate ester based SBF under natural conditions by the indigenous microbial flora of the Gulf of Guinea sediments were examined under natural environmental conditions over a 120 day period. Incubation at hydrostatic pressure was not necessary because previous research have shown that incubation at such deep offshore pressure had no effect on the hydrocarbon substrate degradation (Benka-Coker and Olumagin, 1995, Alan *et al*,2006, Deborah and Alan ,2006).

The present study have demonstrated that the Gulf of Guinea sediments used in the study harboured considerable population of microorganisms with the capability to utilise and degrade the ester based fluids. Analytical study on the background nutrient composition of the sediment used in the study equally showed that the sediments had fairly good nutrient composition that can sustain microbial growth and proliferation. Previous investigations have shown that the Gulf of Guinea sediments have significant background nutrient composition and are populated with wide variety of microorganisms with the capability to utilize the organic carbon in the sediment (Okoro, 2010a). In the present study, the indigenous microbial populations of the sediment ranged between 1.20 -3.10 x $10^6$ cfu/g and only about 0.92-3.30% of the total heterotrophic population have the capability to utilize and degrade the ester based SBF in the sediment. Deborah and Alan (2006) have demonstrated that the indigenous aerobic microbial populations in the Gulf of Mexico sediments ranged between 1.0 x $10^8$ – 1.4 x $10^9$ cfu/g but less than 10% of the total heterotrophic population have the capability to utilize the SBF as their sole carbon and energy source.

In the present study, it was observed that the populations of microorganisms in the sediment that are capable of utilizing the ester based fluids increased in population when the ester based fluids were introduced but the populations declined gradually upon the degradation of the ester based fluids. A similar study conducted by OGD (2003) showed that biodegradation of esters increased with higher concentration of fluids in the sediment, the study also went further to demonstrate that sediment type (Sandy Vs Clay or Silt) for instance effects degradation rate and that degradation occurs more rapidly in Silt/Clay sediments than in sandier sediments. The sediment that was used in this study was sandy.

An investigation carried out Cavellier *et al*, 1999 with the Gulf of Mexico sediments showed that under aerobic and anaerobic conditions, about 50% of the esters in the sediments were degraded after 28 days and the residual concentration of esters were reduced to zero after 120 days of exposure. In the present study, only aerobic degradation was monitored and the average % degradation of the Ester based fluids in the sediment was, about 43% for a 30 day period and 94% for the 120 day period. A similar investigation carried out by OGD(2003) revealed that

loss of the SBF deposited on the cuttings which was measured over a period of 150-187 days was about 80%. Other investigators like Prince (1993), Swannel *et al*,(1996), Kjeilen (1997) and Paulsen *et al* (1997) have equally demonstrated that the organic ingredients in the SBF are readily biodegradable over a considerable length of time.

**Table 2. Population dynamics of Hydrocarbon utilizing bacteria during biodegradation of Ester based fluids (Bacterial Population x $10^6$ cfu/g)**

| SEDIMENT | DAY 0 | DAY 30 | DAY 60 | DAY 90 | DAY 120 |
|---|---|---|---|---|---|
| SE-BR-EST | 0.011 | 0.580 | 1.260 | 0.360 | 0.086 |
| SE-CH-EST | 0.080 | 0.620 | 1.560 | 0.840 | 0.460 |
| SE-PFB-009 | 0.023 | 0.180 | 1.040 | 0.960 | 0.210 |
| SE-PFE-008 | 0.041 | 0.186 | 1.132 | 0.840 | 0.110 |

**Table 3. Microorganisms isolated from Gulf of Guinea Sediments with the capability to utilize the SBF**

| | SE-BR-EST | SE-CH-EST | SE-PFB-009 | SE-PFE-008 |
|---|---|---|---|---|
| **DAY 0** | *Micrococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Corynebacterium* sp.,*Actinomycetes* sp., *Achromobacter* sp., *Pseudomonas mallei, Aspergillus niger, Candida sp., Penicillium* sp., *Rhizopus* sp. | *Acinetobacter lwoffii, Pseudomonas* sp., *Enterobacter* sp., *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger Aspergillus fumigatus Penicillium crysogenum* | *Vibrio* sp., *Pseudomonas* sp., *Acinetobacter lwoffii. Alkaligenes* sp. *Pseudomonas mallei. Penicillium* sp.,*Actinomycetes, Enterobacter, Penicillium crysogenium, Aspergillus niger* | *Micrococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Corynebacterium* sp., *Achromobacter* sp., *Pseudomonas mallei Rhodotorula* sp. *Candida* sp., *Cladosporium* sp., *Fusarium* sp |
| **DAY 30** | *Micrococcus* sp., *Pseudomonas sp.,Actinomycetes* sp., *Achromobacter sp., Pseudomonas mallei, Aspergillus niger, Penicillium* sp., | *Pseudomonas* sp, *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger Penicillium crysogenum* | *Pseudomonas* sp.. *Alkaligenes* sp., *Pseudomonas mallei. Penicillium* sp., *Penicillium crysogenium, Aspergillus niger* | *Micrococcus* sp., *Pseudomonas* sp.,., *Corynebacterium* sp., *Achromobacter* sp., *Rhodotorula* sp. *Candida* sp., *Cladosporium* sp., *Fusarium* sp. |
| **DAY 60** | *Micrococcus* sp., *Pseudomonas sp.,Actinomycetes* sp., *Achromobacter* sp., *Aspergillus niger, Penicillium* sp., | *Pseudomonas* sp, *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger Penicillium crysogenum* | *Pseudomonas* sp.. *Alkaligenes* sp,. *Penicillium* sp., *Penicillium crysogenium, Aspergillus niger* | *Micrococcus* sp., *Pseudomonas* sp., *Achromobacter* sp., *Rhodotorula* sp,. *Candida* sp., *Cladosporium* sp., *Fusarium* sp. |
| **DAY 90** | *Micrococcus* sp., *Pseudomonas* sp.,*Actinomycetes* sp., *Achromobacter* sp., *Aspergillus niger, Penicillium* sp., | *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger Penicillium crysogenum* | *Pseudomonas* sp.. *Alkaligenes* sp,. *Penicillium* sp., *Penicillium crysogenium, Aspergillus niger* | *Micrococcus* sp., *Pseudomonas* sp., *Achromobacter* sp.,. *Candida* sp., *Cladosporium* sp., |
| **DAY 120** | *Micrococcus* sp., *Pseudomonas* sp., *Actinomycetes* sp., *Achromobacter* sp., *Aspergillus niger, Penicillium* sp., | *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger Penicillium crysogenum* | *Pseudomonas* sp.. *Alkaligenes* sp,. *Penicillium* sp., *Penicillium crysogenium, Aspergillus niger* | *Micrococcus* sp., *Pseudomonas* sp., *Achromobacter* sp.,. *Candida* sp., *Cladosporium* sp., |
| **% of Heterotrophs utilizing SBF** | **0.92** | **3.30** | **1.64** | **1.32** |

One important issue in mixed culture microbial degradation is the issue of dominance of some microbial species over others. The population dynamics and the species diversity of the indigenous microorganisms that have the capability to utilize the ester based fluids as their sole carbon source was monitored and the dominant microbial flora in the Gulf of Guinea sediments were identified as *Pseudomonas* sp., *Alkaligenes* sp., *Micrococcus* sp., and *Achromobacter* sp. among the bacterial species and *Penicillium* sp., *Aspergillus niger* and *Penicilluim crysogenum* among the fungal species. A similar study in the Gulf of Guinea sediments by Benka-Coker and Olumagin (1995) showed the predominant microbial flora of the Gulf of Guinea sediments with the capability to utilize the SBF as *Alkaligenes* and *Micrococcus*, among the Bacterial species and *Penicilium* and *Cladosporium* sp. among the Fungal species. The study also showed that 6% of the total heterotrophic population possess the capability to utilize the SBF as their sole carbon and energy source.

**Conclusion:**

The present study have clearly demonstrated that all the ester based fluids used in the study are readily biodegradable and the Gulf of Guinea sediment is populated with diverse microbial flora with adequate nutrient composition which made the biodegradation of ester based SBF easier. The literature reviews conducted as part of this research revealed that ester based SBF are more readily degradable than others such as paraffins and olefins. In conclusion therefore, it can be deduced from the present study that the 4 ester based SBF used in the study are readily biodegradable by the Gulf of Guinea sediment and as such can be safely discharged into the ocean. The recommended ocean discharge is safer and far more economical than other disposal options like onshore thermal treatment process and land farming.

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# Community Participation for Poverty Reduction in Iran

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**Abstract:** This study assesses the level of community participation for poverty reduction in rural areas of Iran. Data were collected using focus group discussions. Results indicate that although there is sense of community towards poverty reduction between the rural people; but rural communities still face challenges and constraints which hinder their participation in poverty reduction.
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**Keywords:** community participation, rural community, poverty reduction

## 1. Introduction

The term 'participation' has recently come to play a central role in the discourse of rural development practitioners and policy makers (United Nations, 2009). Participation is a dynamic process. Participation is considered as an important factor for successful and prosperity of local development (Aref et al., 2010). At the same time, people's interpretations of the term – and criticisms of other people's interpretations – have multiplied, and the intentions and results of much participation in practice have been questioned or even denounced. In other words, participation has become a hotly contested term, in a debate with deep implications for the ways in which community, society, citizenship, the rights of the poor and rural development itself are conceived, and for the policies that are formulated about and around some of these concepts and the social realities to which they refer (United Nations, 2009).

Community participation refers to peoples' engagement in activities within the community. It plays an essential and long-standing role in promoting quality of life (Putnam, 2000). Participation is recognized as an essential strategy to strengthen the well-being of individuals, families and communities, government and non government agencies (Aref, 2010a). Community participation is one of the mechanisms to empower people to take part in community development. Increased participation is a means to achieve community capacity to resolve the community problems (Lasker, Weiss, & Miller, 2001). Community participation also is the mechanism for active community involvement in partnership working, decision making and representation in community structures for poverty reduction (Aref, 2011; Chapman & Kirk, 2001). It should be noted that community participation often means the involvement of people or community with the government to solve the community problems(Aref, 2011).

In Iran there are some local organization for poverty reduction; but there are many challenges that face organizations who make it their goal to rural participation (Narayan, 2002). Hence, this paper addresses the specific challenge that is faced by participation for poverty reduction in rural area of Iran.

## 2. Literature review

Community participation is a concept that attempts to bring different stakeholders together for problem solving and decision making (Talbot & Verrinder, 2005). The World Bank (1993) recognized the lack of community participation as a reason for failure of many community development attempts in developing countries (Aref, 2011). Community participation was measured in this study as a means of determining the level of community involvement in poverty reduction.

Poverty being a rural phenomenon where the majority of the people live in most developing countries, the mechanisms to be used should target the recipients. One of these methods which are used widely today is to organize people in form of associations or collaboration (Adebayo, Chinedum, Dabo, & Pascal, 2010). According to World Bank poverty is hunger. Poverty is lack of shelter. Poverty is powerlessness, lack of representation and freedom (Drinkwater, 2005). Whereas poverty is a multi-faceted phenomenon that hinders the satisfaction of basic life requirements, the tendency has been for some analysts to conceptualize it in narrow economic terms by insinuating that it is simply the lack of

money (Smith & Ross, 2006). Poverty has been defined as the "denial of opportunities and choices most basic to human development to lead a long, healthy, creative life and to enjoy a decent standard of living, freedom, dignity, self-esteem and respect from others" (Hirschowitz et al.,2000, p. 54). Poverty has been defined as the "denial of opportunities and choices most basic to human development to lead a long, healthy, creative life and to enjoy a decent standard of living, freedom, dignity, self-esteem and respect from others" (Hirschowitz et al., 2000). Poverty can be reduced through community participation. Hence this study provides an approached for enhancement of community participation for poverty reduction in Iran.

In despite of sense of community in rural areas, there is a number barrier for poverty reduction. For example lack of government programs and organizational capacity to respond to the opportunities provided (Jamieson & Nadkarni, 2009). Lack of formal education and skills and planning (Bushell & Eagles, 2007, p. 154). As a consequence, community facilities and services may be unacceptable for rural areas. Hence building local participation in rural communities is necessary for stakeholders involved in rural development (Bushell & Eagles, 2007).

## 3. Methods

The rural areas of Marvdasht in Fars province, Iran was selected as a case study area because it provided many opportunities to develop rural development; This study is based on quantitative methodology to investigate the barriers of community participation related to poverty reduction. The participants in FGD were educated people that were engaged in government and non government institutes. To achieve the objectives of this study, the researcher uses quantitative method. Focus group discussion (FGD) was performed to collect data from local residents.

FGD conducted in a group setting and was used for obtaining a better understanding of participants' attitudes (Aref, 2010b). There is no consensus among qualitative researchers on the optimal number of participants in FGD. But the ideal number of participants in each FGD is six to ten. The respondents were participated in 10 groups. They ranged in age from 22 - 45 years. The researcher explained to them the objectives of the study and what questions would be asked. The researchers examined, categorized participants responses from each focus group of villagers that were recorded in video tapes

## 4. Result

Information for this study was gathered from educated people through FGD. A qualitative analysis was undertaken to determine viewed the current level of local participation for poverty reduction and also barriers of participation for poverty reduction. There were overall 55 participants with an average of 33 years old. The FGDs held on in 10 convenient centers in Marvdasht, Fars, Iran. They were chosen because of their knowledge. The questions were asked about the local particiaption in poverty reduction and barriers of local participation for poverty reduction.

In terms of local participation in poverty reduction, they believe that rural residence does not have important role in their communities especially on poverty reduction. The findings showed that rural residence are without any certain planning for poverty reduction. Although the FGD respondent referred to variety barriers in terms of participation for poverty reduction in their communities, the study refer to some common barriers which have been discussed in majority of FGD groups. The most barriers in terms of local participation for poverty reduction were including:

***Lack of local conditions:*** The majority of FGD participants believed there are no suitable conditions in their village for participation in social and political participation and decision making.

***Lack of training:*** FGD respondents believed the lack of training; especially was behind the failure of participation for poverty reduction.

***Lack of skill and knowledge:*** The participants in all groups mentioned to this issue as one barrier of community participation for poverty reduction in their communities. Moscardo (2008) also argues that a lack of skill and knowledge has been used in many developing countries to justify the exclusion of local residents to resolve this problem (Aref, 2011).

***Community structural barriers:*** They respondents also referred to structural barriers for local participation in poverty reduction. Structural barriers are usually associated with institutional, power structures, legislative, and economic systems. Tosun (2000) describes a few of the relevant barriers including: attitude of professionals, lack of expertise, elite domination, lack of an appropriate legal system, lack of trained human resources, relatively high cost of community participation and lack of financial resources (Aref, 2011).

***Cultural barriers:*** Through FGD respondents believed that there are some cultural barriers towards community participation. There seem to be some cultural factors including limited capacity of poor people to handle development effectively, apathy and

low level of awareness in the local community (Aref, 2011; Moscardo, 2008).

Overall the results of this study indicated that in most rural area local participation is limited by the some cultural restrictions that limit their access to education and health services, and these impose serious constraints on their autonomy, mobility, and on the types of livelihoods that are available to them. Their lack of access to education and resulting low-skill levels limits their opportunities for employment further.

## 5. Conclusion

This paper addresses the specific challenge that is faced by community participation for poverty reduction in Marvdasht, Iran. This study has identified the barriers of participation for poverty reduction. Lack of capable organizations, lack of resources, and cultural restrictions were an important element contributing to limited rural areas for poverty reduction.

Overall the findings indicated that residents have negative attitude towards contribution of rural residence towards poverty reduction in their communities. They refereed to government policy and lack of local organizational capacity as main barriers related poverty reduction. Clearly, the described barriers may not be only specific to rural areas; some of them may also be considered as common general problems in urban communities in Iran. Base on the findings for community participation, any project should include, include the below items:

-The integration of procedures and principles aimed at enhancing and promoting the role of local people as creators of development,

- The enhancement of the image of rural people as guardians of the traditional know-how so as to favor and promote their involvement in rural economic activities, and management processes.

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# Production, Purification and Characterization of Alkaline and Thermostable Protease by *Shewanella putrefaciens*-EGKSA21 Isolated from El-Khorma Governorate KSA

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**Abstract:** Proteases catalyzes the hydrolysis of protein. Among the various proteases, bacterial proteases are the most significant when compared with animal and fungal proteases. The aim of the present study was to production of alkaline thermostable bacterial protease for application in biodetergent technology. Screening studies were carried out for twenty one thermophilic bacterial isolates with respect to their ability to produce both protease and lipase when grown on mineral salts medium supplemented with gelatin as only source and carbon and energy at 50°C and pH 9. The most potent thermophilic bacterial isolate for production of two enzymes was identified as *Shewanella putrefaciens*-EGKSA21. The optimum incubation temperature and pH for maximum alkaline–thermostable protease production were 50°C and 9 under fermentation conditions. Optimum substrate concentrations for protease production were 3 % gelatine. The best carbon sources that induce protease production by *Shewanella putrefaciens*-EGKSA21 was D (+) arabinose and D-xylose. Potassium nitrate, sodium nitrate and ammonium chloride were the optimal nitrogen sources for alkaline–thermostable protease production by *Shewanella putrefaciens*-EGKSA21. Maximum protease production was observed at the end of 48hrs. The overall steps protocol resulted in raising the purification fold to 411.9 times. Optimum incubation temperature and thermal-stability were 50 and 50-55 ºC for the purified protease. The activities of the purified enzymes increased gradually with the increase of time up to 48 h incubation of the reaction mixture. The activities of the purified enzyme increased gradually by the increase of enzyme concentrations. The effect of different metalic ions on the purified enzyme activities recorded that Sodium azide (50 ppm), Lead acetate (50 ppm) and EDTA (50ppm) exhibited maximal activities while cadmium chloride and magnesium chloride inhibited the purified enzyme activities. The purified enzymes exhibited good stability towards organic solvents. The crude and purified protease of produced by *Shewanella putrefaciens*-EGKSA21 bacterial strain with a potential to be a candidate for the application in the detergent industry.

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**Key words:** Thermostable protease, Biodetergent, *Shewanella putrefaciens*, Submerged fermentation; EL-Khorma Governorate.

## 1. Introduction

Proteases constitute one of the most important groups of industrial enzymes and have applications in different industries such as detergent, food, feed, pharmaceutical, leather, silk and for recovery of silver from used x-ray films. Proteases are one of the most important classes of biocatalysts from an industrial point of view, occupying a major share of 60% of the total enzyme market (Pandey, 2003). These biocatalysts hydrolyze peptide bonds in proteins and hence are classified as hydrolases and categorized in the subclass peptide hydrolases or peptidases (Ellaiah *et al.*, 2002). Because of this functional property, they are widely used in laundry detergents, leather processing, protein recovery or solubilization, meat tenderization, and the biscuit and cracker industries (Johnvesly and Naik, 2001). However, other application potentials of these enzymes depend on the nature of catalytic activity with respect to reactant medium, which led to the classification of proteases as acidic, neutral, and alkaline. Among these different biocatalysts, alkaline proteases have wide application spectra and novel properties due to their exotic catalytic nature. Hence, these proteases and their producing organisms attracted attention of scientific community to understand the protein chemistry and protein engineering to enhance their utilization niche (Germano *et al*., 2003).

Though several microorganisms such as bacteria, fungi, yeast, plant, and mammalian tissues are known to produce alkaline proteases (Ellaiah *et al*., 2002; Prakasham *et al*., 2005 a&b), with increasing industrial demand for proteases it is expected that hyperactive strains will emerge and that the enzymes produced by new exotic microbial strains could be used as biocatalysts in the presently growing biotechnological era. Available literature information indicates that, among all protease-producing microbial organisms, the *Bacillus* genus assumes importance because of its potential for production in large amounts

(Kumar *et al*., 1999; Mabrouk *et al*., 1999; Mehrotra *et al*., 1999). Moreover, several medium components such as nitrogen and carbon sources, physiological factors such as pH, incubation temperature and incubation time, and biological factors such as the genetic nature of the organism influences the metabolic/biochemical behavior of the microbial strain and subsequent metabolite production pattern (Kumar *et al*.,1999; Ellaiah *et al*., 2002; Prakasham *et al*.,2005 a&b). Hence, in commercial practice, the optimization of medium composition is one of the essential steps to maintain a balance between the various medium components to minimize the amount of unutilized components at the end of fermentation and have cost-effective metabolite yield (Kumar *et al*., 1999; Prakasham *et al*., 2005a&b).

In general, no defined medium has been established for the best production of any metabolite because the genetic diversity present in different microbial sources causes each organism or strain to have its own special conditions for maximum product production (Ellaiah *et al*., 2002). Therefore, it is essential to have a detailed investigation on newly isolated microbial strain for production pattern under different environmental conditions and in an optimized pattern to achieve maximum production benefit. For effective triggering of alkaline protease production, it is highly imperative to optimize all fermentation conditions including medium composition, which further facilitates economic design of the full-scale operation system. However, it is impractical to optimize all parameters and to establish the best possible conditions by interrelating all parameters, as this involves numerous experiments to be carried out with all possible combinations (Prakasham *et al*., 2005 a&b; Sreenivas Rao *et al*., 2004). Experimental design based on statistical tools is known to provide economic and practical solutions in such cases.

Optimization procedures developed to optimize the biotechnological processes consist of an empirical modeling system developed on a full factorial central composite technique for evaluation of the relationship among the parameters that influence the production process. The Taguchi method of statistical procedure is mainly based on orthogonal arrays to provide a systematic, simple, and efficient approach (Phadke and Dehnad, 1988). It allows a more realistic arrangement of the experimental sets working with the understanding system, parameter, and tolerance designs (Phadke and Dehnad, 1988). Importance of this procedure has been evaluated in several microbial secondary metabolite production processes (Prakasham *et al*., 2005 a&b; Sreenivas *et al*., 2004).

Alkaline proteases assume significant importance in laundry, food, leather and silk industries (Priest, 1977; Turk, 2006; Subba, 2008 a &b).However, novel proteases with high activity profile at versatile environments have major application potential in pharma, diagnostic, detergent, tannery, amino acid production, contact-lens cleaning agents, effluent treatment, enzymic debridement and support the natural healing process in the skin ulcerations (Subba, 2009).

Proteases properties like physical, biochemical, thermal, molecular and catalytic, vary with the genetic nature of the producing organism (Geok *et al*., 2003). Each enzyme is specific and their use depends on stability and robustness against solvents, surfactants and oxidants.Hence, basic catalytic knowledge is one of the pre-requisites for evaluation of its potential for biotechnological application (Prakasham *et al*., 2006; Subba *et al*., 2008a&b).

This study aims (i) isolation of thermophilic and alkaliphilic bacterial isolates from local region in KSA (ii) Production of the thermoalkaliphilic proteases from the potent bacterial isolate under investigated optimal nutritional and environmental conditions and (iii) characterization of the purified enzyme.

## 2. Materials and Methods

### Construction of standard curves

### A stock enzyme preparation:

A stock solution of (50,000 µg/ml) purified protease enzyme supplied by Sigma chemicals Co. was prepared in Tris– buffer (0.2 M) at pH 9.0. Descending dilutions were prepared.

### Construction of the standard curves:

After preparing the required dilutions for protease enzyme, only 0.1 ml of each dilution was transferred to each well in the assay plate. Three wells were used for each dilution. Incubation was performed at 55ºC for 18 h. Then mean diameters of clearing zones (mm) were determined for protease concentration. A standard curve was constructed relating protease concentrations applied against their corresponding mean diameters of clearing zones (mm).

The obtained standard curves were used for estimating the protease activities in terms of µg/ml and then units (U). One unit is defined as the amount of enzyme protein (mg) required to exert one unit of clearing zone (mm) in one unit of time under all the specified conditions of protease assay (clearing zone technique).

### Protein determination:-

Protein of protease preparations was determined by the method of Lowry *et al.* (1951) using Bovine Serum Albumin as standard.

**Growth and maintenance medium:**

**Nutrient agar (NA) medium:**

This medium contained (g/l): Peptone, 5; sodium chloride, 5; beef extract, 3; agar-agar, 15; and distilled water up to 1000 ml. The ingredients were dissolved by heating, pH was adjusted at 7, and sterilized at 121ºC for 15 min (Shiriling and Gottlieb, 1966).

**Production media:**

The basal medium (BM) was prepared according to Vincent (1970). It contained the following (g/l): Sucrose, 10; $KNO_3$, 0.6; $KH_2PO_4$, 1; $MgSO_4$, 0.25; and $CaCl_2$, 0.1 was found most convenient for the production of different enzymes. It was modified to include the following constituents: (g/l) NaCl, 6; $(NH_4)_2SO_4$, 1; yeast extract, 1; $KH_2PO_4$, 0.5; $MgSO_4.7H_2O$, 0.1; $CaCl_2$, 0.1, and distilled water up to one litre.

**Isolation of thermophilic bacteria:-**

Thermophilic bacterial isolates were isolated from different localities alkaline desert soil samples collected from different localities of El-Khorma governorate, Taif, Kingdom of Saudi Arabia (KSA). Soil samples were applied using the soil dilution plate technique.

**Qualitative screening test media, methods and conditions (First survey):**

**Proteolytic enzyme production:**

The same ingredient of BM was used in addition to 1% gelatin and 1.5% agar and was adjusted at pH 9. It was autoclaved at 1.5 atm. for 15 minutes. Plates of the same size were poured with equally amounts of agar medium in each Petri dish. After cooling, each plate was inoculated in the center with bacterial isolate onto the surface, then incubated at 55ºC for 24 h. Bacterial growth and clearing zones of the medium after addition of acidic mercuric chloride solution were investigated and taken as criteria for determining the proteolytic activity.

**Media used in screening test for selecting the most potent bacterial isolates:**

For screening protease(s) activity, the BM was used and a gelatin waste was used as a substrate by a concentration of 5%. Enzyme activity was detected by gelatin clearing zone (GCZ) technique.

**Assay media:**

This medium was prepared according to gelatin clearing zone (GCZ) technique according to Ammar *et al*., (1991). The assay plates containing 1% (w/v) gelatin and 1.5% (w/v) agar for solidification, to be dissolved up to 100 ml of Tris-buffer (pH 9).. At the end of incubation period, protease(s) activity was detected by flooding each plate with 10 ml freshly prepared acide mercuric chloride solution (Barrow and Feltham, 1993). Mean diameters of clearing zones were recorded, calculated and then taken as indication for proteolytic activities.

**Identification of the most potent thermophilic bacterial isolates:-**

The most potent bacterial isolate EGKSA21 was identified by examination of their morphological physiological and biochemical characteristics.

**Reagent:**

Acid mercuric chloride reagent was prepared as the following: Mercuric chloride, 12g; distilled water, 80 ml; and conc. HC1 16 ml. $HgCl_2$ mixed with water, the acid added, and the mixture mixed well until solution was completely prepared(Barrow and Feltham, 1993).

**Preparation of cell-free-filtrate (CFF) from the thermostable protease production medium:**

This was performed by preparing the previously mentioned production medium under all investigated optimal nutritional and cultural conditions. At the end of incubation period, the bacterial growth was harvested by centrifugation at 5,000 r.p.m. for 20 min. The supernatant was obtained and preserved into the refrigerator as a crude enzyme and/or assayed at the same times.

**Parameters controlling the thermostable protease productivity:**

The effect of different temperatures; pH values; substrate concentrations; carbon and nitrogen sources and different incubation periods on protease production by EGKSA21 were determined by growth of organisms in fermentation medium. Protease productivity was measured through assay as previously mentioned.

**Purification of protease enzyme:**

The following steps were performed during the course of production, and purification of thermostable protease. *Shewanella putrefaciens*-EGKSA21 was allowed to grow under the optimal fermentation conditions for protease production.

At the end of incubation period, the bacterial growth was harvested by centrifugation at 5,000 rpm for 15 min. The supernatant was filtrated, and the obtained cell-free filtrate was preserved in the refrigerator as a crude enzyme according to Ammar *et al*. (1985).

**Ammonium sulphate fractionation:**

The chart of Gomori (1955) as mentioned by Dixon and Webb (1964) was applied to calculate the solid ammonium sulphate to be added to achieve any given concentration of the cell free filtrate under investigation.

**Applying on column chromatography technique on sephadex G 200 :**

The dialyzed-partially purified-protease preparation was applied onto a column packed with sephadex G 200. This was equilibrated with Tris-buffer (0.2 M) adjusted at pH 9, then eluted with the same buffer. Preparation of the gel column and the fractionation procedure was carried out as mentioned by Soliman (2003).

**Factors affecting the purified thermostable enzymes activities:**

**Effect of incubation temperatures:**

This was carried out by incubation of purified protease after pouring in assay medium at different incubation temperatures viz. 35, 40, 50, 55, 60, 65 and 70ºC respectively.

**Effect of temperature stability:**

This experiment was designed to determine the range of temperature within which the enzymes maintained their activities. The experiment was carried out by incubating the purified enzymes for 2 h at different temperatures viz., 50, 55, 60, 65, 70, 75, 80, 85 and 90ºC, respectively. At the end of incubation temperature, the replicate tubes were cooled and assayed for each purified enzyme to determine the retained enzyme activity as previously mentioned.

**Different pH values:**

The purified enzyme was incubated at different pH values viz., 6, 7, 8, 9, 9.5, 10 and 11 by using phosphate, Tris- or glycine buffers. After sterilization, pouring and solidification of the assay plates, three wells were made in each plate and then 0.1 of ml purified enzymes were inoculated in the well. Then the plates were incubated at 55ºC for 18 h. The protease activity was determined as previously mentioned.

**Metallic ions as enzyme activators and /or inhibitors:**

In this experiment, the purified enzymes were supplemented with different separated metallic ions in the form of cadmium chloride, magnesium chloride, sodium azide, lead acetate and EDTA. Different metallic ions concentrations were applied viz., 50, 100, 250, 500 and 1000 ppm for each metallic ion. Enzymes activities were assayed as previously mentioned.

**Stability of the purified enzymes in the presence of organic solvents:**

The aim of this experiment is to study the computability of the purified protease with organic solvent.

**Amino acid analytical data of the purified enzyme**

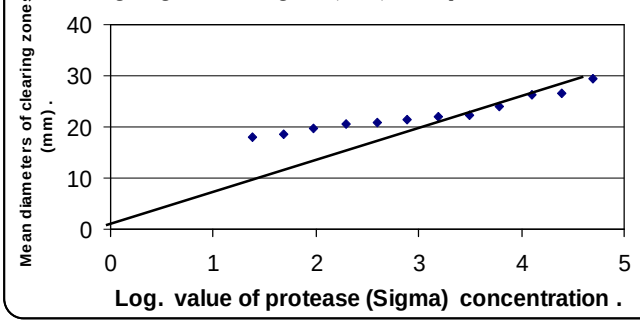
The hydrolyzed protein amino acids have been determined in the central Lab. for Food, Agricultural Research Center according to the methods described by Pellet and Young (1980). LKB Alpha plus high performance Amino Acid Analyzer LKB Biochrom. LTD England was used for this purpose. Retention time and area was determined using Hewlett Pakard 3390 recording integrator. The concentration of each amino acid GM/16 G.M., nitrogens was calculated by special designed program.

**3. Results:**

**Construction of standard curve for determination of enzyme activity:**

The productivity of protease was estimated in term of mean diameter (MD) of clearing zones (mm) using a special standard curves, prepared for such a purpose and covering the range of (24.4–50 000 µg protein /ml) as shown in figure (1). The standard protease (Sigma) was used by using GCZ plate techniques. Mean diameters of clearing zones in term of (mm) and their corresponding log. values of enzyme concentrations were recorded.

Fig.(1):The relation of Log. Value of standard protease concentrations to their corresponding mean diameters of clearing zone (mm) using the gelatin clearing zone(GCZ) technique..




**Qualitative screening test for selection of the most potent thermostable protease producers:**

Twenty one thermophilic bacterial isolates were isolated from different localities of different alkaline soil samples collected from khorma governorate , Taif, Kingdom of Saudi Arabia (KSA) . These isolates were purified, and subjected to a screening program in order to evaluate their proteolytic and lipolytic activities by measuring (observing) the hydrolysis of gelatin and tributyrin around the bacterial colonies. Out of twenty one isolates there was found that thirteen bacterial isolate gave proteolytic

productivity and isolate number 21 was found to be the best protease producer while eighteen isolates considered to have a good lipolytic activity and isolate number 21 also was found to be the best lipase producer (Table 1).

**Quantitative screening for selection of the most potent protease producer thermophilic bacterial isolates:**

Data recorded in table (2) showed that bacterial isolate number 21 gave a higher protease productivity by gelatin clearing zone quantitatively. Bacterial isolate number 21 gave the highest proteolytic activities where it reached up to 25 (mm) compared to other isolates. From the previous results concerning the qualitative and quantitative screening of two enzymes qualitatively and protease quantitatively production, bacterial isolate number 21 were selected for their ability to produce the two enzymes in high enzyme production. The most potent thermophilic bacterial isolate was subjected to identification.

**Identification of the two most potent bacterial isolates:**

The most potent bacterial isolate 21 was subjected to an identification program to the species level.

**Table (1): A screening test for the selection of the most potent two hydrolytic thermostable enzymes producers out of twenty one pure thermophilic bacterial isolates**

| Bacterial isolate code no. | Bacterial Code No. | Extracellular thermostable hydrolytic enzymes production | |
|---|---|---|---|
| | | Protease(s) | Lipase |
| 1 | 15 | 0 | 22 |
| 2 | 18 | 0 | 26.5 |
| 3 | 20 | 18 | 17 |
| 4 | 21 | 42 | 28 |
| 5 | 22 | 34 | 26 |
| 6 | 24 | 31 | 18.5 |
| 7 | 41 | 0 | 18 |
| 8 | 42 | 26 | 21.5 |
| 9 | 50 | 38 | 16.5 |
| 10 | 111 | 0 | 19.5 |
| 11 | 112 | 29 | 22 |
| 12 | 121 | 25 | 16 |
| 13 | 122 | 32 | 22 |
| 14 | 131 | 0 | 0 |
| 15 | 132 | 0 | 0 |
| 16 | 191 | 0 | 25 |
| 17 | 212 | 29 | 22 |
| 18 | 221 | 40 | 20 |
| 19 | 2221 | 32 | 17 |
| 20 | 2222 | 29.5 | 17 |
| 21 | SH1 | 0 | 0 |

**Table (2): Relation of thermostable protease production by most potent bacterial isolates at 55ºC for 48h. using GCZ techniques**

| Bacterial isolate code no. | Bacterial Code No. | Extracellular thermostable protease production |
|---|---|---|
| 1 | 20 | 19.5 |
| 2 | 21 | 25 |
| 3 | 22 | 18.5 |
| 4 | 24 | 18 |
| 5 | 42 | 18 |
| 6 | 50 | 20 |
| 7 | 121 | 19 |
| 8 | 122 | 18 |
| 9 | 212 | 18.5 |
| 10 | 221 | 21 |
| 11 | 2221 | 20 |
| 12 | 2222 | 12 |

**General characteristics of the most potent thermophilic bacterial isolates :**

All morphological characteristics and stain reaction led to the fact that the bacterial isolate under identification are suggestive of being belonging to the genus *Shewanella* , Gram-negative aerobes to facultative anaerobes, non Endo-spore formers. Amylase, cellulase, protease, lipase and catalase positive while, urease and caseinase negative. The cells were able to grow between (30-60°C) temperature interval. The cells were able to grow in the presence of (0-2%) of NaCl at pH 9.0

**Specific characteristics of the most potent thermophilic bacterial isolate:**

This isolate appears waxy in colour on nutrient agar medium, rod shaped, nitrate reduced, assimilate of N-acetyl –glucosamine and malic acid. Produced cyctochrome oxidase. *Shewanella* was able to grow facultative anaerobically, it is suggested to belong to the species putrefaciens. It could be given the tentative name *Shewanella putrefaciens*.

**Parameters controlling the four thermostable enzymes production**

Data recorded in table (3) showed a summary of the optimal nutritional and environmental conditions for thermostable hydrolytic protease production by ***Shewanella putrefaciens*-EGKSA21**.

**Purification of protease produced by *Shewanella putrefaciens*-EGKSA21 allowed to grow on BM under fermentation conditions.**

In this section enzyme produced by ***Shewanella putrefaciens*-EGKSA21** previously grown on BM supplemented with gelatin as a preferable substrate supplemented with mineral salts under the optimum nutritional and environmental conditions recorded in table (3) were purified to homogeneity as previously mentioned by performing ammonium sulphate fractionation, dialysis, and applying column chromatography on sephadex G200. The obtained purified enzymes were further investigated for some factors affecting its activity.

**Table (3): A summary of the optimal nutritional and environmental parameters controlling four thermostable hydrolytic enzymes productivities by *Shewanella putrefaciens*-EGKSA21 under solid or semi solid fermentation conditions.**

| No | Parameter | *Shewanella putrefaciens*-EGKSA21 |
|---|---|---|
| | | Protease(s) |
| 1 | Temperature(ºC) | 50 |
| 2 | pH value | 9 |
| 3 | Substrate concentration | 3% |
| 4 | Carbon source | L(+) Arabinose |
| 5 | Nitrogen source | Potassium nitrate |
| 6 | Inoculme size (ml) | 1 ml |
| 7 | Incubation period(hours) | 48h |

**Purification of thermostable protease:-**

The following steps were performed during the course of the purification of enzyme under study. where protease was produced by *Shewanella putrefaciens*-EGKSA21 allowed to grow on BM supplemented with gelatin under submerged fermentation condition at 50ºC under all the optimal conditions.

**Enzyme production and preparation of CFF :-**

The most potent bacterial strain was allowed to grow on the BM supplemented with gelatin under all the previously mentioned optimal submerged fermentation conditions as shown in table (3) for production of protease by *Shewanella putrefaciens*-EGKSA21. At the end of incubation period, 1000 ml of protease production medium was extracted and collected separately. Centrifugation of the obtained extracts was done at 5000 rpm for 15 min at 10ºC. The precipitate was collected and tested for determination of protease activity and protein content and corresponding specific activity was calculated.

**Fractional precipitation by ammonium sulphate:**

Results recorded in table (4) indicated that the most active enzyme protein preparation was obtained at an ammonium sulphate level at 60-80 % for protease was at 80%, where specific activity was 21658.5 U/mg $^{1}$ proteins. Only 42 ml were obtained at the end of the process of dialysation against tap water of protease.

**Concentration by dialysation against sucrose:-**

The most active ammonium sulphate fractions previously obtained at the best saturation, (50 ml) protease was dialyzed against distilled water followed by dialysis against sucrose crystals until a volume of 4.5 ml was obtained (Table 4).

**Table (4): Ammonium sulphate precipitation pattern of the protease produced by *Shewanella putrefaciens*-EGKSA21 allowed to grow on BM supplemented with gelatin at 50ºC.**

| $(NH_4)_2SO_4$ saturation level (%) | Protease activity (U/ml) (x) | Protein content (mg/ml) (y) | Specific activity (U/mg$^{-1}$protein) (x/y) | Purification fold |
|---|---|---|---|---|
| CFF | 1304.936425 | 0.48 | 2718.617557 | 1.0 |
| 20 | 1867.96537 | 0.622 | 3003.159759 | 1.1 |
| 40 | 3827.61067 | 0.88 | 4349.557 | 1.59 |
| 60 | 5479.07473 | 1.3 | 4214.6728 | 1.55 |
| **80** | **7843.0868** | **1.2** | **6535.9007** | **2.4** |
| 100 | 911.611698 | 0.82 | 1111.72157 | 0.4 |

**Preparation of sephadex G-200 gel filtrate column and applying the enzyme sample:**

Data recorded in table (5) showed that, there were one active peaks. It was found that the first beak (fractions 10-18) has the highest specificity and the fraction number 11 was reached to the maximum specific activity up to 5095.221 U/mg$^{-1}$ protein.

**Table(5): Fractionation pattern of protease produced by *Shewanella putrefaciens*-EGKSA21 at 50 ºC using sephadex G-200 column chromatography technique.**

| Fraction No. | Protease activity (U/ml) (x) | Protein content (mg/ml) (y) | Specific activity (U/mg$^{-1}$protein) (x/y) |
|---|---|---|---|
| 1-9 | UD | UD | UD |
| 10 | 217.116 | 0.0236 | 9199.830 |
| 11 | 1936.184 | 0.380 | 5095.221 |
| 12 | 1618.292 | 0.353 | 4584.398 |
| 13 | 1304.946 | 0.428 | 3048.9392 |
| **14** | 684.205 | 0.477 | 1434.6868 |
| 15 | 848.504 | 0.396 | 2142.6868 |
| 16 | 761,938 | 0.179 | 4256.6368 |
| 17 | 761.938 | 0.112 | 6803.0178 |
| 18 | 571.869 | 0.123 | 4639.34114 |
| 19-40 | UD | UD | UD |

**Characterization of the purified protease produced by *Shewanella putrefaciens*-EGKSA21 at 50$^{O}$C.**

The aim of the present series of experiments was to investigate some properties of the partially purified enzyme produced by ***Shewanella putrefaciens*-EGKSA21** allowed to grow on BM and incubated under all optimal nutritional and environmental submerged fermentation conditions. These properties include:- Effect of incubation temperature, pH values, pH stability, inhibitors and/or activator and stability with organic solvent on hydrolytic thermostable purified protease activity.

**1- Effect of incubation temperature:**

Results recorded in table (6) showed that the maximum activity of thermostable protease was obtained at 55ºC, where it reached up to 1867.96537 U/ml.

**Table (6): Different incubation temperatures in relation to the activity of purified protease produced by *Shewanella putrefaciens*-EGKSA21 .**

| Incubation temperature (ºC) | Protease activity (U/ml) |
|---|---|
| **35** | 715.092 |
| **40** | 1304.946 |
| 50 | 3199.175 |
| 55 | 1867.96537 |
| 60-70 | 0 |

**2-Effect of different pH values on the activity of purified enzymes:**

Data recorded in table (7) emphasized that the best pH value that fulfill the highest protease activity was 9 where it reached up to 5479.074 U/ml.

**Table (7): Different pH values in relation to the activity of the purified protease produced by *Shewanella putrefaciens*-EGKSA21.**

| pH | Protease activity (U/ml) |
|---|---|
| **Control** | 2673.919 |
| **7** | 74.02077 |
| 8 | 2673.919 |
| 8.5 | 3827.610 |
| 9 | 5479.074 |
| 9.5 | 3827.610 |
| 10 | 217.116 |
| 11 | 74.0207 |

**3- Effect of metallic ions (activators and / or inhibitors) on the purified protease activities:**

Results shown in table (8) that nearly all of the tested metallic ions exhibited inhibition effect on the purified protease activity.

**4-Stability of the purified enzymes in the presence of organic solvents:**

The aim of this experiment is to study the computability of the purified enzyme with organic solvent. This is an important observation because of the fact that enzyme incompatibility with organic solvent is the reason for its stability. Data recorded in table (9) showed that 40% acetone concentration did not exert any inhibition for protease activity.

**Table (8): Different inhibitors and/or activator in relation to the activity of the purified protease produced by *Shewanella putrefaciens* EGKSA21**

| Activator and /or inhibitor | Concentra-tion (ppm) | Protease activity (U/ml) | Inhibition (%) |
|---|---|---|---|
| **Control** | **0.0** | **32930.97301** | **0.0** |
| **Cadmium chloride** | 50 | 322.1435 | 99.02 |
| | 100 | 0 | 0 |
| | 250 | 0 | 0 |
| | 500 | 0 | 0 |
| | 1000 | 0 | 0 |
| **Magnesium chloride** | 50 | 241.78305 | 99.265 |
| | 100 | 0 | 0 |
| | 250 | 0 | 0 |
| | 500 | 0 | 0 |
| | 1000 | 0 | 0 |
| **Sodium azide** | 50 | 3199.1751 | 90.285 |
| | 100 | 2872.7919 | 91.276 |
| | 250 | 2673.919 | 91.880 |
| | 500 | 2488.8137 | 92.442 |
| | 1000 | 2234.9023 | 93.213 |
| **Lead acetate** | 50 | 3827.6106 | 88.376 |
| | 100 | 3562.639 | 89.181 |
| | 250 | 2234.9023 | 93.213 |
| | 500 | 1936.1846 | 94.120 |
| | 1000 | 1677.3935 | 94.906 |
| **EDTA** | 50 | 3562.639 | 89.181 |
| | 100 | 2977.708 | 90.957 |
| | 250 | 2579.706 | 92.166 |
| | 500 | 1936.18 | 94.120 |
| | 1000 | 1304.946 | 96.037 |

**5- Amino acids analysis of the purified enzymes:**

Data recorded in table (10) showed that, 12 amino acids were detected in addition to ammonia. Alanine and glutamic acid represented the highest value i.e 305.28 and 180.3 µg/ml respectively.

**Table (9): Stability of the purified protease produced by *Shewanella putrefaciens* EGKSA21in the presence of acetone.**

| Acetone concentration (%) | Protease activity | Protein content | Volume(ml) |
|---|---|---|---|
| 20 | 8129.51 | 0.312 | 10 |
| 40 | 21412.590 | 0781 | 15 |
| 60 | 11227.063 | 1.4 | 10 |
| 80 | 5479.074 | 1.1 | 5 |
| Before precipitation | 23005.1513 | 0.821 | 9.5 |
| After precipitation | 13432.470 | 1.2 | 40 |

**Table(10):A summary of amino acids analytical data of *Shewanella putrefaciens*-EGKSA21 purified protease.**

| No. | R.T | Amino acid | (µg/ml). | (µg/ml).X4 |
|---|---|---|---|---|
| 1 | 11.62 | **Aspartic** | 67.17 | 268.68 |
| 2 | 14.75 | **Threionine** | 20.59 | 82.36 |
| 3 | 16.17 | **Serine** | 39.70 | 158.8 |
| **4** | **18.27** | **Glutamic acid** | **180.3** | **721.2** |
| 5 | 27.03 | **Alanine** | 305.28 | 1221.12 |
| 6 | 32.62 | **Valine** | 48.94 | 195.76 |
| 7 | 35.45 | **Methionine** | 7.02 | 28.08 |
| 8 | 38.47 | **Leucine** | 20.47 | 81.88 |
| 9 | 42.93 | **Phenylalanine** | 67.94 | 271.76 |
| 10 | 50.97 | **Histidine** | 6.23 | 24.92 |
| 11 | 53.85 | **Lysine** | 70.09 | 280.36 |
| 12 | 62.75 | **Arginine** | 90.77 | 363.08 |

R.T.: Retention time

## 4. Discussion

Enzyme production is a growing field of biotechnology and the world market for enzyme is over $1.5 billion and it is anticipated to double by the year 2008 (Lowe, 2002). The main object of the present work was an investigation of screening, production, purification and characterization of thermo-alkalostable enzymes for application in detergent technology has been undertaken.

In this regard twenty one bacterial isolates were isolated from different soil and water samples collected from different localities in Khorma Governorate, Taif, Kingdom of Saudia Arabia (KSA). These bacterial isolates were grown at 50°C and at pH 9 to be able to produce a thermostable and alkalophilic enzymes which favorable to be used as additive to bio-detergent formulations(Bayoumi *et al*., 2011; Bayoumi and Bahobil,2011).

A screening test of proteolytic and lipolytic productivities of all bacterial isolates resulted in the fact that, only thirteen and seventeen bacterial isolates gave a good proteolytic and lipolytic productivities respectively.

*Shewanella* spp. are widespread Gram-negative bacteria that have been isolated from many different habitat. This adaptability is supported by a flexible metallic capability , particularly in the choice of respiratory electron acceptors (Gordon *et al*., 2000).

The present investigation was also aimed at optimization of medium components which have been predicted to play a significant role in enhancing the production of proteases (Gupta *et al*., 2002). Hence proper combination of various cultural conditions can be established in order to *Shewanella putrefaciens*-EGKSA21 for high secretion of alkaline protease. *Shewanella putrefaciens*-EGKSA21 was allowed to grow in media of different pH ranging from 7-11. Maximum enzyme activity was observed in medium of pH which was the optimum pH 9 for *Shewanella putrefaciens*-EGKSA21. The majority of microorganisms producing alkaline proteases show growth and enzyme production under alkaline conditions ( Tsujibo *et al*.,1990; Dunaevsky *et al*., 1996).

Enzyme activity recorded at different temperature revealed that the *Shewanella putrefaciens*-EGKSA21 yielded maximum protease production at 50. The temperature was found to influence extracellular enzyme secretion; possibly by changing the physical properties of the cell membrane (Rahman *et al*.,2005).

Various sources of carbon such as D(-) glucose, L(+) arabinose, D-xylose, D(-) lactose, maltose, D(-) mannitol, dextrin, cellulose, starch, inulin , Myo-inositol and D-sorbitol were used to with the original carbon source in growth media . Results obtained were showed that, L(-) arabinose brought the highest protease production compared to other carbon sources at 50ºC,pH 9 and 48 h of incubation. For commercial production , sugars like fructose, lactose, lactose, mannitol , sucrose will be prohibitive due to their cost (Suresh *et al*., 2008).

Production of extracellular protease has been shown to be sensitive to repression by different carbohydrate and nitrogen sources (Levisohn and Aronson,1967; Haulon *et al*., 1982). The effect of nitrogen source was studied in the growth medium , where sodium nitrate, potassium nitrate, ammonium oxalate, ammonium molybedate, peptone, urea ammonium chloride. Among the various nitrogen sources tested potassium nitrate was found to be the nitrogen source for alkaline protease production.

The application in the detergent industry does not require high-purity of enzymes and generally require use of the crude or partially purified enzyme preparation. However, it is significant to obtain enzymes with higher specific activity for their kinetic characterization. Since *Shewanella putrefaciens*-EGKSA21 proved to be the most potent proteolytic and lipolytic bacterial strain, they were selected for the

purpose of production, purification and investigating properties of proteases biosynthesized by this particular strain. However, there was no universal prescription for various techniques which could be applied for enzyme purification (Wilson and Walker, 1994).

Purification process includes essential steps as precipitation of protein using ammonium sulphate or other precipitants as low molecular weight alcohols and gel filtration using different column chromatography. Needless to say, that, most of the enzyme purification schemes described in the literature focused on purifying small amounts of the enzyme to homogeneity to characterize it. Little information has been published on large-scale processes for commercial purification. Most commercial applications of enzymes does not require highly pure enzyme. Excessive purification is expensive and reduces over all recovery of the enzyme. In the present study, the purification procedure included preparation of cell free filtrate; applying precipitation technique, dialysis; and then passing the enzyme preparation through Sephadex G-200 column chromatography techniques.

Fractional precipitation of enzymes was carried out firstly by ammonium sulphate since it is highly soluble in water, cheap and has no deleterious effect on structure of protein, so for all these reasons, precipitation by ammonium sulphate was selected as a first step of purification program. Many investigators used ammonium sulphate precipitation processes (Omar, 2000; Roushdy, 2001; Sodhi *et al.*, 2005).

In a trial to precipitate enzymes by ammonium sulphate, results revealed that, increasing the concentration of ammonium sulphate resulted in an increase in specific activity of amylase up to 80% saturation, a decrease in specific activity was recorded above this value. On the other hand, 80% saturation with ammonium sulphate was proved to be the best concentration for maximal specific activity for protease.

In complete accordance with the present results for protease, Chitte *et al.* (1999) found that, the crude protease enzyme was concentrated by precipitation with 80% saturation of ammonium sulphate. Hutadilok *et al.* (1999) used 80% saturation of ammonium sulphate for protease purification. Uchida *et al.* (2004) recorded that, 75% ammonium sulphate saturation for *B.subtilis* CN2.

Gel filtration of the thermostable protease on sephadex G-200 showed that, the enzyme activity was detected firstly, in fractions 10-18 where the highest specific activity 5095.221 $U/mg^{-1}$ protein was recorded in fraction 11. The major peak was in fraction no. 11.

Moharam *et al.*, (2003) purified protease from two strains of *B.sephaericus* (IS 2362) and NRC 69 by 58 and 126 folds respectively by using ammonium sulphate fractionation and sephadex G-100 column chromatography while, Nilegaonkar *et al.*, (2002) recorded 10 folds increase in specific activity of protease when purified using gel filtration on sephadex G-100. Uchida *et al.*, (2004) purified protease produced by *B.subtilis* $CN_2$ by 272 folds with an over all yield of 9.2% from the culture supernatant.

In the present study, amino acids analytical data of the purified protease produced by *Shewanella putrefaciens*-EGKSA21 indicated a total number of 15 amino acids with glutamic acid and alanine showing the highest value. Similarly, Mahmoud, (2004) found that, glutamic acid gave the highest value for purified protease produced by *Pseudomonas aeruginosa* B8.

Concerning the purified protease, it was found the temperature and pH optima 60ºC and 10.5 respectively, and the enzyme was thermostable up to 50-55ºC and pH stable at 6 and 10 where the maximal activity was obtained.

Interestingly, in the present work, it was found that, $Pb^{+2}$, $Na^{+}$ and EDTA stimulated alkaline-thermostable protease activity produced by *Shewanella putrefaciens*-EGKSA21 while $Cd^{+2}$ and $Mg^{+2}$ make inhibition at high concentration (100-1000 ppm). Confirming that, these cations take part in the stabilization of the protease structure and are required for protection against thermal denaturation (Paliwal *et al.*, 1994), and play a vital role in maintaining the active confirmation of the enzyme at high temperatures (Gupta *et al.*, 2002).

Similar results was recorded by Yang *et al.*, (2000) who found that, the activity of purified protease enzyme produced by *B.subtilis* was increased in the presence of $Mn^{+2}$, $Fe^{+2}$, $Zn^{+2}$, $Mg^{+2}$, $Co^{+2}$ ions, but it was inhibited by $Hg^{+2}$ ions.

**5. Conclusion**

The aim of this research work was to isolate and identify high protease production from local habitat. *Shewanella putrefaciens*-EGKSA21 was produced maximum yield of alkaline protease and it was selected as a potent strain for further studies. The optimum temperature and pH were determined as $50^{O}$C and 9 and best carbon and nitrogen sources were L(-) arabinose and potassium nitrate. This information has enabled the ideal formulation of media composition for maximum protease production by this organism. After optimization, the mass production was carried out in one liter of optimized media at 50 ºC for 48 h at pH of 9 on basal medium. In conclusion, results of the present study suggested the possibility of *Shewanella putrefaciens*-EGKSA21 to produce enzymes by BM using cheapest substrates for enzyme production. This enzyme was stable over a wide range of pH and temperature. Considering the overall properties of different alkaline enzymes of microbial origin and the thermostable alkaline enzyme from our strain

*Shewanella putrefaciens*-EGKSA21 is better as regards to pH and temperature**.**

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# Employment of rural women

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**Abstract:** Women as an effective member of society, can crystalline their lead roles in various responsibilities formations. These responsibilities include promoting the concept of participation and employment in life and building the suitable areas for freely activity and introduce the right of economic management, ownership and.... This requires that all fees and necessary training for women to be considered. Due to the fact that the concept of women's participation, is not necessarily the female employment, although certainly part of the participation of women will be crystallized in their employment, but in this context, home and family affairs by women and their role in nutrition and child growth and Their education are also many responsibilities that women often are responsible for them. Throughout history we have always been seen that women have always been active but in culture and tradition, this mentality largely exists that if the job exists, it would be for men. Because they are responsible for their families Economic or wherever there is a good opportunity for participation, men have a prior right.

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## Introduction:

Reason that women are less important in the development is this thought and action. Because women are in occurred opportunities in the second stage, or even sometimes do not come into account. Zanjani in the article "Women's Empowerment" according to economic, social and cultural characteristics, one of the important subjects that have investigated is the effect of number of children in female employment in urban and rural communities. In Iran urban, employment opportunity population continually reduces by increasing the number of children. This reduction is weak, up to the third child and then takes the intensity. So that the employment opportunities of women decrease in pay to first child to the second 3 / 2 percent and the second child to the third 9 / 6 percent, while this reduction from third child to the quarter is 3 / 27 percent. But in rural society due to the household problems, type of activity and employment, increasing numbers of children not only make no reduction in women employment opportunities so with increasing the number of children, women's job opportunities is also growing and by having 7 child reaches its peak. Since then relegated to minor finds, in a way that employment opportunities of rural women that has nine child is equal to the job opportunities of a woman with one child. Thus children are effective on women employment so that increasing the number of children in urban society has negative effect and in rural society has positive effect (Zanjani, 2002). Lhsay Zadeh in a research by the name that (considering the role of Iranian rural women in the economic scene), first specified the women's place in job structure, and then compared it with the job site of rural men. His study demonstrated that the employment of rural women is important as men. Because the rural economy includes three separated and also related parts, namely agriculture, industry and services and the author, with the share of women in agricultural activities come to the conclusion that in addition to their considerable added value contribution in agriculture, unfortunately, the real value of their activity is not known has been formed in the article. (Lahsaeezadeh, 2004)

## Factors associated with employment of rural women:

Women's share of the total lot of manpower required in the agricultural sector worldwide, and Iran form. Facts and figures and statistics in relation to women in productive activities are offered much less than real, because the statistics many times, often including seasonal employment, part time and unpaid, and housekeeping activities Women are not considered (lahsaeizadeh, 2001). Perhaps the most fundamental problems brought on participation rate of women in rural agricultural economy; this is a topic that participation and employment of women more than men, influenced by economic conditions and various factors - social, cultural and ecological is. As a result of how women's employment in different areas or within a country is different. Here are some important factors are mentioned:

**1 - Structure of agricultural and social classes:**

Women as the first group are known to have paid agricultural work, and evidence shows that women farmers have been the first. Important factor causing women's participation in agricultural activities has been, among them we can mention the following (Fami, 2003):

A - Seasonal agricultural employment, and intensified the need for labor in certain seasons.

B - Men migrate to find better jobs and to assume responsibility for home and farm and agricultural work and its management by women:

In some countries men migrate to cities, or on bringing those to wage jobs have led to women's responsibility for 30 to 40 percent of agricultural plants and are responsible, in some areas this figure reaches 70 percent (lahsaeizadeh, 2001).

C - Effect of cultural - social conditions on women work:

Sociological experience shows that kinship networks status and community practices determine that who and in which areas women can have activity and employment. Several Kinship networks provide different economic roles for women based on age, marital status and their place in father and husband family (Movahedi, 2005).

**2 - cultivation and diversity of products system:**

The decisions to change cultivation products, whether the products have domestic consumption or export aspects are taken, can have an important effect of working pressure on women.

The fact that women traditionally reserve can't have the means that they don't have any ability or interest to generate cash products. If they feel that a cash product yields is higher than a livelihood one, they cultivate it and play the role in its production that have much more widespread that it is thought. For example, 70 percent of coffee production activities are performed by women in Rwanda (Saleh Nasab, 2004) the role of women in rice and tea production is very important in Iran. Based on research carried, 76 percent of rice productions in some Lahijan villages (in Iran) are carried by women. The effect of improving the plantation related to rural poverty is different. Food and increase production affected farmers and workers increasing income. Rising agricultural and production incomes, lead to labor force employed by them are and this reduce the work pressure on women and make some free time for them(fani, 2001).

**3 - agricultural modernization**

Towards expansion and agricultural intensification activities, rural activity rates also increased. Thus it need for a tool which reduces the men and women working pressure. Agricultural Modernization and technology development, lead to business development orientation, and investments are seeking more money. In capitalist development, monetary employment takes the money force and the separates the workforce and capital. Overcome these two variables have interaction effect on the type and amount of work that women should spend for plantation. If the Modernization development doesn't increase women's agricultural participation, it leads to separation of housework from productive activities. In some cases, with the agricultural trading and the technology boom, men have more responsibilities that previously were done by women. Some development theorists, believe that with technology development and application of agricultural machinery, employment of women, have been affected (Mehrabi Basharabady 2000) Of course, this theory can be discussed from different angles and study, but what can affect on agricultural labor force modernization structure, is that the every day dependence of farmers to new agricultural and investment methods, small farmers are likely to get out from stage. The reason can search in increasing costs and decreasing prices of agricultural products that the petty peasants were forced to sell their land. This led to inequality rural employment in (Azkia and imani, 2006) that lead to reduced wages and in this case is whether women as agricultural workers or housewives impact by politics.

**4 - Family status**:

Women are considered as labor in the family, for example, every woman in the animal economy, can bleed a few sheep and goats and this implied that the number of women in families is high. By considering that in developing countries, the economic power is in men hands, men for supply their required labor, married again and in some cases, women go to woo for their husbands second marriage, because it reduces their exploitation (Aly and et al, 2000).

young families with many children in villages often are an obstacle for agricultural and non-farm employment of women and diminish their working time, but with the growth of children their free times increase to acquire more working on the farm(zanjani, 2003) .Being Households head is being one of the important factors determining the participation rate of women. For example, in Colombia when a woman is household's head, her entering to market, increases to 47 percent, but for women who are not heads of households, entering the job market is only 21 percent. So the family status is one of the factors affecting rural women's work and leads their participation or non participation.

**5 - participation rate of women in decision making:**

a positive relationship between women's participation in agricultural and non-agricultural

employment of men can be seen, so that in some countries men migration to cities or bringing them on a day wage jobs has led them responsibilities in the absence of their husbands take charge of 30 to 40 percent of work related to home and agriculture. In some areas this figure reaches to 70 percent. Number of factors also led to a kind of common gender division of labor, especially in rural societies and one the most veteran of these factors is a particular power and ability of women to provide sustenance (Ghaffari, 2005).

**Results:**

Razavi during a study has shown those women's achievements in academic and social areas in the past 30 years; according to their status in the labor market has not improved. Women's participation rates are low and their non- employment rates increase in these years their and their career options are still limited (lahsaeizadeh, 2004).

Hashemi (2000) with the employment status of women in Iran has shown that the rate of economic participation of women in Iran were similar with developing countries, while their literacy and education rate are comparable with advanced countries. He believes that formal institutions, namely laws and regulations have the most effective on women's employment levels that in their turn are under the social and cultural effects.

Bamdad during his study on socio - economic status of women has shown that social and economic improvement of society is associated by increasing employment rate of women. There are also differences in cultural and social discrimination between men and women, is a serious obstacle in increasing the economic participation of women. Finally, increasing women's economic participation is the function of social development – economic factor (Banihashem, 2002).

The positive effect of government spending in women employment indicates the fact that, there are limitations and discrimination for women in the labor market that the market mechanism can not destroy it thus recognizing these limits, discrimination and government intervention in the market (of course in cooperation with people) is necessary to eliminate them.

Today there is this belief that communities rather than, affected by mood men and environmental conditions, affect by personality and education of women. Thus in the process of economic and social development, women affects are more than men, and the non-developed countries have understood the undeniable fact that to achieve the economic development should employ women creative and effective forces. Structure of female employment in different countries shows that there is a direct relationship between population growth and increasing employment rates of women. In other words, in countries where female employment rate is lower, the population growth and economic development is slower.

So if the state goal and the country's development policies, be the attention to women's active participation in society as half of the labor community, the cultural, social, political and economic area of their presence should allow to provide till we can use their intellectual power, creativity, innovation and The large number of workforce innovation for family and society economic development, otherwise, with the slogan and write policies and strategies and using no proper tools and executive Migration, like the former, manpower of this huge group saw little presence in the various community activities.

Different economic sectors (particularly industry and service sector) have the capacity to create many job opportunities for active participation of rural women that can be more benefit in more employment opportunities. Some variables such as marriage to divorce ratio, the share of government expenditure of GDP, the degree of development and Underdevelopment, number of children born and household expenditure are impressive on rural women's employment rates. Thus, if policy makers intend to predict the employment status of rural women, they should attend to affective factors on this group employment.

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# Challenges of information and communication technology (ICT) in education

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**Abstract:** Technologies (ICT) during the past two decades have had many points of contact with education and training. The development of technology is placing new demands on expertise, and it is also leading to the increased use of information technology (IT) in instruction and learning. As early as in the 1970s discussions of the future of school systems started to pay attention to the opportunities provided by ICT. Now with the approach of the new millennium, IT is playing an increasingly central role in almost all future planning of schools and instruction. With the help of state and local funding, information technology has been purchased for schools ever since the 1980s. The state has also found many ways to support teacher training in the use of IT, and it has also allocated funds for the production of IT programs. Instruction in the use of IT has also played an important role in teacher training organized by local school authorities. It is against this background that the need arose to find out how far we have progressed in the application of ICT in education and what impacts these significant economic investments have had. It is also time to start a value-oriented discussion of how strongly the future of the Iran society will be linked to the vision of an information society brimming over with technology.
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**Keywords:** ICT, education

## Introduction

There are Fundamental challenges about the role of information and communication technology (ICT) in education. This has led to serious skills shortages in many countries. In turn this has put increasing pressure on policy makers, universities and other training institutions to come up with approaches to inspire young students to choose ICT for their studies. There is also a strong argument for retraining many people who already have pre-service and in-survive education, whether in the workforce or not, to overcome to looming ICT skills crises. This paper reports on the examination of these points. It will also explore appropriate ways to combat this problem through analysis and identification of real prospects for ICT education.

Although valuable courses may around the world be learned best practices, there is no formula to determine the best level of ICT integration in education system. The main challenges for policymakers, planners, managers, coaches and other stakeholders that should consider include, is a comprehensive educational policy and planning, infrastructure, language and capacity building and financial affairs. (Collis, 2002).

## 1- The challenges to educational policy and planning:

To achieve promotion and reform in education through ICT, should be considered explicit and clear objectives, guidelines, mobilize the required resources and political requirements for understanding the primary goal in all levels. Some essential elements in planning for ICT are listed below:

1-1-A correct analysis of the current state of education system. ICT impacts should be considered institutionalized as current methods, respectively, and especially "those ICT to drive forward and the barriers should be recognized, as well as those related to education and training programs, infrastructure, capacity building, language and content and finance.( Collis, 2004).

1-2-Educational objectives at different levels of education, as well as various aspects of ICT applications that can best meet these goals in the state be used. Policymakers must understand the potential of ICT in various different goals when the concepts are used.

As well as may alert best practices around the world, about the priority educational needs, financial and

Human resources and capacity bottlenecks the country and how these experiences can be adapted to the specific needs of the country (Hakkarainen, 2000).

1-3- Identifying stakeholders and coordinating actions among different interest groups.

1-4- Conducting chosen model based on ICT, should are tested on a small scale, best design models or those who proved they can be used in other areas. Such guidance is essential for identifying, correcting, feasibility, etc.

1-5- Preparation of available financial resources and identify strategies to generate financial resources for strengthening the application of ICT in the long run. (Harris, 1999).

**2- Infrastructural challenges in education of based on ICT:**

Before any program of based on ICT to run, an Educational technology infrastructure is placed above infrastructure of information and telecommunications. Policy makers and planners should carefully take into account the following:

2-1- At first, is there suitable rooms and buildings for placing technology? Building schools in countries that they are too old, is required to ensure an extensive repair of electrical wiring system, building, cooling and heating, ventilation and safety.( Swaminathan, 2002).

2-2- are there electricity and phone? Developing countries, vast areas still lack adequate power and several miles away their nearest phone station. In some African countries are using wireless technology, although expensive approach, but other developing countries with poor telecommunications can try this solution.

2-3- Policy makers must are examined also attending a variety of ICT in the country in general and the educational system (all levels) in particular. For example, "a primary need in education of based on ICT (using a computer and via online) access to computer and Internet services at the community level, especially schools and host families (Virgo, 2008).

**3- Challenges of Capacity building:**

Various attempts should be occur throughout the educational system integration for success of ICT.

3-1- professional development of teachers should be have five-axis: (Dadgaran, 2002)

- Skills in specific applications
- merging in existing curriculum
- curriculum changes regarding the application of IT (including changes in instructional design)
- Changes in the teacher's role
- to support educational theories

Ideally these should be served in pre-service training of teachers and be upgraded in in-service. In some countries, like Singapore, Malaysia and England, is required to recognize the application of ICT training courses. ICT will change speedily technologies and in this regard even the most elite teachers need to promote ICT skills and are welcome the latest developments and best practices.

Although the first focus is skills with specific applications but other four focus is importance. Research on ICT application in different fields as education and uniform over the years show disability as a barrier to teachers successfully plan, understand why they should use ICT and how to properly get the best teaching aid.( Falk and Wolfmayr, 2008).

Unfortunately, most teacher professional development in ICT has been the emphasis on teaching tools and their application in education. If learning process being Student centered, anxiety of teachers from being struck by the technology or the loss of authority in the classroom, can be prevented and as a deep understanding and feeling a severe change in their role than do not have to be raised.

Whether ICT will replace teachers? Answer is "no". In fact, with promoting ICT in the classroom, teacher's role in learning process is even more important. What can and should change is the role of teacher. Likewise the role of students "developed since the ICT can be opened classroom doors to the outside world, the community could be a new role in class. (Mohseni, 2003).

Since education is transferred in model centered-teacher to centered-student model, the unique authority of teachers was low and are known more than as facilitators, observers and trainers (of the absolute ruler to guide the way).

Primary task of the teacher is teaching students how to ask questions and to discuss the issue, make hypotheses, and then if necessary to reach Information about finding the issues raised in relation to the assessment. (FAO, 2000).

Because of improved ICT training a new experience, even for teachers, teachers learn educational process and new things are discovered among the students.

Plus this is not unusual to see students in a class based on ICT undertake formal and informal roles of teacher to younger friends and students and sometimes even for teachers. (Saadan, 2001).

Teachers and students from different schools, experts, parents, community and business leaders, politicians and other stakeholders are involved in the educational process areas as resource persons, critic, observer and encouraging,.

They also are essential and general customers for student published work on the Web or other media. Not many teachers reluctant to use ICT are especially

"computer and internet usage. Hannafin and Savenye were found several reasons for this reluctance:

- Poor design of software,
- pessimism towards Computer effects of increasing efficiency in teaching,
- lack of managerial support,
- the time and efforts to increase technology and learn how to use for training
- Fear of losing authority in the classroom, as class is centered student.

These are points that should be served in pre-service training and professional development programs in in-service training of teachers. In in-service training about professional development of ICT teachers, should in the long run, be flexible and possible. (Cecchini and talat, 2002).

For many teachers lack the necessary conditions, and with less rights in developing countries, adaptation of ICT effectively subject to granting the necessary opportunities for learning things that they need to learn according to their own experience. Motivation of teachers and supporting teachers to pursue professional development plan is necessary. That can be promoted as with ICT initiatives for teachers who are classroom teachers or ensure adequate access to technology is after training.

**4- Current challenges within the language and content:**

English is the dominant language on the Internet. One estimate shows that 80% of online content is English. Also a large share of educational software produced in the world market is in English. A serious obstacle to maximize the use of World Wide Web in developing countries and regions outside the major cities is that English is not prevalent. (Mohseni, 2003).

Even in countries where English is a secondary language (such as Singapore, India, Philippines and Malaysia) is essential that materials the needs of national courses and meet the local content of the curriculum, rather "to create local language be.

Must ensure that the web is a multicultural environment with people of different cultures, namely have a role and a voice in education online communities. Therefore, is essential according to the specific needs of remote and rural segments of cultural and linguistic minorities in general.

**5- Challenges related to financing the cost of ICT:**

One of the biggest challenges in application of ICT in education, balancing educational objectives with economic realities. ICT in educational programs requires massive investment in developing countries that should decide on what models about the current usage of ICT and be cautious and remain vigilant about keeping the economic balance. **(**Annan, 1997**)**

Finally, this issue is raised whether application of ICT value added costs to balance or not, the other for any effective ICT-based teaching strategies intended for educational purposes or not, and if there is and scale requirements that can be implemented regardless of existing human and financial resources than that, what does it support? (Dadgaran, 2002)**.**

Whyte offers potential sources of financial and ICT applications in following:

1. grant aid
2. the public subsidies
3. private sector funds
4. Support Equipment and volunteers
5. community support (i.e. to putting the house without receiving rent)
6. Members membership fees
7. revenue derived from the central and main tasks:
    a. Connections (telephone, fax, internet and web page)
    b. direct access to computer users
    **c.** administrative services (photocopiers, audio-visual aids and scan)
8. Subsidiary activities income
    a. Different services (word processing, preparing financial statements, the preparation, printing and adoption services)
    b. Educational Services (non face to face training and educational courses)
    c. social services (conference rooms, social events, local information)
    d. Works distance and consultation
    e. specific activities (telemedicine)
    f. Sale

**4. CONCLUSION**

A common strategy in higher education ministries in developing countries is public and private sector partnership in strategy or pursue rapid ICT projects is based. This partnership has different forms such as grant aid private sector interaction with public assistance, donated educational equipment and components by companies to public schools, providing technical assistance for planning, management and consolidation tools and human resources at the local level. But after financial aid, testing programs based on ICT is critical.

Many of the ICT training programs based on the charitable agencies aid have been unable to have high durability. Because the government has failed in its financial assistance in this situation none of the local

communities to provide resources do not needed to continue these programs. Two strategies in here "to support government and local communities to move" are important. Since the 21st century, is century of education support about youth in Asia, to find sustainable ways to bridge the digital age in Asian countries is a real priority. And work through partnership that local leaders and guides are experts it can be lasting forever.

Several recommendations that emerged from the discussions emphasized on the need to think of ICT in education beyond computer aided learning and investigate the potential other technologies like community radio and other medium. These mediums could not only be cost effective but also has a greater outreach potential. It was also pointed out that low cost software solutions for e-learning that have scopes for innovation, should be incorporated in large scale projects. With an indication to open source solutions, the sessions recommended that such solutions should become a part of the overall policy for implementating technology supported education interventions.

Sustainability and scalability of project are also issues that needed serious considerations. While moving beyond the pilot and experimental phase, projects especially those that needs a considerable financial contribution should have a viable sustainability model for up scaling. It was also recommended that implementers needs to be cautious when selecting areas for implementing ICT in education projects.

Projects should also not lose priority of the education objectives. In some cases ensuring school accountability system and teachers attendance may be more important that investing time and resources in ICT integration in schools. One fact that emerged in the sessions was that ICTs effectively computers, initiated in government department and schools were being used as decision support in education. Essentially, clear criteria, norms and standards needs to be developed for the information that was being used for decision-making.

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# Field study on Cadmium pollution in water and Crustacean gill parasites in freshwater cultured *Tilapia zilli* fish

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**Abstract:** The aim of this study is to explain the relationship between Cadmium pollution in water and Crustacean gill parasites in freshwater cultured *Tilapia zilli* fish. A total of 375 adults cultured *Tilapia zilli* were studied the effect of water cadmium pollution on clinical examination and the prevalent seasonal crustacean gill parasitic infestations in the period 2009-2010. This investigation revealed the appearance of the parasites during spring, summer and autumn and their disappearance during winter. Clinical signs were pale skin, blood spots with cognation of gills, as well as post mortem lesions and isolation of infested parasites. *Ergasilus sp* and *Lamproglena sp* were decreased in gills with high concentration of cadmium. The present study was concluded that, there were inversely proportion relationship between cadmium concentration pollution in aquaculture and the prevalence of gill crustacean infestation during spring, summer and autumn seasons while infestation was disappeared during winter season. Also, there was a relationship between cadmium residues in *Tilapia zilli* gills and its concentration in the water, the obtained results showed that the cadmium concentration in the gills were higher than that in the water.

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**Keywords**: *Tilapia zilli*, gills, cadmium, *Ergasilus* sp., *Lamproglena* sp., pollution

## 1. Introduction

Over the last few decades; aquatic pollution is still a problem in many freshwater and marine environments as it causes negative effects for the health of the respective organisms (Farombi et al. 2007). Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial mining and agricultural activities (Kalay and Canli 2000). Aquatic organisms such as fish accumulate metals to concentrations many times higher than present in water (Olaifa et al. 2004 and Noor El Deen et al.2010). Permissible limits of cadmium are 0.05 ppm Egypt E.O.S.Q.C. (1993), but in environments impacted by man, concentrations can be several micrograms per liter or greater (Annune et al. 1994). They can take up metals concentrated at different levels in their different body organs. Target organs such as gills, have a tendency to accumulate heavy metals in high values by (Khaled 2004 and Yilmaz 2005). Cadmium may have toxic effects, altering physiological activities in gills and fish blood (Mona Zaki et al. 2010). The relationship of parasitism and pollution is not simple and in essence involves a double edged phenomenon in which parasitism may increase host susceptibility to toxic pollutants or pollutants may result in an increase or decrease in the prevalence of certain parasites. Pollutants may affect an intermediate or alternate hosts in parasite life cycle and on free-living life cycle stages of parasite invasion (Sindermann 1990). Pollution stress can influence the prevalence of parasites directly or indirectly,or the parasite infestation may decrease the host resistance to toxic pollutants (Khan and Thulin 1991) . *Ergasilus* sp which exposure to pollution considerably reduced in zone of pollution (Kuperman 1992). The prevalence of crustacean parasite infesting *Oreochromis niloticus* in Lower Egypt (Kafre El–Shiehk Governorate ( 1998-1999) fish farms were 30 % and the highest the prevalence was obtained during summer (56.6%.) and 44.8% in spring, while was 20.5 % in autumn and 0% in winter in ( Noor El Deen 2000). The less polluted water can allow for or cause parasite proliferation,whilst higher level of contamination can have a negative effect on the survival of *Lamproglina clariae* ( Avenant-Oldewage (2003). There is more awareness of the importance of studying fish parasites as one of the major obstacles in fish production. About 80% of fish diseases are parasitic especially for warm water fish (Eissa et al. 2000). This study was undertaken to investigate the correlation between cadmium concentration in water and the prevailing crustacean gill parasites in cultured *Tilapia zilli* which were collected from Kafr El sheikh governorate fish farms.Clinical picture, correlation between crustacean parasitic infestation and concentration of cadmium in

water and tissues of cultured *Tilapia zilli* are also considered.

## 2. Materials and methods

### Fish:

A total number of 375 cultured adult *Tilapia zilli* fish were collected with average length of 15-20 cm and of body weight ranged from 85-100 gm and subjected to clinical examination for detection of the prevalent parasitic infestations during the different seasons over a period of a year (2009 - 2010).

### Water samples

A total number of 27 water samples simultaneously with fish specimens and equally distributed through out the different seasons, were collected from the different fish ponds from Kafr El sheikh governorate (Lower Egypt).

### Clinical Examination

Alive fish were clinically examined for general behaviors, changes in colour, respiratory manifestation, feeding and any clinical abnormalities of the gills according to the methods described by Noga (1996).

### Parasitological examination

Crustaceans were refrigerated then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70, 90,95% and absolute) then cleared in xylol, mounted in Canada balsam or by clearing in lacto phenol and mounted in glycerin gelatin according to (Lucky 1977) and identified according to Paperna (1996).

### Estimation of cadmium in Tilapia zilli gills

Each 0.5gm of different gill fish samples were well digested using Conc, H2sO4 according to the method outlined by Cottenie (1980).

### Statistical analysis

The results of prevalance performances were statistically analyzed using analysis of variance procedure in SAS (Duncan 1955).

## 3. Results

### Clinical picture

The first sign observed in *Tilapia zilli* exposed to cadmium pollution was swam rapidly in circles manner of the affected fish in which the fishes aggregate in groups around the water inlet. Most of these fishes showed dark discolourtion of the skin, emaciation, loss of appetite and eventually loss of escape reflex. The gills appeared pale in colour with numerous noduler like as white to yellowish colouration and appear as V or inverted V-shaped of the egg sacs on the attached gills in some examined fish during post mortem examination.

### Parasitological examination

The microscopical examination the white nodule appear as V or inverted V-shaped of the egg sacs on the attached gills revealed as ergasillid female copepods (Figure1). Also, other parasites appears as cylindrical consists of three distinctive parts, cephalothorax that is oval and externally unsegmented are present revealed as Lamproglid female attached firmly to gill filament by the aid of two powerful claws alone (Figure2). While in few tilapia fish both infestations (Figure3).

### Cadmium residues in water fish farms and Tilapia zilli gill tissues

As shown in Table 1 , the mean concentration of cadmium in water of *Tilapia zilli* farms were 0.02± 0.009, 0.02± 0.002 and 0.02± 0.009 ppm in location 1, 2 and 3 respectively during winter season and the mean concentration of cadmium in water of tilapia farms were 0.05± 0.003, 0.03± 0.001 and 0.02± 0.003 ppm in location 1, 2 and 3 respectively in autumn season while the mean concentration of cadmium in water of *Tilapia zilli* farms were 0.07± 0.005, 0.04± 0.003 and 0.03± 0.007 ppm in location 1, 2 and 3 respectively in spring season and the mean concentration of cadmium in water of *Tilapia zilli* farms were 0.04 ± 0.009,0.03± 0.002 and 0.04± 0.009 ppm in location 1, 2 and 3 respectively in summer season.

Table 2, showed that the mean concentrations of cadmium in gills of *Tilapia zilli* were 0.19±0.002, 0.72±0.002 and 0.99±0.004 in location 1, 2 and 3 respectively in spring season and the mean concentration of cadmium in gills were 0.12±0.002,0.63±0.002 and 0.85±0.004 ppm in location 1, 2 and 3 respectively in summer season. While the mean concentration of cadmium in gills of *Tilapia zilli* were 0.11±0.004, 0.53±0.004 and 0.65±0.004 ppm in location 1, 2 and 3 respectively in autumn season and the mean concentration of cadmium in gills were 0.08±0.003, 0.35±0.004 and 0.45±0.002 ppm in location 1, 2 and 3 respectively in winter season. Cadmium residues were significantly increased in gills of *Tilapia zilli* than water fish farms. Also, the crustacean gill infestation increased parallel to increase cadmium pollution in examined water and gills of *Tilapia zilli* was observed. On opposite no infestation in winter season was observed.

**Table 1:** Cadmium concentration in water of *Tilapia zilli* farms (three localities) in summer, spring and autumn seasons was done.

| Metal | Areas | In summer water samples (mg/L) | | | In spring water samples (mg/L) | | | In autumn water samples (mg/L) | | | In winter water samples (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Mean ± SE | Min. | Max. | Mean ± SE | Min. | Max. | Mean ± SE | Min. | Max. | Mean ± SE |
| Cadmium | 1 | 0.001 | 0.082 | 0.05± 0.03 | 0.003 | 0.058 | 0.07 ± 0.005 | 0.001 | 0.082 | 0.04± 0.009 | 0.001 | 0.042 | 0.02± 0.009 |
| | 2 | 0.001 | 0.062 | 0.03±0.01 | 0.002 | 0.042 | 0.04 ± 0.003 | 0.001 | 0.062 | 0.03± 0.002 | 0.002 | 0.037 | 0.02± 0.002 |
| | 3 | 0.001 | 0.042 | 0.02± 0.003 | 0.001 | 0.072 | 0.03± 0.007 | 0.001 | 0.082 | 0.04± 0.009 | 0.001 | 0.082 | 0.02± 0.009 |
| Total average | | 0.0013 | 0.07 | 0.03±0.01 | 0.002 | 0.052 | 0.03 ±0.51 | 0.001 | 0.072 | 0.07 ±0.51 | 0.001 | 0.072 | 0.02± 0.007 |

Means within the same column of different letters are significantly different at ($P < 0.05$).

**Table 2: Residue of Cadmium and the prevalence of crustacean gill *Tilapia zilli* fishes parasites at different seasons.**

| Season and samples location | Autumn, 125 *Tilapia zilli* fish | | | | Summer,125 *Tilapia zilli* fish | | | | Spring,125 *Tilapia zilli* fish | | | | Winter,125 *Tilapia zilli* fish | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cd in gills (mg/kg dry wt.) | No of parasites | | | Cd in gills(mg/kg dry wt.) | No of parasites | | | Cd in gills | No of parasites | | | Cd in gills(mg/kg dry wt.) | No of parasites | | |
| | | E | L | E&L | | E | L | E&L | | E | L | E&L | | E | L | E&L |
| 1 | 0.11±0.004 | 27 | 13 | 3 | 0.12±0.004 | 18 | 9 | 3 | 0.19±0.002 | 1 | 6 | 2 | 0.08±0.003 | 0 | 0 | 0 |
| 2 | 0.53±0.004 | 25 | 10 | 3 | 0.63±0.007 | 17 | 7 | 2 | 0.72±0.002 | 8 | 4 | 0 | 0.35±0.004 | 0 | 0 | 0 |
| 3 | 0.65±0.004 | 23 | 7 | 3 | 0.85±0.003 | 10 | 5 | 1 | 0.99±0.004 | 4 | 2 | 1 | 0.45±0.002 | 0 | 0 | 0 |
| Total average | 0.43±0.004 | 25 | 10 | 3 | 0.53±0.0038 | 15 | 7 | 2 | 0.63±0.006 | 8 | 3 | 1 | 0.33±0.004 | 0 | 0 | 0 |
| % | | 20 | 9. | 2.4 | | 12 | 5.3 | 1.7 | | 6 | 2. | 1.3 | | 0 | 0 | 0 |

Cd: Cadmium E: *Ergasilus* L: *Lamproglena* $Chi^2 = 17.53$ * = Significant at ($P < 0.05$). (The number of infested fish differ significantly among localities

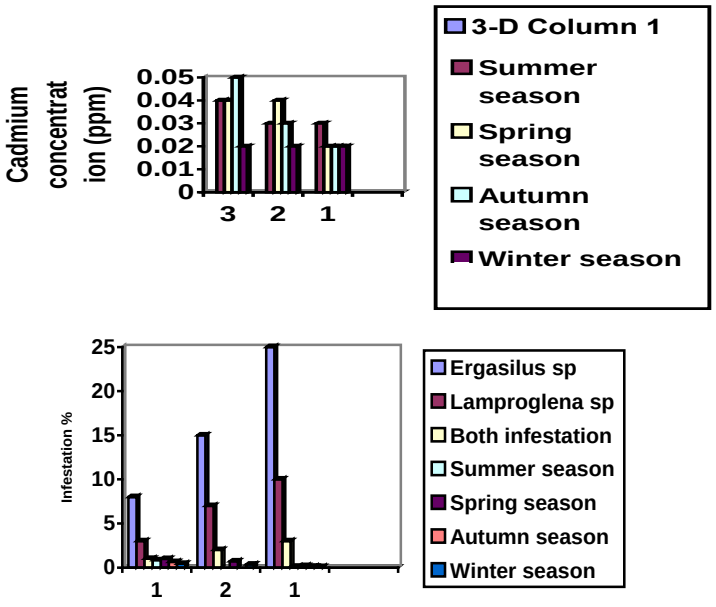


**Figure 1: Showing *Tilapia zilli* was suffering from cognation and white dot (*Ergasilus* sp.) on gills.**

**Figure 2: Showing *Tilapia zilli* was suffering from paleness and white dot (*Lamproglena* sp.) on gills.**

**Figure 3: Showing *Tilapia zilli* with mixed infestation with *Ergasilus* and *Lambroglena* sp. on gills.**

**4. Discussion**

The fish farms in Egypt are probably polluted by Cd pollutant mainly through drainage system of irrigation water which is designed to flow directly to the fish farms and from by products of industry (Eissa et al. 2011). The relationship of parasitism and pollution was not simple and the parasitization might result in an increase or decrease in the prevalence of Ergasilus and Lamproglina sp among Tilapia zilli . Regarding to clinical signs of infested Tilapia zilli and exposed to natural Cd showed that respiratory distress and slimy pale skin. These may be attributed to prolonged exposed to Cd who affect on osmoregulation. These results were in agreement with (Allen,1994).

Concerning, the postmortem of examined Tilapia zilli and infested with Ergasilus sp and Lamproglena sp, there were increase of mucus producing cells in the gills and presence of white dots. These results may be attributed to harmful effect of parasites. These results agree with those recorded by Rani and Ramamurthi 1987 and Eissa 2004 who found that the postmortem examination of gills of tilapia sp revealed pale gill appearance with white dots.

Dealing with parasitological examination of Tilapia zilli naturally exposed to Cd, infested with Ergasilus sp revealed that the Ergasilus appear as V or inverted V-shaped of the egg sacs on the attached gills. The results were coincided with those recorded by Eissa et al 2010. Also, Lamproglina sp parasite was appeared as cylindrical consists of three distinctive parts, cephalothorax that is oval and externally unsegmented are present revealed as Lamproglid female attached firmly to gill filament by the aid of two powerful claws alone . The results were coincided with those recorded by Eissa 2004.The results obtained from Tilapia zilli natural exposed to Cd in water of tilapia farms ,it was slightly increased gradually from winter season (0.02± 0.007 ppm) and (0.03± 0.001ppm) in autumn season to (0.03± 0.005ppm) in summer season and (0.04± 0.005ppm) in spring season and the prevalence of infestation was decreased gradually from autumn season (20, 9.5 and 2.4 % of Ergasilus sp., Lamproglena sp. and mixed infestation respectively) and (12, 5.3 and 1.7 % of Ergasilus sp., Lamproglena sp. and mixed infestation respectively) in summer season to (6, 2.5 and 1.3 % of Ergasilus sp., Lamproglena sp. and mixed infestation respectively) in spring season. While, the parasitic infestations in winter

season were absence result to sharp decrease of temperature which effect on crustacean parasites life cycle. These results revealed that were correlation between the prevalence of crustacean gill infestation and cadmium pollution was inversely. These results revealed that there were inversely proportion between cadmium concentration and prevalence of infestation where the number of *Ergasilus* sp. and *Lamproglena* sp decreased with increased cadmium concentration. The increase cadmium pollution and decrease of infestation in spring and summer season may be attributed to industrial and agriculture activity. These results were agreement with that reported with (Rehab 2004) who revealed that inversely proportion between cadmium concentration and parasitic infestation. These results in agreement with that recorded by (Kuperman, 1992) who found that the number of highly sensitive ectoparasites of *Abramis brama*, *Ergasilus* sp (Crustaceans) reduced in Rybbinsk reservoir (Volga basin) polluted with heavy metals.

Regarding the results of cadmium concentration in gills of examined *Tilapia zilli* was decreased gradually from winter season (0.33±0.0004 ppm) and autumn season (0.43±0.004 ppm) to (0.53±0.003ppm) in spring season and (0.63±0.006 ppm ) in summer season. These concentrations were increased in spring and summer than autumn and winter season may be attributed to direct contact of examined fish to polluted water. The recorded results of cadmium concentrations in fish were higher than the permissible limits intended by FAO/WHO (1992) (0.05 ppm) and Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C" (1993) (0.1 mg /1). These results was nearly agreement with those reported by Celik and Oehlenschlager (2007) who recorded Cd concentration with levels varied from 0.1 to 0.8 ppm. The high levels of Cd in gills may be attributed to direct contact to polluted water fish farms. The result was revealed that inverted correlation between cadmium concentration and prevalence of crustacean gill parasites in different seasons and the infestation decrease gradually with increase of cadmium concentration, these results attributed to the harmful effect of cadmium on crustacean gill parasites. These results revealed that inversely proportion between cadmium concentration and crustacean gill parasites. From the point of view, one could attribute the result of effect of cadmium on crustacean gill parasites. These results in agreement that reported with (Sinderman 1990) who recoded that the relation of parasitism and pollution was not simple and the parasitisation might result in an increase or decrease in the prevalence of certain parasites and the effects of pollution on parasites may be positive or negative i.e. pollution may increase parasitism and on the other hand it may be fatal for certain parasite species leading to a decrease in parasitism. The effects of simultaneously occurring parasites and pollutants can be additive, synergistic or antagonistic and that they can not be predicted easily. These results obtained from crustaceans gill parasites of *Ergasilus sp* and *Lamproglena sp* infested *Tilapia zilli* were decreased gradually with cadmium concentration above the permissible limit in water and exposure time. These were attributed to the parasites with direct life cycles are normally ectoparasites. These results were agreement with that recorded by Khan and Thulin (1991) who recorded that ectoparasites directly exposed to water may be more sensitive to contamination, thereby reducing there survival and reproductive rates. Also,(Kuperman,1991) who reported that the abundance of crustacean parasites changes under different environmental conditions and decrease considerably in polluted areas. Avenant-oldewage(2003) who suggested the less polluted water can allow for or cause parasite proliferation , whilst higher level of contamination can have negative effect on the survival of *Lamproglena clariae*.

The results of seasonal prevalence of cultured investigated fish revealed that the highest percentage of infestation was in summer followed by spring and the lowest in autumn while no infestation in winter due to these crustacean parasites disappear at low temperature. On the other hand, cadmium concentration in gills of examined fish was the lowest in winter season and the crustacean gill parasites were absent. These may be attributed to absence of crustacean parasites with low temperature under 18C. These results were agreement with that recorded by Bruton (1979) who recorded that the suitable environmental condation for spawning, which usually takes place at water temperatures above 18 °C.

The present study was concluded that, there were inversely proportion relationship between cadmium concentration pollution in aquaculture and the prevalence of gill crustacean infestation during spring, summer and autumn seasons while infestation was disappeared during winter season. Also, there was a relationship between cadmium residues in *Tilapia zilli* gills and its concentration in the water, the obtained results showed that the cadmium concentration in the gills were higher than that in the water.

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**5/8/5011**

# Benazepril Inhibits the Formation of Abdominal Aortic Aneurysms in Rabbits

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**Abstract Background:** The purpose of this study was to observe the effect of benazepril on the formation of abdominal aortic aneurysm (AAA) in rabbits.**Methods:** Male New Zealand white rabbits were randomly divided into six groups according to the perfusion solution (saline, elastase, and elastase combined with benazepril intervention) and postoperative observation time (two days and seven days). Morphological changes of the abdominal aorta after perfusion and blood pressure changes were observed. The expression of matrix metalloproteinase-9 (MMP-9) and nuclear factor kappa B (NF-κB) were measured. **Results:** Among the three groups at postoperative day two, there was no significant difference in the mean dilation rate of the abdominal aorta ($P$=0.055). At postoperative day seven, the mean dilation rates were 7.50% (saline perfusion), 120.62% (elastase perfusion), and 39.20% (benazepril intervention). Blood pressure is not significantly correlated with the mean dilation rates of the abdominal aorta (r=-0.137). Benazepril partially reduce degradation of elastic fibers and inhibit inflammatory cell infiltration ($P$<0.01). In the benazepril intervention groups, the expression of MMP-9 were decreased in each time group compared with that in the elastase groups ($P$<0.01), and the intranuclear expression of NF-κB p65 was also decreased compared with that in the elastase groups ($P$<0.01). **Conclusion:** Benazepril can significantly inhibit AAA formation in rabbit models; the mechanism may be related to inhibition of inflammatory infiltration, multilevel down regulation of degradation of extracellular matrix, and protection of elastic fibers.
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**Key words:** AAA, Inflammation, MMP-9, NF-κB

## 1. Introduction

Abdominal aortic aneurysm (AAA) is a common life-threatening arterial degenerative disease; currently, the main treatment of AAA is elective surgery based on imaging monitoring, and typically, an AAA diameter greater than 5.5 cm is considered to be an indication for surgery. A large-scale randomized controlled trial found that early elective surgery for small AAAs (diameter 4.0-5.5 cm) could not improve the survival time of the patients [1, 2]. Therefore, drugs that can inhibit AAA formation and growth would greatly improve the current treatment of AAA.

Studies have found that chronic transmural inflammation and destructive remodeling of structural proteins in the medial aortic layer are the main pathophysiological changes of AAA [3-5]. Activation of matrix metalloproteinases (MMPs) is the main cause of structural protein degradation in the process of AAA formation. Large amounts of data have shown that macrophage-derived gelatinase MMP-9 is the main enzyme for elastase degradation and plays a critical role in the formation of AAA [6, 7]. As a junction of the signal transduction pathway, nuclear factor kappa B (NF-κB) is closely related to the development of inflammation. In its non-activated state, NF-κB (p50/p65 dimer) is bound by its inhibitory protein IκBα in the form of a trimer and is sequestered in the cytoplasm. With stimulation of external signals, IκBα is phosphorylated by specific kinases and subsequently degraded, which results in the release of a p50-p65 dimer. The free p50-p65 dimer then translocates to the nucleus, binds to κB sites in the promoter region of its target genes, and regulates the expression of its target genes. These target genes include many inflammatory mediators, including interleukin-1 (IL-1) and interleukin-6 (IL-6), and some matrix metalloproteinases, includingMMP-1,3, and 9[8-13]. Pyrrolidine dithiocarbamate (PDTC, a specific inhibitor of NF-κB) inhibits the activity of MMP-9 and can effectively inhibit the formation of AAA in experimental rabbits [14].

Angiotensin-converting enzyme inhibitors (ACE-I) not only have an anti-hypertensive effect but can also directly inhibit cardiovascular remodeling[15,16]. A retrospective analysis of 15, 326 patients with AAA in Canada showed that the incidence of rupture was significantly lower in patients treated with ACE-I, while other anti-hypertensive drugs did not have this

effect [17]. In order to explore the effect and mechanism of ACE-I on the formation of AAA, we established an elastase perfusion AAA rabbit model.

## 2. Material and Methods

**2.1 Surgical procedures** After obtaining approval from the Laboratory Animal Science Committee of Central South University, we selected 42 healthy male New Zealand white rabbits (1800-2200 g) and perfused the infrarenal abdominal aorta with elastase [18]. After entering the rabbit abdominal cavity and exposing the abdominal aorta and inferior vena cava, a segment of the abdominal aorta (approximately 1 cm long) without branches in the anterior and lateral wall was selected (if the branches could not be avoided, they were ligated with #2-0 silk sutures); lumbar arteries in the posterior wall of this segment did not need to be fully mobilized. Then, the proximal end of this segment of the abdominal aorta was blocked with a noninvasive bulldog clamp, the common iliac artery on one side was punctured with a BD intima-II closed intravenous catheter (0.7*19 mm), and the needle after the catheter entered this segment of the abdominal aorta. Next, the catheter was advanced until it was 2 cm away from the bulldog clamp, the abdominal aorta was ligated at the site close to its branches by tying a slipknot with #0 silk suture to fix the catheter, and the lumbar artery was blocked behind the abdominal aorta occluded with a bulldog clamp (Figure 1). The catheter was connected with a microperfusion pump with the perfusion pressure maintained at 100 mmHg. After 30 minutes of perfusion, the catheter was withdrawn, and the punctured site on the iliac artery was sutured with #6-0 silk suture to restore blood flow in the abdominal aorta. Then the abdominal cavity was closed layer by layer with #0 silk suture. Animals were kept in separate cages after surgery.

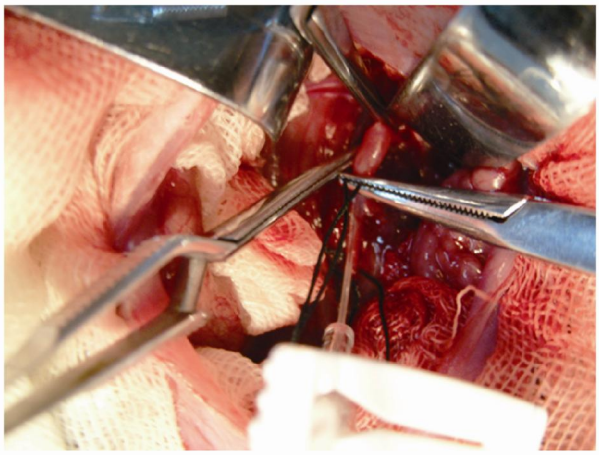

Figure 1. A blocked segment of the infrarenal abdominal aorta for catheterized perfusion.

During surgery, we measured the diameter of the abdominal aorta before perfusion (AD pre) and after perfusion (AD post, 5 minutes after blood flow resumption) using an operating stereomicroscope (Leica, Deerfield, Ill) and a calibrated ocular grid. The second surgery was performed in rabbits two days or seven days later, and the final abdominal aortic diameters (AD final) were measured. After the second surgery, the perfused segment of abdominal aorta was harvested and divided into two segments; one segment was placed into liquid nitrogen and then immediately transferred into a -70°C freezer for preservation, and the other segment was placed into 10% neutral formalin and fixed overnight for routine paraffin-embedding.

**2.2 Animal grouping and treatment** Rabbits were numbered and randomly divided into the following groups: saline perfusion control group (group A, 10 rabbits), elastase perfusion control group (group B, 16 rabbits), and benazepril experimental group (group C, 16 rabbits). After surgery, rabbits in each group were randomly and equally divided into two groups, the two-day group and the seven-day group. The perfusion solution in group A was saline and that of groups B and C was type I porcine pancreatic elastase solution (E1250, Sigma, US). Rabbits in group C were administered 3 mg/kg body weight/day benazepril intragastrically from one day before perfusion until one day before harvesting the samples.

**2.3 Measurement of blood pressure** OMP-7201 Life Scope monitor was used to measure the systolic pressure and diastolic pressure of three rabbits per group when punctured with a catheter before the first surgical perfusion, and then the mean arterial pressure was calculated. Mean value of three times of measurement in five minutes was calculated as blood pressure before perfusion (BP pre). The same method was employed in the second surgery to determine the mean arterial pressure as the final blood pressure (BP final).

**2.4 Histological staining** Aortic tissue cross sections (5 μm) were examined with hematoxylin-eosin (HE) and verhoeff-van gieson (VVG) staining. Inflammation was detected by HE staining. Sections from three animals in each group were scored using a one to five-point scale for the extent of inflammation by three observers blinded to the experimental design. Elastic fiber was stained to black by VVG staining and quantitatively evaluated for changes in the aortic wall. The percentage of elastin content in the entire aortic wall was calculated by a morphometry system (MacSCOPE, Version 2.2, Mitani Corporation, Japan).

2.5 **Western blot** Tissue MMP-9 total protein and NF-κB nucleoprotein were extracted using protein extraction kits (Pierce Chemical, Lot No:78503 for MMP-9, Lot No:78833 for NF-κB) and quantified by

Coomassie brilliant blue staining. The nucleoprotein was extracted as follows: frozen issue was dissolved in PBS and homogenized, after 5 minutes standing on ice, the mixture was centrifuged for 2 minutes at 500 rpm under 4°C and the supernatant was discarded; then Cytoplasmic Extraction Reagent and protease inhibitors were added and the solution underwent 15 seconds high-speed vortex and 10 minutes standing on ice; after that, 5 seconds high-speed vortex and 5 minutes centrifugation at 16000 rpm under 4°C were performed and the supernatant was discarded. Nuclear Extraction Reagent and protease inhibitor were added in precipitation and 15 seconds high-speed vortex was done. The mixture was placed on ice for 40 minutes and 15 seconds high-speed vortex was done every 10 minutes. After 5 minutes centrifugation at 16000 rpm under 4°C, the supernatant was collected, which was the nuclear protein. Samples were subjected to SDS-polyacrylamide gel electrophoresis for 1 hour, and then transferred to polyvinylidene difluoride (PVDF) for 70 minutes. Blots were blocked overnight with 1% Casein, sectioned, and incubated with appropriate primary antibodies for 2 hours. Primary antibodies included rabbit anti-NF-κB p65 (polyclonal antibody, 1:500, BD, NJ) and rabbit anti-MMP-9 (polyclonal antibody, 1:500, Chemicon, US). GAPDH (1:1000, Sigma-Aldrich, US) and PCNA (1:500, Santa Cruz, US) were used as internal controls for MMP-9 and NF-κB p65, respectively. The blots were then incubated for 1 hour in the appropriate secondary antibodies. Proteins were visualized using an enhanced chemiluminescence system (ECL, Pierce Chemical). Bands were quantified by densitometry.

**2.6 Statistical analysis** The data was analyzed using a SPSS 17.0 software package. Data in each group was shown as mean ± standard deviation (mean ± SD); the differences of means between two groups were compared using independent sample *t* tests and were compared using single factor analysis of variance (ANOVA) among multiple groups. The related samples were analyzed by paired *t* test. Person test was used to analyze correlation. $P<0.05$ was selected as the standard for significant difference.

## 3. Results

### 3.1 Dilation rate of the abdominal aorta

The mean dilation rates of abdominal aortic diameter after perfusion are shown in Figure 2. There were approximately 50% dilation rates in each group immediately after perfusion, and the rates were not significantly different among the groups (F=1.401, $P=0.257$). In the two-day groups (A2, B2, C2), the aortic diameters in each group decreased two days after perfusion compared with those immediately after perfusion, but still increased compared with the diameters before perfusion. The mean dilation rates of group A2, B2, and C2 were 19.18%, 29.58%, and 24.94%, respectively, and there was no significant difference among the three groups (F=5.330, $P=0.055$). In the seven-day groups (A7, B7, C7), the mean abdominal aortic dilation rates were 7.50%, 120.62%, and 39.20%, respectively. The mean aortic dilation rate of group B7 at the time of sampling was statistically different than those of A7 and C7 ($P<0.01$). An abdominal aortic dilation rate of more than 100% was selected as the diagnostic criteria for AAA. Accordingly, all the rabbits in group B7 had AAA, while there was no AAA formation in rabbits in group C7.

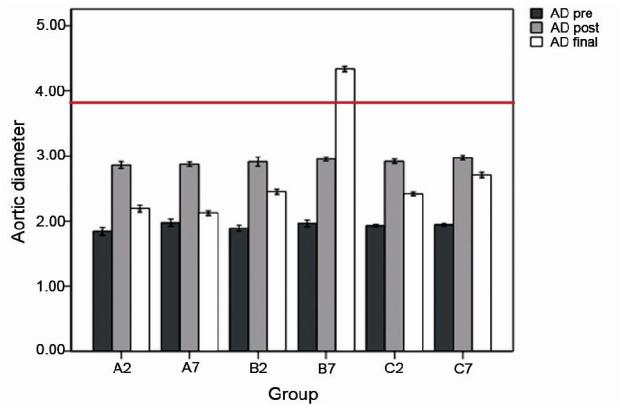


Figure 2. The aortic diameters

The aortic diameters at different times in each group are shown as the mean ± standard error by bar graph. The red line represented the mean preoperative aortic diameter of all experimental rabbits* (1 +100%), which was also the criteria for AAA in this study.

### 3.2 Changes in arterial pressure

The rabbit blood pressure was monitored to find out whether the antihypertensive effect of benazepril was involved in the inhibiting of benazepril of AAA formation. Our results showed that there was no significant difference in BP pre between each group of rabbits (F=0.164, $P=0.974$, Table 1). Paired *t* test showed that BP final was not significantly different from BP pre in each group ($P>0.05$). Person correlation analysis showed that BP final was not obviously correlated with the final abdominal aortic dilation rates ($r=-0.137$, $P=0.456$).

The systolic pressure and diastolic pressure were measured directly by a catheter puncturing into the iliac artery before first surgical perfusion. Mean arterial pressure = (systolic pressure + 2*diastolic pressure)/3. Mean value of three times of measurement was calculated in three rabbits from each group. Data were shown as mean ± SD.

### 3.3 Histological staining

In the saline infusion groups (groups A2 and A7), there was no significant inflammatory cell

infiltration, and the structures of elastic fibers were continuous and intact. In groups B and C, there was significant inflammatory cell infiltration of the arterial walls after elastase perfusion. However, there was no significant difference in the degree of inflammation between group B2 and group C2 (*P*=0.297), while there was a significant difference between group B7 and group C7 (*P*=0.028). The content of elastic fibers in group B2 decreased after elastase perfusion and significantly decreased in group B7, characterized by elastic fiber rupture and degradation and vacuole formation. In the benazepril intervention groups (group C2 and C7), the content of elastic fibers also decreased in group C2, and there was no significant difference compared with group B2 (*P*=0.176). The content of elastic fibers in group C7 decreased further; however, when compared with group B7, the content and structural integrity of elastic fibers in group C7 were superior (*P*<0.01). (Figure 3)

Table 1. Measurement of mean arterial pressure of rabbits of each group.

| Mean BP (mmHg) | Group A2 | Group A7 | Group B2 | GroupB7 | Group C2 | Group C7 |
|---|---|---|---|---|---|---|
| BP pre | 88.9±3.0 | 89.4±4.7 | 89.5±3.5 | 90.8±3.3 | 89.4±3.1 | 90.1±3.6 |
| BP final | 86.8±2.8 | 89.0±2.9 | 88.3±3.3 | 87.5±2.6 | 88.4±3.6 | 86.8±2.0 |

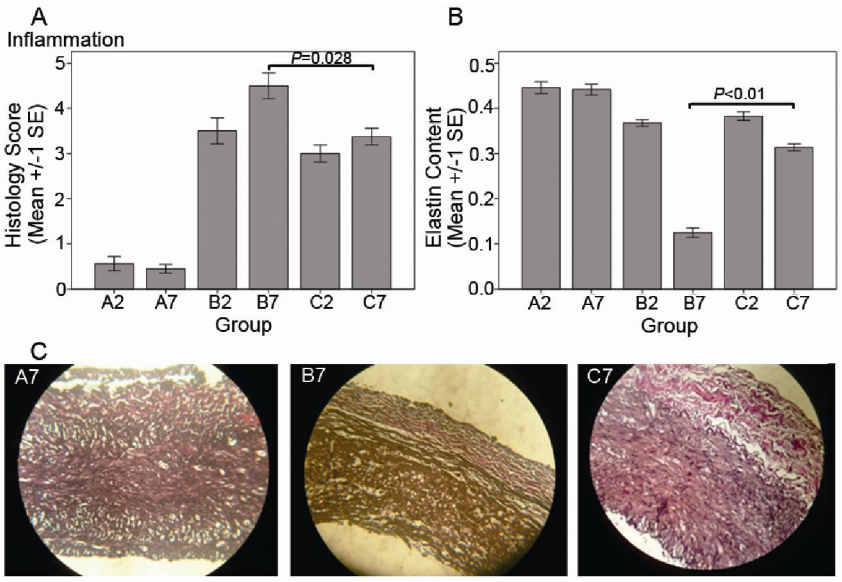


Figure 3. the degree of inflammation

A. There was no significant difference in the degree of inflammation between group B2 and group C2 (P=0.297), while there was a significant difference between group B7 and group C7 (P=0.028). B. The content of elastic fibers decreased in group C2, and there was no significant difference compared with group B2 (*P*=0.176). The content of elastic fibers in group C7 decreased further,and there was a significant difference between group B7 and group C7(*P*<0.01). C. VG staining in each group (VG*100): Group A7- The elastic fibers were continuous and intact. Group B7- Elastic fibers in the medial arterial wall were significantly injured, noncontinuous and had a large amount of vacuole formation. Group C7- Continuous elastic fibers can be seen in the medial arterial wall. The content and structural integrity were both significantly better than those of group B7.

### 3.4 Western blotting

The results of MMP-9 expression in each group are shown in Figure 4. There was no MMP-9 protein expression in either of the two saline perfusion groups. The expression of MMP-9 in elastase perfusion groups increased two days after surgery (group B2) and increased significantly seven days after surgery (group B7). This was significantly different compared with group B2 (*P*<0.01). After benazepril intervention, MMP-9 expression in each time group (group C2 and C7) showed a significant decrease (*P*<0.01).

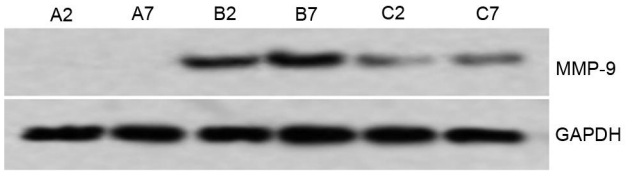


Figure 4. Expression of MMP-9 in each group

The intranuclear expression of NF-κB p65 was also detected using western blot analysis. Non-activated NF-κB p65 is bound in the cytoplasm by its inhibitor

IκBα. After phosphorylation and subsequent degradation of IκBα, NF-κB p65 is activated and then translocates to the nucleus. Therefore, the detection of intranuclear expression of NF-κB p65 is equivalent to detection of its activity. The results are shown in Figure 5. There was no intranuclear expression of NF-κB p65 in either of the two saline perfusion groups. In the elastase perfusion groups, the intranuclear expression of NF-κB p65 after perfusion was increased in both the two-day group (B2) and the seven-day group (B7); however, there was no significant difference between the two groups ($P$=0.092). In the benazepril intervention groups, the intranuclear expression of NF-κB p65 in the two-day group (C2) and the seven-day group (C7) both decreased compared with those in group B, and there were significant differences between groups B2 and C2 ($P$<0.01) and between group B7 and C7 ($P$<0.01). These results suggested that the activation of NF-κB was inhibited after intervention with benazepril.

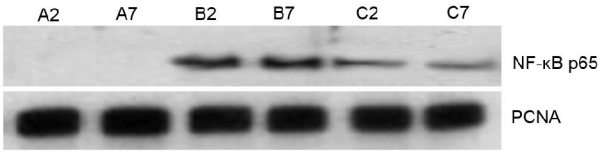


Figure 5. Intranuclear expression of NF-κB p65 in each group

**4. Discussions**

Abdominal aortic aneurysm (AAA) is a common life-threatening disease. Currently, chronic transmural inflammation and destructive remodeling of structural proteins in the medial layer of the aorta are considered to be the most significant pathophysiological changes associated with AAA. Some studies indicate that ACE-Is may inhibit the formation and development of AAA, but there have been no further studies on its mechanism. In this study, we focused on dynamic changes of MMP-9 and NF-κB as indicators, and for the first time, we found that benazepril could protect the medial aortic structural proteins and partially inhibit the infiltration of inflammatory cells by inhibiting the expression of MMP-9 and the activity of NF-κB, thereby inhibiting AAA formation.

The results of this study showed that the content of elastic fibers started to decrease two days after elastase perfusion, and a large amount of inflammatory cells infiltrated the arterial wall, accompanied by increased MMP-9 expression and NF-κB activation. However, there was no significant change in aortic diameter. This may be due to the considerable amount of elastic fibers that can resist arterial pressure. ACE-Is could partially inhibit MMP-9 expression and NF-κB activation but could not prevent the decrease of elastic fiber content and inflammatory cell infiltration completely. In the later stage of this study, in groups without benazepril intervention, the expression of MMP-9 in the abdominal aorta continued to increase, NF-κB remained in an activated state, and the number of infiltrated inflammatory cells increased further. Additionally, the degraded fragments of elastic fibers could further induce inflammatory cell infiltration and increase the release of MMP-9, thereby causing a cascade that would significantly decrease aortic elastic fibers and lead to an AAA. ACE-Is could inhibit the continued expression of MMP-9, provide more protection to elastic fibers, inhibit activation of NF-κB, reduce inflammatory cell infiltration, and block this cascade effect.

The current study shows that benazepril did not lower the rabbit blood pressure while inhibiting AAA formation, suggesting AAA suppression may not correlate with blood pressure decrease. However, no blood pressure reduction after a relatively short period of drug treatment in this study can not exclude the possibility of lowering blood pressure by long-term application of benazepril in rabbits. Besides, the dosage of benazepril required to suppress tumor, determined by preliminary experiments, may be lower than that decreasing blood pressure. Rabbits with normal blood pressure rather than those with hypertension were used in this study. Suguru[19] found that AAA diameter in rats with hypertension increased more rapidly than that in rats with normal blood pressure after perfusion with elastase. In any case, these results at least indicate that the function of benazepril in lowering blood pressure is not the main mechanism of its effect in AAA inhibition in rabbits.

The inhibitory effect of ACE-I on MMP-9 may be through the following mechanisms. (1) Inhibition of MMP-9 activity by direct binding. Angiotensin-converting enzymes (ACE) are zinc-dependent endopeptidases produced by macrophages and smooth muscle cells. Some researches found that ACE-I inhibits MMP-9 activation in rat kidney and human myocardium in in vitro experiments [20, 21]. Another study found that, because there was a zinc binding structure (histidine side chain) in the activation center of MMP-9, which was similar to ACE, ACE-I could bind MMP-9 via two pathways to inhibit MMP-9 [22]. (2) Inhibition of the expression of MMP-9 through inhibiting NF-κB. Nakashima et al. [23] found that formation of AAA and expression of MMP-9 in aortic tissues in rats could be effectively inhibited by inhibiting the activity of NF-κB using oligodeoxynucleotide. A study of Lawrence et al. [24] used the immunosuppressant rapamycin to inhibit the formation of AAA in experimental rats and found a similar effect. In this study, the results showed that inhibition of NF-κB activation was accompanied by down regulation of MMP-9 expression levels after

benazepril intervention. NF-κB is an important component of Ras and vascular inflammation. Angiotensin II activates the nuclear factors in macrophages and smooth muscle cells through one subtype of AT-1 receptor. After dephosphorylation, these activated nuclear factors translocate into the nucleus and up-regulate the expression of cytokines, adhesive molecules, and other inflammatory mediators, such as MCP-1, IL-6, and a variety of growth factors [25]. In a rabbit model of atherosclerosis, quinapril was found to reduce NF-κB-mediated expression of pro-inflammatory response factors [26]. (3) Direct inhibition of inflammatory cell infiltration to reduce the synthesis and release of MMP-9. (4) A possible direct protective effect on elastic fibers. An experimental animal model developed by Isenburg [27] was used to show that the protective agent of elastic fibers could inhibit protease-mediated elastolysis simply through binding the hydrophobic area in elastic fibers, thus inhibiting formation of AAA without downregulation of MMP-9 and inflammation inhibition. However, further research is needed to explore whether ACE-I has this effect.

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**Competing interests**

The author declare that they have no competing interests.

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5/10/2011

# The Observations of Cytokines and Coagulation for Patients after Operation of Peripherally Inserted Central Catheter

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**Abstract:** Fifty cancer, blood disease and non-cancer patients after operation of peripherally inserted central catheter were observed to analyze TNF-α, IL-6, IL-10, blood coagulation in blood which may correlated with the formation of venous thrombosis. Basically, for TNF-α and IL-6 by radioimmunoassay, IL-10 by enzyme-linked immunosorbent assay and the blood coagulation by automatic coagulation instrument were measured in plasma. The experimental results revealed that operation of peripherally inserted central catheter may cause inflammatory cytokines in plasma levels of TNF-α increased and levels of IL-6 decrease. It also anticipate the possibility of the formation of venous thrombosis.

[Zhang Zhenxiang, Liu Ying, Wang Yanli, Yang Qiaofang, Cheng Ruilian, Gao Feng, Lin Beilei. **The Observations of Cytokines and Coagulation for Patients after Operation of Peripherally Inserted Central Catheter.** Life Science Journal. 2011;8(2):613-615] (ISSN:1097-8135). http://www.lifesciencesite.com.

**Keywords:** PICC; vein; thrombosis; inflamation; factor

## Introduction

PICC (Peripherally inserted central catheter) is basically a safe, low cost and less causing side-effect skill of making a central venous pathway for patients [1]. In China, this skill was imported from USA in the year around 1990 and widely used for patients [2]. However, the side effect of causing of thrombosis by inflammatory stimulation of the venous wall, high blood coagulation for cancer patients, drug adhesion and bad sealing of the catheter is becoming serious concerned. Clinic experimental evidences have shown that the variation of the inflammatory cell factor highly correlates to the blood thrombosis [3]. We have tested seven indicators including TNF-α、IL-6、IL-10, plasma prothrombin time (PT), international standardization ratio (INR), activation part blood coagulation time (APTT) live enzyme, thrombin time (TT) and plasma fibrinogen (FIB) to recognize the difference between the patient and normal person. The experimental results revealed the high correlation for all indicators with blood thrombosis.

## Method

Eighty eight patients with and without PICC were selected as statistical samples for analysis from February to September 2009. According to the disease catalogue, it was divided into four groups. The records of 38 healthy people were used as control. Records of 20 not cancer patients were used as on experimental group I. Cancer patients with PICC were in group II. 16 Hematological patients were in group III. Meanwhile, in group I, there were three subgroups were considered, two patients for esophageal carcinoma subgroup, five for lung cancer and three for gastric cancer patients. The materials for PICC were not found any significant difference. The blood samples from the vein were taken for all patients and the agreement following the experimental standards were signed. All the inflammatory factors TNF-α、IL-6、IL-10 and four indexes of blood coagulation were tested including PT, APTT, FIB and TT.

The regular method of RIA for checking the levels of TNF、IL-6 and ELLSA for testing IL-10 were performed as well as using auto blood coagulation instruments to test the blood coagulation. The steps are all referred to the instruction guide.SPSS17 were used for statistical analysis at $\alpha = 0.05$.

## Experimental Results

The test result of PT、APTT、FIB、TT and control for PICC with non cancer patients (group I) is listed in Table 1. The *p*values are.136、0.866、0.127、0.451，respectively ($p>0.05$) (Table 1).

Other tests for PICC patients and control are listed in Table 2. Comparitively, least square anaysis is performed. For test of TNF-α, $p$ is equal to $0.000<0.01$ for group II icomparied with control, $p=0.002<0.01$ for group I compared with control，$p=0.007<0.01$ for group III compared with control. For test of IL-6, $p$

=0.000<0.01 for group II icomparied with control, *p* =0.000<0.01 for group I comparied with control，*p* =0.000<0.01 for group III compared with control. However, for test of IL-10, no significant difference was observed (Table 2).

Table 1. PICC test result-group I（±s）

| | n（samples） | PT | APTT | FIB | TT |
|---|---|---|---|---|---|
| PICC group I | 20 | 11.85±1.43 | 31.68±4.75 | 4.01±1.83 | 18.34±3.45 |
| control | 38 | 10.97±0.69 | 30.76±3.68 | 3.28±1.02 | 18.29±1.61 |

note：* *p*<0.05， ** *p*<0.01

Table 2. Test Results of TNF-α、IL-6、IL-10 and controlfor of PICC (±s）

| | n（samples） | TNF-α | IL-6 | IL-10 |
|---|---|---|---|---|
| Group I | 20 | 0.85±0.25▲ | 97.09±44.39▲ | 0.20±0.40 |
| Group II | 14 | 1.07±0.38▲ | 76.10±32.51▲ | 0.19±0.04 |
| Group III | 16 | 0.82±0.23▲ | 128.13±50.70▲ | 0.21±0.10 |
| Control | 35 | 0.61±0.25 | 207.04±124.86 | 0.32±0.64 |

note：n comparison with non-PICC patients, significant difference is demonstrated

**Discussion**

PICC to the clinical treatment of intravenous infusion bring great convenience, but also appeared to phlebitis, venous thrombosis mainly of a series of complications, which reported the incidence of venous thrombosis have very different at home and abroad, mainly in the 1.9% volatility between ~ 38.0% [6-9]. PICC operator's Dongzuoguoda injury in vein; catheter as a foreign body floating in the blood vessels within the movement; catheter tip to the vessel wall to stimulate; treatment of drug stimulation; patients after catheter tube side of the body over activities, will directly or indirectly caused by stimulation of vein wall intimal response to local inflammation, causing the release of inflammatory cytokines, resulting in inflammatory cytokines in the blood increased.

Vein thrombosis is an acute non-suppurative venous inflammation and thrombosis associated with secondary disease [10]. Cancer patients in a hypercoagulable state itself, the endogenous synthesis of tumor cells and mononuclear macrophages can lead to anti-tumor effects of tissue factor in the increase of TNF-α, activates prothrombin directly involved in venous thrombosis [5]. IL-6 is a multifunctional inflammatory cytokine, is a key component of the network of inflammatory mediators in the inflammatory response play an important role. As a long-term anti-inflammatory cytokine or cytokines may be a balance of proinflammatory cytokines or the damaging effects of early cytokines, play a protective role [11]. PICC in this study three groups of serum TNF-α compared with the control group, respectively, higher than average, and the difference was statistically significant (p <0.01); of PICC three groups of serum IL-6, respectively, compared with the control group, lower than the mean PICC group, and the difference was significant (p <0.01). TNF-α and IL-6 level changes, suggesting that catheter and vein wall inflammation have great relevance.

Studies have shown [12], hypercoagulable state in cancer patients can lead to self-vein thrombosis, blood, often accompanied by a series of patients with a coagulopathy [13], FIB prothrombotic state as molecular markers, their levels easy marks the occurrence of thrombosis or thrombosis. Disease, cancer and blood disease itself may affect the value of the significance of coagulation. The coagulation Africa mean tumor group compared with the control group, although both were no significant differences between the results (p> 0.05), but higher than the mean, indicating that PICC catheter for non-cancer patients have increased Four levels of clotting tendency, but the effect was not significant; PICC also shows that the main factors in patients with thrombosis may be caused by vein catheterization inflammatory response, rather than a direct impact on blood viscosity, but still needs further experimental studies are needed to confirm this.

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