An International Journal

Nature and Science

ISSN 1545-0740

Volume 9 - Number 1 (Cumulated No. 46), January 1, 2011

Marsland Press

PO Box 180432, Richmond Hill, New York 11418, USA

http://www.sciencepub.net
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naturesciencej@gmail.com

347-321-7172

Nature and Science

ISSN: 1545-0740

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Websites: http://www.sciencepub.net;

ISSN: 1545-0740

Nature and Science

ISSN: 1545-0740

Volume 9 - Number 1 (Cumulated No. 46), January 1, 2011

Cover Page, Introduction, Contents, Call for Papers, ns0901

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	Rachana Mishra and D. L. Verma Department of Chemistry, Kumaun University, SSJ Campus, Almora-263601 (Uttarakhand) India. Email: 09411102476m@gmail.com **Abstract:** Fern fronds (about 500gm) of *Cheilanthes dalhousiae* Hook. Vouch. Sp. No. 21 was collected from Pindari glacier routes (2200-2800m) of Almora district of Uttarakhand state. It was extracted with acetone-water (1:1, V/V) and extract was concentrated under reduced pressure until H_2O layer (up to 50ml) remained. The H_2O layer was partitioned with CH_2Cl_2, EtOAc and BuOH Successively. The CH_2Cl_2 fraction gave antibacterial tests against *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Escherichia coli* by the standard method of disc-diffusion using DMSO-d_6 solution of CH_2Cl_2 residue impregnated on Whatman No. 3, paper disc (6 nm) and base plates containing 10ml MH agar. Antibacterial activity was expressed as the ratio of the inhibition zone (nm) produced by CH_2Cl_2 extract and the inhibition zone caused by the reference, neomycin (2µg). No antibacterial activity was observed in ethyl-acetate and n-butanol fractions. EtOAc fraction was evaporated to dryness and residue obtained was dissolved in MeOH. The MeOH soluble of EtOAc fraction was fractionated on Whatman No. 3 chromatographic papers using BAW (n- BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. Two blue UV fluorescent flavone-5-O-glycosides: Quercetin-3-methyl ether-5-O-glycoside and Kaempferol-5-O-(6"-O-malonyl)-glycoside were isolated by RPPC from EtOAc fraction of acetone-H_2O (1:1) extract of fern fronds of *Cheilanthes dalhousiae.* The structural elucidation of the compounds was carried out by UV, 1HNMR and MS spectral studies. [Rachana Mishra and D. L. Verma. **Flavone-5-O-Glycosides from *Cheilanthes dalhousiae* (Hook)**. Nature and Science 2011;9(1):34-38]. (ISSN: 1545-0740). http://www.sciencepub.net. **Keywords:**Kumaun Himalaya, *Cheilanthes dalhousiae* (Hook), Medicinal plants		
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	Abstract: An experiment was conducted in 2008 in Asaba, Delta State, Nigeria to evaluate the eco-toxicological implications of crude oil pollution on *Rhizophora racemosa* seedlings. Five crude oil levels of crude oil (0.0, 12.0, 18.0, 24.0 and 30.0%) per 1.5kg of flood soils served as the treatments. The experiment was laid out in a randomised complete block design with four replications. The results showed that oil pollution at 18.0, 24.0 and 30.0% significantly affected ($P \geq 0.05$) the seedlings of the test plant in terms of plant height, number of leaves, leaf area, collar diameter and root, growth at the 5% probability level when compared with the seedlings grown in the unpolluted soils and those exposed to 12.0% of the oil. Root growth of the seedlings was significantly reduced ($P \geq 0.05$) with increasing oil levels. At 30.0% oil treatment, root hairs were totally absent. The study has established that *R. racemosa* seedlings tolerated all the crude oil concentrations used. No death was recorded throughout the trail period although significant reductions were noticed with increasing oil levels and this may have implications on the growth and establishment of the red mangrove. Conclusively, *R .racemosa* seedlings conserve as a bio-indicator of pollution and can be recommended for use in area of low levels of pollution for environmental clean-up or bioremediation. **[**Agbogidi, O.M. **Eco-Toxicological Implications Of Crude Oil Pollution On *Rhizophora Racemosas* (G.F.W. Meyer).** Nature and Science 2011;9(1):45-49]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: Toxic implications, crude oil pollution, *Rhizophora racemosa*, ecosystem | | |
| 7 | **Effect Of Particulate Materials On Lactic Fermentation Of New Local White Variety Cassava ("Bianbasse") Using Both Spontaneous And Starter Culture**

Adetunde L. A., Onilude A. A., Adetunde I. A.
*Adetunde L. A., University For Development Studies, Faculty Of Applied Sciences Applied Biology, Department Of Botany And Microbiology, Navrongo Campus. Ghana. Uer
Onilude A. A., University Of Ibadan, Department Of Botany And Microbiology, Ibadan. Oyo State
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ABSTRACT: Lactic acid bacteria isolated in the fermentation of cassava for fufu were Lactobacillus plantarium, Lactobacillus sp and Leuconostoc mesenterodes. L plantarium was identified as the most predominant lactic acid bacteria and was used as a starter culture for the fermentation of 'fufu' production. The mean value counts during spontaneous fermentation, the total dissolved loads in all the samples, the total reducing sugars of all samples, the microbial loads in all the samples, the contents of crude protein, crude father, ash, crude fidbe, phytic acid and Tannin were determined. The mean value counts during spontaneous fermentation process from zero hour to 72hours were found to increase 0.67 x 1012cfu/ml to 3.56 x 1012 in lactic acid bacteria than total bacteria with an increase from 0.69 x 1012 to 2.94 x 1012cfu/ml and yeasts which increased from0.07 x 1012 to 2.06 x 1012cfu/ml. There was corresponding increase in total dissolved solids of sample from 600mg/l to 2500mg/l, when varying the concentration of particulate materials for 72 hours and from 500mg/l to 1400mg/l when varying the concentration of Osmoregulators. The total reducing sugar for all the samples ranged from 5.8mg/l to 5.7mg/l at zero hour. At 24 hours, it ranged from 3.0mg/l to 5.4mg/l, at 48 hours it ranged from 3.5mg/l to 6.2mg/l and 72 hours, it ranged from 4.8mg/l to 6.4mg/l. Sample A inoculated with starter culture highest counts of Lactic acid bacteria ranged from 3.35 to 5.50 x 109cfu/ml while total bacterial counts ranged from 1.23 to 1.32 x 109cfu/ml. Other samples with supplemented materials had lactic acid bacterial counts ranged from 2.60cfu/ml to 3.92 x 109cfu/ml while bacterial counts ranged from 3.15 to 3.80 x 109cfu/ml. Control had LAB counts ranged from 2.52 to 3.04 x 109cfu/ml while total bacterial counts ranged from 2.48 to 2.80 x 109cfu/ml.
[Adetunde L. A., Onilude A. A., Adetunde I. A. **Effect Of Particulate Materials On Lactic Fermentation Of New Local White Variety Cassava ("Bianbasse") Using Both Spontaneous And Starter Culture**. Nature and Science 2011;9(1):50-56]. (ISSN: 1545-0740). http://www.sciencepub.net. | Full Text | 7 |

	Research Centre, Dokki- Cairo, Egypt during the winter season of 2006/2007 to evaluate the effect of available water depletion before irrigation (AWDBI) and boron foliar spray on growth and photosynthetic pigments of fodder beet plants c.v. Red Forshenger. The experiment contained 3 levels of AWDBI in combination with 2 boric acid treatments in addition to the control treatment *i.e.* 9 treatments in 6 replicates arranged in split plot design. Negative relationship was found between leaf area, and fresh and dry weights of fodder beet plants and AWDBI. The whole fresh weight/plant showed the same response while the dry weight of whole plant with the two drought treatments showed approximately the same values. Top, root and whole plant fresh or dry-weight gave their higher values when plants received 75 ppm boric acid which exceeded than those received 150 ppm boric acid or sprayed by fresh water. However, leaf area and shoot/root ratio increased as the boric acid concentration increased up to 150 ppm. Plant height and number of leaves/plant did not significantly affect by boron spraying. Top/root ratio increased with boron application under different AWDBI. The highest percentages of Chl a, Chl b, carotenoids and total chlorophyll were obtained by spraying 75 ppm boric acid compared to spraying with 150 ppm or control plants. This was true for Chl a / Chl b and total chlorophyll / carotenoids ratio. Positive relations were found among the concentration of N, K, Ca and Zn and drought treatments. Phosphorus, Mg and Na concentrations did not affect. Either Fe or Cu concentration decreased by both drought treatments, however, the concentration of Mn decreased with the 50 days period AWDBI and tended to increase to be more than the control treatment. Increasing the period of available water depletion before irrigation induced positive effect on N and Ca uptake, while, K, Mg, Na, Fe, Mn and Cu uptake showed opposite trend. In the same time the dose 75 ppm boric acid increased both concentration and uptake of macro and micro-nutrients by the plant tops; however the higher dose (150 ppm) led to a reverse effect. [Hussein, M.M.; Shaaban, M.M., El-Saady, A.M. and El-Sayed, A.A. **Growth And Photosynthetic Pigments Of Fodder Beet Plants As Affected By Water Regime And Boron Foliar Fertilization.** Nature and Science. 2011;9(1):72-79]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. **Keywords**: Fodder beet, Available water depletion, Boron, Growth, Pigments, Mineral status		
11	**Evaluation Of Garhwal Springs Water For Drinking Purpose By Using Water Quality Index** Avnish Chauhan[*], Suman Chauhan[#], Amit Pal Singh, Neha Chamoli and Krishna Kumar Pande *Dept. of Applied Sciences, College of Engineering, Teerthanker Mahaveer University, Moradabad, UP-India, 244001 [#]Research Officer, Paramhans Sanstha (N.G.O.), Dehardun, Uttarkhand, India Dept. of Biotechnology, H.N.B. Garhwal University, Srinagar, Uttarakhand, India Director, College of Engineering, Teerthanker Mahaveer University, Moradabad-UP, India. avnishchauhan_phd@aol.in, sumansingh_in@aol.in, pandekk@gmail.com, **Abstract:** A very few studies have been carried out on natural springs of Garhwal Himalayas which is the main source of potable water in Garhwal Himalayas. This paper based on water quality status of these springs, for this purpose parameters like alkalinity, acidity, DO, BOD, free CO_2, nitrate, chlorides, hardness, pH and coliform number were studied. The study elucidates that the water quality of selected natural water springs is suitable for drinking purpose. **[**Avnish Chauhan, Suman Chauhan, Amit Pal Singh, Neha Chamoli and Krishna Kumar Pande. **Evaluation Of Garhwal Springs Water For Drinking Purpose By Using Water Quality Index.** Nature and Science. 2011;9(1):80-84]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. **Keywords: -** Spring water, DO, BOD, Alkalinity, Hardness, coliform, Garhwal	Full Text	11
12	**Physiological Studies on the Effect of Inoculation with Arbuscular Mycorrhizae (AM) Fungi on**	Full	12

	Superior Grape Rootings under Salt Stress Conditions **Abd El-Wahab, M.A.*[1]; El-Helw, H. A.[1] and Tolba, H. I.[2]** [1]Viticulture Res. Dept., Hort. Res. Instit., Agric. Res. Center, Giza, Egypt [2]Microbiology Res. Dept., SWE Res. Instit., Agric. Res. Center, Giza, Egypt *mohamedabdelaziz2003@yahoo.com **Abstract:** This study was carried out to disclose the effect of soil inoculation with arbuscular mycorrhizal fungi under different water salinity levels (1000, 2000 and 3000 ppm) in an attempt to improve vegetative growth parameters, nutritional acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings through two successive seasons (2008 & 2009).The results indicated that increasing levels of water salinity, particularly in case of high salinity concentration (3000 ppm) decreased survival percentage and vegetative growth parameters (i.e. shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2), total leaf area/plant (cm^2), coefficient of wood ripening, shoot and root biomass, total biomass and root/shoot ratio). Leaf total chlorophyll, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur content and shoot total carbohydrate content decreased with increasing salinity concentration. On the contrary, leaf proline amino acid, sodium, and chloride content increased with increasing levels of salinity. Concerning the microbial and enzyme activity in the rhizosphere of Superior grape rootings, it was noticed that populations of total microbial count, spore numbers of AM fungi, the percentage of infection of AM fungi, dehydrogenase enzyme activity in the rhizosphere were also decreased with increasing levels of water salinity. Superior grape rootings strategy for salt stress tolerance could be achieved by AM fungi colonization. AM fungi inoculation benefits the plants by avoiding the undesirable effects of saline water and improving of survival percentage, vegetative growth parameters, nutrient acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings under low to medium level salt concentrations (1000-2000 ppm). However, AM fungi inoculation didn't protect the plants at the highest salt concentration (3000 ppm) used in this experiment. [Abd El-Wahab, M.A.; El-Helw, H. A. and Tolba, H. I. **Physiological Studies on the Effect of Inoculation with Arbuscular Mycorrhizae (AM) Fungi on Superior Grape Rootings under Salt Stress Conditions.** Nature and Science. 2011;9(1):85-100]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. **Keywords:** Inoculation, Arbuscular Mycorrhizae, Superior Grape	Text	
13	**Cytologenetical Study of some Wild Plants from Taif, Saudi Arabia** **Soliman, M.S.A.[1]*, El-Tarras. A.[2] and El-Awady, M. A.[2]** Biotech. & Genet. Eng. Res. Unit, Taif University, Taif, KSA **Permanent Address**: *[1]Botany & Microbiology Dept., Fac. of Science, Helwan Univ., Helwan, Egypt (*Corresponding author); [2]Genetics Dept., Fac. of Agriculture, Cairo Univ., Egypt. prof.msoliman@yahoo.com **Abstract:** Saudi Arabia is the largest country of the Arabian Peninsula which has a diverse higher plant flora in its varied landscapes with more than 2243 plant species which has a valuable economic importance due to its usage as pharmaceuticals, nutritional, fire wood suppliers as well as its use in popular remedy. Due to the scant of wild plant species studies of Arabian in literatures, the present study aim to report the chromosome numbers of 8 taxa belonging to 4 families of angiosperms collected from Taif province, Saudi Arabia flora. These taxa are: *Solanum villosum* Mill., *Datura stramonium* L., *Aerva javanica* (Burm.f.) Juss. Ex Shult, *Calotropus procera* (Aiton) W.T. Aiton, *Acacia tortilis* subspecies tortilis (Forssk.) Hayne, *Acacia oerfota* (Forssk.) Schweinf, and *Acacia gerrardii* Benth. [Soliman, M.S.A., El-Tarras. A. and El-Awady, M. A. **Cytologenetical Study of some Wild Plants from Taif, Saudi Arabia.** Nature and Science. 2011;9(1):101-104]. (ISSN: 1545-0740).	Full Text	13

Application of GA_3 and NAA as a Means for Improving Yield, Fruit Quality and Storability of Black Monukka Grape Cv.

Rizk-Alla, M.S.[1], Abd El-Wahab, M.A.[*1] and Fkry, O.M.[2]

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Abstract: This study was carried out for two successive seasons: 2009 & 2010 in a private vineyard located at El-Khatatba, Menoufiya governorate; to study the possibility of increasing yield, improving cluster quality, reducing berry shattering and enhancing storability of Black Monukka grapes through spraying with GA_3 and different doses of NAA either in the single or in the combined form. The chosen vines were ten-year-old, grown in a sandy loam soil, spaced at 2 X 3 meters apart, irrigated by the drip system, and cane-pruned and trellised by the double "Y" shape system. Eight treatments were applied as follows; spraying with tap water (control), spraying with 20 ppm GA_3, spraying with 25 ppm NAA, spraying with 50 ppm NAA, spraying with 75 ppm NAA, spraying with 20 ppm GA_3 + 25 ppm NAA, spraying with 20 ppm GA_3 + 50 ppm NAA and spraying with 20 ppm GA_3 + 75 ppm NAA. All treatments were applied after fruit set stage (at 2-3 mm berry diameter). Spraying with 20 ppm GA_3 + 75 ppm NAA gave the best results in comparison with control. This treatment resulted in the best yield and its components as well as the best physical properties of cluster and improved physical and chemical characteristics of the berries. Histological studies showed the existence of a negative correlation between pedicel diameter and shattering through the increase in thickness of the cortex and xylem layers in all treatments specially that of spraying with 20 ppm GA_3 + 75 ppm NAA. Concerning the effect of GA_3 and/or NAA on clusters during cold storage for four weeks at 0°C, RH 90-95%, it was noticed that spraying with 20 ppm GA_3 + 75 ppm NAA was the best treatment on enhancing storability, since it reduced wastage resulting either from disease infection or physiological disorders and inhibited the rate of deterioration of physical and chemical properties of grapes during cold storage by reducing weight loss (%), decay (%), shattering (%), total spoilage (%) and the decrease in firmness, it also increased berry colour, TSS and TSS/acid ratio and decreased acidity compared to control. The economical study indicated that spraying clusters with 20 ppm GA_3 + 75 ppm NAA resulted in the highest net income of Black Monukka grape as compared to the control.

[Rizk-Alla, M.S., Abd El-Wahab, M.A. and Fkry, O.M. **Application of GA_3 and NAA as a Means for Improving Yield, Fruit Quality and Storability of Black Monukka Grape Cv**. Nature and Science 2011;9(1):1-19]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Application; GA_3; Improving Yield; Fruit Quality; Storability; Black Monukka Grape

1. Introduction:

Black Monukka is one of the table grape cultivars; ripens in mid July to late August. This cultivar holds a significant promise for commercial purpose due to its seedless, sweet, crisp, purplish-black colour and skin tenderness. However, the production of small to medium berries, loose clusters and high berry shattering are negatively reflected on productivity, cluster quality and storability (Harry *et al*., 1991).

The plant growth regulators (PGR) act as messengers and are needed in small quantities at low concentrations. Generally their site of action and biosynthesis are different. Most of the plant growth regulators exhibit a broad spectrum and thus a single PGR may influence several entirely different processes (Kassem *et al*., 2010). Berry size and cluster conformation of seedless grapes are customarily improved through the application of growth regulators (Reynolds *et al*., 1992).

Gibberellic acid (GA_3) applied at fruit set is used extensively to increase berry size of Vitis vinifera seedless table grapes. Gibberellins primarily affect growth by controlling cell elongation and division, which is reflected on yield and its components and fruit quality of various grape cultivars (Omar and El-Morsy 2000 and Omar and Girgis 2005).

NAA application affects fruit formation through cell division and elongation (Dutta and Banik 2007). Also, Iqbal *et al*. (2009) reported that NAA significantly reduced fruit drop, increased yield and improved fruit quality. There are some reports indicating that the use of a combination of GA x NAA is more effective than the use of each compound alone in improving size of seedless grapes (Luckwill, 1959, El-Hammady & Abd El-Hamid, 1995 and El-Morsy, 2001).

Concerning the effect of preharvest treatments

on storability, spraying GA_3 and/or NAA reduced weight loss (%), decay (%), shattering (%), total spoilage (%) and acidity (%) while it increased berry colour, TSS and TSS/acid ratio compared to control after 45 days of cold storage at 0°C, RH 90-95% (Fatma and Aisha, 2005 on Roumy Ahmer grapes; Rizk –Alla and Meshreki, 2006 and Mohamed *et al*., 2007 on Crimson Seedless grapes) working on GA_3 spraying. Also, El-Abbasy and El-Morsy, 2002 on Thompson Seedless grapes and Tecchio, et al., 2009 on 'Niagra Rosada' grapes who worked on NAA spraying. Therefore, the main objective of this study was to raise the yield/vine and its components, to improve cluster and berry characteristics and storability of "Black Monukka" grapes through the spraying of GA_3 and different doses of NAA either in the single or in the combined form.

2. Materials and Methods:

This investigation was conducted for two successive seasons (2009 & 2010) in a private vineyard located at El-Khatatba, Menoufiya governorate; on mature Black Monukka grapevines to study the effect of spraying with GA_3 and NAA on yield, fruit quality and storability of Black Monukka grapevines. The chosen vines were ten-year-old, grown in a sandy loam soil, spaced at 2 X 2.5 meters apart, irrigated by the drip system, and cane-pruned and trellised by the double "T" shape system. The vines were pruned during the second week of January with bud load of 60 buds/vine. Ninety six uniform vines were chosen. Each four vines acted as a replicate and each three replicates were treated by one of the following treatments.

Clusters were sprayed as follows:

1. Spraying with tap water (control).
2. Spraying with 20 ppm GA_3.
3. Spraying with 25 ppm NAA.
4. Spraying with 50 ppm NAA.
5. Spraying with 75 ppm NAA.
6. Spraying with 20 ppm GA_3 + 25 ppm NAA.
7. Spraying with 20 ppm GA_3 + 50 ppm NAA.
8. Spraying with 20 ppm GA_3 + 75 ppm NAA.

The following parameters were adopted to evaluate the tested treatments:-

Representative random samples of 6 clusters/vine were harvested at maturity when TSS reached 16-17% according to Tourky *et al*., (1995).

1. Yield and physical characteristics of clusters:

Yield/vine (kg) was determined as number of clusters/vine X average cluster weight (g). Also, average cluster weight (g) and average cluster dimensions (cm) were determined.

2. Physical characteristics of berries:

These characteristics included the determination of the following:

Berry weight (g), berry size (cm^3), berry dimensions (length and diameter) (cm), berry firmness (g/cm^2) (using 1fra texture analyzer instrument), berry adherence strength (g) (using Shatilons's instrument) and berry shattering (%), this estimate was calculated by dividing weight of the shattered berries by the initial cluster weight.

3. Chemical characteristics of berries:

Determination of total soluble solids in berry juice (T.S.S.) (%) by hand refractometer and total titratable acidity as tartaric acid (%) (A.O.A.C. 1985). Hence TSS /acid ratio was calculated and total anthocyanin of the berry skin (mg/100g fresh weight) according to Husia *et al*., (1965).

4. Histological studies

Fresh samples of berry pedicels were taken at the end of the experimental period, cleaned from dust and immediately killed and fixed in FAA solution, dehydrated with tertiary butyl alcohol, Infiltrated and embedded in pure paraffin wax of 56-58°C melting point, cross section of 10-15 Microns were prepared using a rotary microtone. The prepared sections were stained with erythrosine and crystal violet (Johanson, 1940), the cross sections were mounted in Canada balsam, air dried, examined and microscopically photographed.

5. Storability

At maturity stage, when TSS reached 16-17% according to Tourky et al., (1995), clusters from treatment were harvested and picked in perforated bags, each bag contained 550 – 650 g, then packed in carton boxes and each box contained three bags.

All treatments were packed into 36 carton boxes (1.5 - 2 Kg/box), stored at ± 0^oC and 90-95% RH for four weeks.

Each treatment had three carton boxes, representing three replicates for following of the changes occurring in physical and chemical properties of the stored grapes.

5.1. Physical properties:

- § Weight loss (%) per box was determined periodically according to the equation (weight loss X 100 / the initial weight of box).
- § Decay (%) per box was calculated periodically according to the equation (weight of decayed X 100 / the initial weight of box).
- § Shattering (%) per box was calculated periodically according to the equation (weight of the shattered berries X 100 / the initial weight of box).

- § Total spoilage percentage (%) was calculated periodically as the sum of weight loss, decay and shattering per box.
- § Berry firmness (g/cm^2) was estimated on ten berries through the use of texture analyzer instrument using a penetrating Cylinder of 1mm of diameter to a constant distance 1 mm inside the berry skin by a constant speed 2mm per sec. and the peak of resistance force of the skin was recorded periodically.
- § Berry colour: Intensity of color was measured by Konick Minolta, Chroma Meter CR-400/410 for the estimation of Hue angle as described by Mcgire, (1992).

5.2. Chemical properties:

- § Percentage of total soluble solids in berry juice (TSS) was recorded periodically using a hand refractometer.
- § Total titratable acidity as tartaric acid (%) was also determined periodically (AOAC 1985).
- § TSS/acid ratio was calculated periodically.

Statistical analysis:

The complete randomized block design was adopted for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Chocran (1980). Averages were compared using the new L.S.D. values at 5% level.

3. Results and Discussion:

1. Yield and cluster physical characteristics:

Yield was significantly increased by the spraying with GA_3 and different doses of NAA either in the single or in the combined form (Table, 1). Spraying with 20 ppm GA_3 and the highest dose of NAA at 75 ppm after shattering resulted in the highest values (15.90 and 15.06 Kg/vine) for both seasons respectively, whereas, the lowest values were obtained from control vines (14.11 and 13.36 Kg/vine) for both seasons respectively.

Cluster weight was appreciably increased due to spraying with 20 ppm GA_3 + 75 ppm NAA (662.4 and 627.5 g) compared with control which had the lowest values (587.9 and 556.7 g) for both seasons respectively.

Effect of spraying with 20 ppm GA_3 and different doses of NAA on cluster dimensions was statistically insignificant.

Yield produced as a result of spraying could be mainly attributed to the positive effect of GA_3 and NAA spray on cluster weight.

The enhancing effect of spraying with GA_3 and NAA on cluster weight can be interpreted in view of that role of GA_3 in stimulating both cell division and cell enlargement which by their turn are reflected on fruit weight increase and consequently yield (Moore 1979), in addition, Wasfy, (1995) reported that GA_3 intensifies an organ ability to function as a nutrient sink; it also increases the biosynthesis of IAA in plant tissues which delays the formation of the separation layer, thus, enhancing fruit retention, consequently fruit yield. Furthermore, the increase in cell size following NAA application possibly indicates its ability to mobilize carbohydrate uptake and thus enlarge the cells considerably. Another possibility is that NAA increases the elasticity of the cell wall, thereby enabling its enlargement due to increasing the rate of fruit growth, eventually leading to an increased yield of large fruit (Arteca, 1996). Application of NAA stimulate cell enlargement in the fruit mesocarp, which in turn, causes an improvement in fruit size and total yield (Stern *et al*., 2007).

The obtained results are similar to those achieved by Omar and El - Morsy (2000), Omar and Girgis (2005) and Omran *et al*., (2005) who found that GA_3 spraying after fruit set significantly increased the vine yield and cluster weight. As for the effect of NAA, Singh *et al*., (1986) on "Khalili" cv. and El-Hammady and Abd El-Hamid (1995) on "Ruby Seedless" found that NAA spraying at 50 ppm significantly increased cluster weight and yield /vine.

2. Physical characteristics of berries:

The positive effects attributed to spraying with GA_3 and different doses of NAA either in the single or in the combined form were obvious on physical characteristics of berries i.e. berry weight, size, length, diameter, firmness, adherence strength and shattering (Table, 2). The highest values of those parameters except shattering which had the lowest percentage were detected in case of clusters sprayed with 20 ppm GA_3 + 75 ppm NAA.

The increase in fruit size may be attributed to the increase in cell division and cell elongation caused by NAA and GA_3 (Cleland, 1995 and Ranjan *et al*., 2003). In addition, Zhang and Zhang (2009) reported that GA_3 and NAA can minimize berry shattering by inhibiting the generation of ABA, inactivating the activities of cellulase and polygalacturonase and delaying the development of abscission layer.

The obtained results are in agreement with those reported by Omar and El – Morsy (2000) and Abd El-Ghany (2001) who reported that GA_3 sprayed after fruit set significantly improved physical berry characteristics. As for the effect of NAA, Singh *et al*., (1986) on "Khalili" cv. and El-Hammady and Abd El-Hamid (1995) on "Ruby Seedless" found that NAA spraying at 50 ppm significantly improved berry physical properties.

Table (1): Effect of different treatments on yield/vine and physical characteristics of clusters in 2009 and 2010 seasons

Characteristic / Treatment	Yield/vine (kg)		Cluster weight (g)		Cluster length (cm)		Cluster width (cm)	
	2009	2010	2009	2010	2009	2010	2009	2010
Control	14.11	13.36	587.9	556.7	31.7	32.1	13.6	13.9
20ppm GA_3	15.44	14.62	643.3	609.4	32.7	32.5	14.1	14.3
25ppm NAA	14.94	14.15	622.3	589.4	32.2	32.7	13.8	14.2
50ppm NAA	15.07	14.27	627.8	594.6	32.4	32.5	14.0	14.4
75ppm NAA	15.17	14.37	632.2	598.8	32.5	32.6	13.7	14.2
20ppm GA_3+ 25ppm NAA	15.54	14.72	647.6	613.5	32.6	32.8	13.8	14.5
20ppm GA_3+ 50ppm NAA	15.70	14.88	654.3	619.8	32.7	32.6	13.7	14.2
20ppm GA_3+ 75ppm NAA	15.90	15.06	662.4	627.5	32.5	32.8	13.9	14.4
new L.S.D. at 0.05 =	**0.17**	**0.15**	**7.8**	**7.5**	**N.S**	**N.S**	**N.S**	**N.S**

Table (2): Effect of different treatments on physical characteristics of berries in 2009 and 2010 seasons

Characteristic / Treatment	Berry weight (g)		Berry size (cm^3)		Berry length (cm)		Berry diameter (cm)		Berry firmness (g/cm^2)		Berry adherence strength (g)		Berry shattering (%)	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Control	2.77	2.61	2.65	2.52	2.13	2.01	1.59	1.51	34.09	32.40	186.90	173.42	5.63	6.47
20ppm GA_3	3.06	2.88	2.95	2.78	2.35	2.22	1.75	1.67	39.98	38.04	206.17	191.48	2.95	3.50
25ppm NAA	2.95	2.78	2.82	2.66	2.26	2.14	1.69	1.61	38.57	36.68	198.86	184.63	2.85	3.41
50ppm NAA	2.98	2.81	2.87	2.71	2.29	2.17	1.71	1.63	38.94	37.03	200.78	186.43	2.78	3.34
75ppm NAA	3.00	2.83	2.87	2.70	2.30	2.18	1.72	1.64	39.23	37.32	202.31	187.86	2.60	3.16
20ppm GA_3+25ppm NAA	3.08	2.91	2.96	2.79	2.36	2.24	1.77	1.68	40.27	38.32	207.67	192.88	2.52	3.09
20ppm GA_3+50ppm NAA	3.12	2.94	3.00	2.84	2.39	2.27	1.79	1.70	40.73	38.75	210.00	195.07	2.41	2.99
20ppm GA_3+75ppm NAA	3.16	2.98	3.05	2.87	2.42	2.30	1.81	1.72	41.27	39.28	212.82	197.71	2.28	2.86
new L.S.D. at 0.05 =	**0.04**	**0.03**	**0.05**	**0.03**	**0.03**	**0.02**	**0.02**	**0.01**	**0.60**	**0.50**	**2.40**	**2.10**	**0.13**	**0.11**

3. Chemical characteristics of berries:

The results presented in (Table 3) revealed that spraying with GA_3 and different doses of NAA either in the single or in the combined form delayed maturity stage represented by berry chemical characteristics; i.e. TSS, acidity, TSS/acid ratio and anthocyanin content of berry skin. Spraying with 20 ppm GA_3 + 75 ppm NAA generally resulted in the lowest values of TSS percentage, TSS/acid ratio and anthocyanin content in berry skin and the highest percentage of acidity in the juice as compared to control.

These results are in agreement with those found by Kataoka *et al*., (1984) and El-Hammady and Abd El-Hamid (1995) who found that GA_3 or NAA spraying decreased TSS percentage, TSS/acid ratio and anthocyanin content in berry skin and increased acidity percentage of the juice as compared to control.

These results are in agreement with those found by Kataoka *et al*., (1984) and El-Hammady and Abd El-Hamid (1995) who found that GA_3 or NAA spraying decreased TSS percentage, TSS/acid ratio and anthocyanin content in berry skin and increased acidity percentage of the juice as compared to control.

4. Histological studies

The positive effects ascribed to spraying with GA_3 and different doses of NAA either in the single or in the combined form were evident in the anatomical structure of berry pedicel i.e. which consists of a narrow pith surrounded by xylem layer followed by cortex layer (Table, 4) and Figure (1).

However, the degree of response varied according to treatment. The transverse sections in the pedicel revealed that the highest values of those parameters were detected in case of clusters sprayed with 20 ppm GA_3 + 75 ppm NAA followed in a descending order by clusters sprayed with 20 ppm GA_3 + 50 ppm NAA and clusters sprayed with 20 ppm GA_3 + 25 ppm NAA while, control gave the lowest values.

There were different effects of GA and NAA on the anatomical development in berry pedicel. GA stimulated xylem thickness, particularly xylem parenchyma cells as reported by Bradley and Crane (1975) in apricot spur shoots, the cell size of pith and cortex was also increased by GA and NAA. Moreover, Naosuke (1986) showed that gibberellin plays an important role in grape xylem differentiation. Also, Rizk-Alla, (2000) found that spraying with GA_3 on Thompson Seedless grape cultivar after fruit set increased pedicel thickness and berry attachment forces to the pedicel which led to less berry drop for the former category.

However, the stimulation of xylem differentiation and development may be due to the interaction between exogenous GA and NAA.

Application of NAA and GA_3 in combination during the development of the grape berry stimulated the growth in thickness of the pedicel by increasing the amount of secondary xylem and tissues outside the xylem i.e. the cortex layer.

5. Storability

5.1. Physical properties:

§ Weight loss (%)

Data in Table (5) show that weight loss (%) increased gradually till the end of the cold storage period. This increase can be probably due to moisture loss from the grapes during cold storage. It can be observed that weight loss (%) was decreased by spraying with GA_3 and different doses of NAA either in the single or in the combined form. The highest weight loss percentage (6.09 & 7.01%) was recorded after four weeks of cold storage for clusters of the control in the two seasons respectively. On the other hand, fruits resulting from spraying with 20 ppm GA_3 + 75 ppm NAA showed the lowest weight loss percentage (5.63 & 6.57%) after four weeks of cold storage in both seasons respectively.

The obtained results are similar to those achieved by Fatma and Aisha, 2005 on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly reduced the increase in weight loss (%) compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly reduced the increase in weight loss (%) in comparison with control during cold storage at 0°C, RH 90-95%.

§ Decay (%)

As shown in (Table 6), a gradual significant increase in fruit decay (%) was observed up to the end of cold storage period. Grapes of the control vines exhibited the highest significant decay percentage (0.57 and 0.62%) for the two seasons respectively. On the other hand, grapes resulting from spraying with 20 ppm GA_3 + 75 ppm NAA showed the lowest decay percentage (0.41 and 0.49%) in both seasons respectively.

These results are in line with those obtained by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found

Table (3): Effect of different treatments on chemical characteristics of berries in 2009 and 2010 seasons

Characteristic / Treatment	TSS (%)		Acidity (%)		TSS/acid ratio		Anthocyanin (mg/100g F.W.)	
	2009	2010	2009	2010	2009	2010	2009	2010
Control	16.41	16.78	0.57	0.54	28.79	31.07	43.7	41.1
20ppm GA_3	16.29	16.67	0.61	0.57	26.70	29.25	41.5	39.0
25ppm NAA	16.37	16.73	0.57	0.55	28.72	30.42	43.1	40.5
50ppm NAA	16.34	16.70	0.58	0.56	28.17	29.82	42.6	40.1
75ppm NAA	16.32	16.69	0.59	0.57	27.66	29.28	42.3	39.8
20ppm GA_3 + 25ppm NAA	16.27	16.63	0.61	0.57	26.67	29.18	41.2	38.7
20ppm GA_3 + 50ppm NAA	16.25	16.60	0.62	0.58	26.21	28.62	40.8	38.3
20ppm GA_3 + 75ppm NAA	16.24	16.58	0.63	0.58	25.78	28.59	40.6	38.1
new L.S.D. at 0.05 =	0.07	0.08	0.01	0.02	1.30	1.10	1.2	0.9

Table (4): Effect of different treatments on the anatomical structure of berry pedicel in 2009 and 2010 seasons

Characteristic / Treatment	Pedicel diameter (µ)		Cortex thickness (µ)		Xylem thickness (µ)	
	2009	2010	2009	2010	2009	2010
Control	1642	1569	804	764	731	695
20ppm GA_3	1738	1661	837	798	746	709
25ppm NAA	1753	1676	819	779	752	715
50ppm NAA	1766	1688	826	785	760	722
75ppm NAA	1797	1718	835	793	762	723
20ppm GA_3 + 25ppm NAA	1809	1729	846	804	770	732
20ppm GA_3 + 50ppm NAA	1827	1747	860	817	782	743
20ppm GA_3 + 75ppm NAA	1850	1769	873	830	794	755
new L.S.D. at 0.05 =	21	17	11	9	7	6

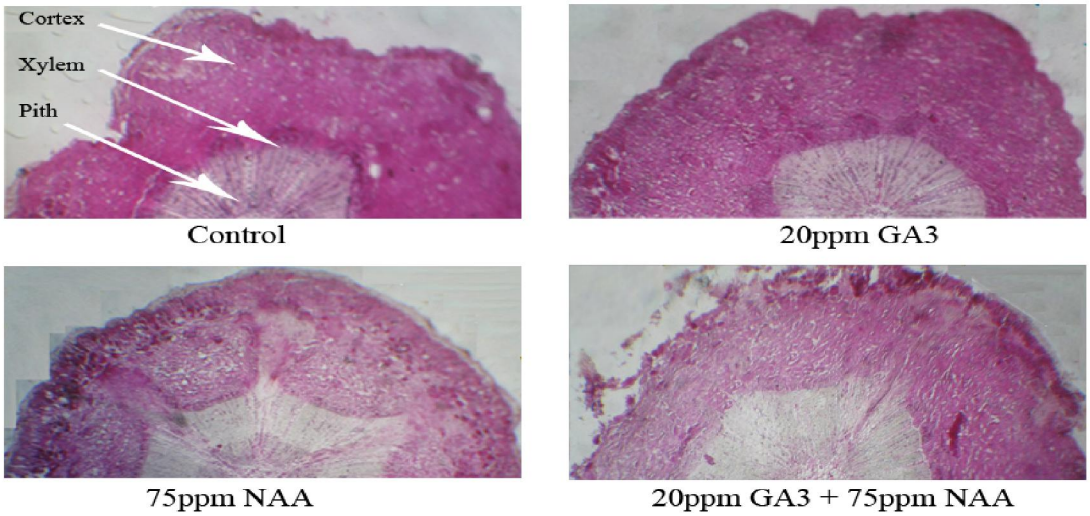

Figure (1): Transverse section (75X) of berry pedicel of Black Monukka grapes treated with GA3, NAA and GA3+NAA

Table (5): Effect of different treatments on weight loss (%) during cold storage in 2009 and 2010 seasons						
2009, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	0.00	1.03	1.41	2.34	6.09	**2.17**
20ppm GA_3	0.00	0.94	1.26	2.12	5.83	**2.03**
25ppm NAA	0.00	0.99	1.35	2.27	5.97	**2.12**
50ppm NAA	0.00	0.97	1.31	2.21	5.91	**2.08**
75ppm NAA	0.00	0.96	1.28	2.15	5.88	**2.05**
20ppm GA_3 + 25ppm NAA	0.00	0.91	1.22	2.07	5.77	**1.99**
20ppm GA_3 + 50ppm NAA	0.00	0.89	1.19	2.05	5.74	**1.97**
20ppm GA_3 + 75ppm NAA	0.00	0.86	1.16	1.99	5.63	**1.93**
MEANS (D)	**0.00**	**0.94**	**1.27**	**2.15**	**5.85**	
new L.S.D. at 0.05 (T) =	**0.04**					
new L.S.D. at 0.05 (D) =	**0.03**					
new L.S.D. at 0.05 (TXD) =	**0.09**					
2010, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	0.00	1.11	1.54	2.62	7.01	**2.46**
20ppm GA_3	0.00	0.97	1.33	2.32	6.71	**2.27**
25ppm NAA	0.00	1.06	1.45	2.51	6.89	**2.38**
50ppm NAA	0.00	1.03	1.40	2.44	6.83	**2.34**
75ppm NAA	0.00	1.01	1.37	2.38	6.78	**2.31**
20ppm GA_3 + 25ppm NAA	0.00	0.95	1.30	2.29	6.65	**2.24**
20ppm GA_3 + 50ppm NAA	0.00	0.91	1.26	2.25	6.60	**2.20**
20ppm GA_3 + 75ppm NAA	0.00	0.88	1.21	2.18	6.57	**2.17**
MEANS (D)	**0.00**	**0.99**	**1.36**	**2.37**	**6.76**	
new L.S.D. at 0.05 (T) =	**0.03**					
new L.S.D. at 0.05 (D) =	**0.02**					
new L.S.D. at 0.05 (TXD) =	**0.07**					

Table (6): Effect of different treatments on decay (%) during cold storage in 2009 and 2010 seasons

2009, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	0.00	0.08	0.13	0.22	0.57	**0.20**
20ppm GA_3	0.00	0.04	0.08	0.15	0.49	**0.15**
25ppm NAA	0.00	0.07	0.10	0.21	0.54	**0.18**
50ppm NAA	0.00	0.05	0.09	0.19	0.54	**0.17**
75ppm NAA	0.00	0.05	0.08	0.16	0.51	**0.16**
20ppm GA_3 + 25ppm NAA	0.00	0.03	0.07	0.13	0.46	**0.14**
20ppm GA_3 + 50ppm NAA	0.00	0.03	0.05	0.12	0.45	**0.13**
20ppm GA_3 + 75ppm NAA	0.00	0.01	0.04	0.09	0.41	**0.11**
MEANS (D)	**0.00**	**0.04**	**0.08**	**0.16**	**0.50**	
new L.S.D. at 0.05 (T) =	**0.05**					
new L.S.D. at 0.05 (D) =	**0.04**					
new L.S.D. at 0.05 (TXD) =	**0.11**					
2010, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	0.00	0.11	0.17	0.25	0.62	**0.23**
20ppm GA_3	0.00	0.06	0.12	0.18	0.55	**0.18**
25ppm NAA	0.00	0.09	0.15	0.22	0.60	**0.21**
50ppm NAA	0.00	0.08	0.13	0.22	0.57	**0.20**
75ppm NAA	0.00	0.06	0.13	0.19	0.57	**0.19**
20ppm GA_3 + 25ppm NAA	0.00	0.04	0.10	0.16	0.53	**0.17**
20ppm GA_3 + 50ppm NAA	0.00	0.03	0.07	0.13	0.50	**0.15**
20ppm GA_3 + 75ppm NAA	0.00	0.01	0.06	0.10	0.49	**0.13**
MEANS (D)	**0.00**	**0.06**	**0.12**	**0.18**	**0.55**	
new L.S.D. at 0.05 (T) =	**0.06**					
new L.S.D. at 0.05 (D) =	**0.05**					
new L.S.D. at 0.05 (TXD) =	**0.13**					

that GA_3 spraying after fruit set significantly reduced the increase in decay (%) compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly reduced the increase in decay (%) compared to control during cold storage at 0°C, RH 90-95%.

§ Shattering (%)

Data in Table (7) revealed that shattering (%) increased gradually till the end of cold storage. It can be observed that shattering (%) increase was decreased by GA_3 and NAA spraying either in the single or in the combined form. The highest shattering percentage (7.54 & 9.22%) was recorded after four weeks of cold storage for fruits of the control vines in the two seasons respectively. On the other hand, spraying with 20 ppm GA_3 + 75 ppm NAA showed the lowest shattering (3.74 & 4.12%) after four weeks of cold storage in both seasons respectively.

Similar results were obtained by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly reduced the increase in shattering (%) compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly reduced the increase in shattering (%) compared to control during cold storage.

§ Total spoilage (%)

Data presented in (Table 8) clearly show that the total spoilage percentage for stored Black Monukka grapes increased gradually and significantly with the extension of the cold storage in both seasons. Clusters of the control had the highest total spoilage percentage (14.20 & 16.85%) recorded at the last sampling date, i.e. after four weeks of cold storage in both seasons respectively. On the other hand, spraying with 20 ppm GA_3 + 75 ppm NAA recorded the lowest total spoilage percentage (9.78 & 11.18%) at the end of storage period in both seasons respectively.

The obtained results are in agreement with those achieved by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly reduced the increase in total spoilage (%) compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly reduced the increase in total spoilage (%) compared to control during cold storage at 0°C, RH 90-95%.

§ Berry firmness (g / cm^2)

As shown in (Table 9), it is obvious that berry firmness decreased gradually till the end of the cold storage period. Berry firmness decrease was reduced by spraying with GA_3 and different doses of NAA either in the single or in the combined form. The lowest berry firmness (19.7 & 18.7 g/cm^2) was recorded after four weeks of cold storage for fruits of the control vines in the two seasons respectively. On the other hand, spraying with 20 ppm GA_3 + 75 ppm NAA resulted in the highest berry firmness (29.1 & 27.7 g/cm^2) after four weeks of cold storage in both seasons respectively.

These results are in accordance with those obtained by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly reduced the decrease in berry firmness compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, *et al*., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly reduced the decrease in berry firmness compared to control during cold storage at 0°C, RH 90-95%.

§ Berry colour

As shown in (Table 10) it is obvious that the improvement of berry colour increased gradually from purplish-black to deep-black up to the end of the cold storage period. Berry colour was enhanced by spraying with GA_3 and different doses of NAA either in the single or in the combined form. The lowest values of hue angle (322.68 & 313.89) was recorded by control grapes at the last sampling date, i.e. after four weeks of cold storage in the two seasons respectively. On the contrary, spraying with 20 ppm GA_3 + 75 ppm NAA resulted in the highest values of hue angle (352.84 & 344.72) in both seasons respectively.

The increase in berry colour during cold storage period may be attributed to the effect of water loss and endogenous sugars which considered being causal agents for synthesis of anthocyamns and other phenol compounds (Pirie and Mullins, 1977 and Ali and El-Oraby, 2004).

Similar results were obtained by Fatma and

Table (7): Effect of different treatments on shattering (%) during cold storage in 2009 and 2010 seasons						
2009, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	5.63	4.36	4.86	6.71	7.54	**5.82**
20ppm GA_3	2.95	2.28	2.87	3.51	4.45	**3.21**
25ppm NAA	2.85	2.21	2.81	3.40	4.36	**3.13**
50ppm NAA	2.78	2.15	2.77	3.31	4.29	**3.06**
75ppm NAA	2.60	2.01	2.69	3.09	4.17	**2.91**
20ppm GA_3 + 25ppm NAA	2.52	1.95	2.57	3.01	3.98	**2.81**
20ppm GA_3 + 50ppm NAA	2.41	1.86	2.54	2.87	3.94	**2.72**
20ppm GA_3 + 75ppm NAA	2.28	1.76	2.41	2.71	3.74	**2.58**
MEANS (D)	**3.00**	**2.32**	**2.94**	**3.58**	**4.56**	
new L.S.D. at 0.05 (T) =	**0.13**					
new L.S.D. at 0.05 (D) =	**0.10**					
new L.S.D. at 0.05 (TXD) =	**0.29**					
2010, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	6.47	5.91	6.36	7.64	9.22	**7.12**
20ppm GA_3	3.50	3.19	3.52	4.13	5.10	**3.89**
25ppm NAA	3.41	3.11	3.37	4.03	4.89	**3.76**
50ppm NAA	3.34	3.05	3.31	3.94	4.80	**3.69**
75ppm NAA	3.16	2.89	3.13	3.73	4.54	**3.49**
20ppm GA_3 + 25ppm NAA	3.09	2.82	3.05	3.65	4.42	**3.41**
20ppm GA_3 + 50ppm NAA	2.99	2.73	2.96	3.53	4.29	**3.30**
20ppm GA_3 + 75ppm NAA	2.86	2.61	2.84	3.38	4.12	**3.16**
MEANS (D)	**3.60**	**3.29**	**3.57**	**4.25**	**5.17**	
new L.S.D. at 0.05 (T) =	**0.16**					
new L.S.D. at 0.05 (D) =	**0.13**					
new L.S.D. at 0.05 (TXD) =	**0.36**					

Table (8): Effect of different treatments on total spoilage (%) during cold storage in 2009 and 2010 seasons

2009, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	5.63	5.46	6.41	9.27	14.20	**8.19**
20ppm GA_3	2.95	3.26	4.21	5.78	10.77	**5.39**
25ppm NAA	2.85	3.27	4.26	5.88	10.87	**5.43**
50ppm NAA	2.78	3.17	4.17	5.71	10.74	**5.32**
75ppm NAA	2.60	3.02	4.05	5.40	10.56	**5.12**
20ppm GA_3 + 25ppm NAA	2.52	2.89	3.86	5.21	10.21	**4.94**
20ppm GA_3 + 50ppm NAA	2.41	2.78	3.78	5.04	10.13	**4.83**
20ppm GA_3 + 75ppm NAA	2.28	2.63	3.61	4.79	9.78	**4.62**
MEANS (D)	**3.00**	**3.31**	**4.29**	**5.89**	**10.91**	
new L.S.D. at 0.05 (T) =	**0.23**					
new L.S.D. at 0.05 (D) =	**0.18**					
new L.S.D. at 0.05 (TXD) =	**0.51**					
2010, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	6.47	7.13	8.07	10.51	16.85	**9.81**
20ppm GA_3	3.50	4.22	4.97	6.63	12.36	**6.34**
25ppm NAA	3.41	4.26	4.97	6.76	12.38	**6.35**
50ppm NAA	3.34	4.15	4.84	6.60	12.20	**6.23**
75ppm NAA	3.16	3.96	4.63	6.30	11.89	**5.99**
20ppm GA_3 + 25ppm NAA	3.09	3.81	4.45	6.10	11.60	**5.81**
20ppm GA_3 + 50ppm NAA	2.99	3.67	4.29	5.91	11.39	**5.65**
20ppm GA_3 + 75ppm NAA	2.86	3.50	4.11	5.66	11.18	**5.46**
MEANS (D)	**3.60**	**4.34**	**5.04**	**6.81**	**12.48**	
new L.S.D. at 0.05 (T) =	**0.19**					
new L.S.D. at 0.05 (D) =	**0.15**					
new L.S.D. at 0.05 (TXD) =	**0.42**					

Table (9): Effect of different treatments on berry firmness (g/cm^2) during cold storage in 2009 and 2010 seasons						
2009, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	34.1	31.7	27.3	24.9	19.7	**27.5**
20ppm GA_3	40.0	37.2	33.1	31.0	27.0	**33.7**
25ppm NAA	38.6	35.9	31.7	27.2	21.4	**30.9**
50ppm NAA	38.9	36.2	32.2	29.6	24.5	**32.3**
75ppm NAA	39.2	36.8	32.6	30.3	26.1	**33.0**
20ppm GA_3 + 25ppm NAA	40.3	37.9	33.7	31.4	27.7	**34.2**
20ppm GA_3 + 50ppm NAA	40.7	38.2	34.2	32.3	28.3	**34.7**
20ppm GA_3 + 75ppm NAA	41.3	38.7	34.5	32.8	29.1	**35.3**
MEANS (D)	**39.1**	**36.6**	**32.4**	**29.9**	**25.5**	
new L.S.D. at 0.05 (T) =	**0.7**					
new L.S.D. at 0.05 (D) =	**0.6**					
new L.S.D. at 0.05 (TXD) =	**1.6**					
2010, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	32.4	30.5	25.9	23.7	18.7	**26.2**
20ppm GA_3	38.0	35.4	31.5	29.5	25.7	**32.0**
25ppm NAA	36.7	34.1	30.1	25.9	23.8	**30.1**
50ppm NAA	37.0	35.2	30.6	28.2	23.3	**30.9**
75ppm NAA	37.3	35.0	31.3	28.8	24.8	**31.5**
20ppm GA_3 + 25ppm NAA	38.3	36.1	32.1	29.9	26.4	**32.5**
20ppm GA_3 + 50ppm NAA	38.8	36.0	32.9	30.7	26.9	**33.1**
20ppm GA_3 + 75ppm NAA	39.3	36.8	32.8	31.4	27.7	**33.6**
MEANS (D)	**37.2**	**34.9**	**30.9**	**28.5**	**24.7**	
new L.S.D. at 0.05 (T) =	**0.6**					
new L.S.D. at 0.05 (D) =	**0.5**					
new L.S.D. at 0.05 (TXD) =	**1.3**					

Table (10): Effect of different treatments on berry color (Hue angle) during cold storage in 2009 and 2010 seasons

2009, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	309.37	312.17	315.22	317.03	322.68	**315.29**
20ppm GA_3	317.27	323.82	328.87	332.31	337.44	**327.94**
25ppm NAA	311.76	316.23	319.56	322.22	327.32	**319.42**
50ppm NAA	313.69	319.25	323.02	326.33	331.05	**322.67**
75ppm NAA	315.99	322.07	326.29	329.68	334.80	**325.77**
20ppm GA_3 + 25ppm NAA	319.81	327.92	332.66	336.79	342.55	**331.94**
20ppm GA_3 + 50ppm NAA	322.53	331.83	337.06	341.09	347.28	**335.96**
20ppm GA_3 + 75ppm NAA	325.44	335.54	341.28	345.81	352.84	**340.18**
MEANS (D)	**316.98**	**323.60**	**327.99**	**331.41**	**336.99**	
new L.S.D. at 0.05 (T) =	**3.87**					
new L.S.D. at 0.05 (D) =	**3.06**					
new L.S.D. at 0.05 (TXD) =	**8.65**					
2010, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	302.37	304.14	305.44	307.67	313.89	**306.70**
20ppm GA_3	309.78	315.54	318.41	322.65	329.56	**319.19**
25ppm NAA	304.81	307.94	309.68	312.57	319.60	**310.92**
50ppm NAA	306.68	310.69	312.87	316.41	323.27	**313.99**
75ppm NAA	308.74	313.64	316.27	320.07	326.77	**317.10**
20ppm GA_3 + 25ppm NAA	312.64	319.36	322.51	327.24	334.59	**323.27**
20ppm GA_3 + 50ppm NAA	315.50	323.18	326.62	331.24	339.24	**327.15**
20ppm GA_3 + 75ppm NAA	318.54	326.81	330.53	335.46	344.72	**331.21**
MEANS (D)	**309.88**	**315.16**	**317.79**	**321.67**	**328.95**	
new L.S.D. at 0.05 (T) =	**3.51**					
new L.S.D. at 0.05 (D) =	**2.77**					
new L.S.D. at 0.05 (TXD) =	**7.85**					

Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly increased colour in the berry skin as compared to control during cold storage at 0°C, RH 90-95%.

5.2. Chemical properties:

§ Percentage of total soluble solids (TSS)

Data in Table (11) revealed that, there was a gradual and significant increase in the berry juice TSS (%) till the end of the cold storage period. This increase is due to the moisture loss. Spraying with 20 ppm GA_3 + 75 ppm NAA recorded the highest TSS (%) at the last sampling date, i.e. after four weeks of cold storage (18.37 & 18.77%) in both seasons respectively. while, the control grapes had the lowest percentages (17.91 & 18.37%) after four weeks of cold storage in both seasons respectively.

Similar results were obtained by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly increased in the juice TSS (%) compared to control during cold storage at 0°C& RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly increased the juice TSS (%) compared to control during cold storage at 0°C, RH 90-95%.

§ Acidity (%)

As shown in (Table 12) it is obvious that berry juice acidity decreased gradually till the end of the cold storage period. Berry juice acidity decrease was reduced by spraying with GA_3 and different doses of NAA either in the single or in the combined form. The lowest berry juice acidity (0.40 & 0.31%) was recorded after four weeks of cold storage as a result of spraying with 20 ppm GA_3 + 75 ppm NAA in the two seasons respectively. On the other hand, berries of the control showed the highest berry juice acidity (0.53 & 0.43%) after four weeks of cold storage in both seasons respectively.

The obtained results are similar to those achieved by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after fruit set significantly decreased in the juice acidity (%) compared to control during cold storage at 0°C, RH 90-95%. As for the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly decreased the juice acidity (%) compared to control during cold storage at 0°C, RH 90-95%.

§ TSS/acid ratio

Results presented in (Table 13) indicate that TSS/acid ratio increased gradually and significantly with the extension of the cold storage period in both seasons. Spraying with 20 ppm GA_3 + 75 ppm NAA recorded the highest TSS/acid ratio (45.93 & 60.55) at the last sampling date, i.e. after four weeks of cold storage in both seasons respectively. On the other hand, grapes of the control had the lowest values of this parameter (33.79 & 42.72) at the end of storage period in both seasons respectively.

These results are in line with those obtained by Fatma and Aisha, (2005) on Roumy Ahmer grapes; Rizk –Alla and Meshreki, (2006) and Mohamed *et al*., (2007) on Crimson Seedless grapes who found that GA_3 spraying after berry set significantly increased in the juice TSS/acid ratio compared to control during cold storage at 0°C, RH 90-95%. As regards the effect of NAA, El-Abbasy and El-Morsy, (2002) on Thompson Seedless grapes and Tecchio, et al., (2009) on 'Niagra Rosada' grapes found that NAA spraying significantly increased the juice TSS/acid ratio compared to control during cold storage at 0°C, RH 90-95%.

6. Economical justification of the recommended treatment (spraying with 20 ppm GA_3 + 75 ppm NAA) compared with control

It can be shown from the data presented in Table (14) that spraying with 20 ppm GA_3 + 75 ppm NAA gave the maximum net profit compared with the control in both seasons. The slight raise in the cost of production/Feddan over control for this treatment is economically justified in view of the higher price of the yield obtained from this treatment.

From the obtained results, it can be concluded that spraying grapes treated with spraying of GA_3 at 20 ppm + NAA at 75 ppm gave the highest yield, improved the physical and chemical characteristics of berries with increased storage life through reducing wastage resulting either from disease infection or physiological disorders and inhibited the rate of deterioration of physical and chemical properties of grapes during cold storage for Black Monukka grapes.

Table (11): Effect of different treatments on TSS (%) during cold storage in 2009 and 2010 seasons

2009, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	16.41	17.03	17.36	17.74	17.91	**17.29**
20ppm GA_3	16.29	17.09	17.49	17.96	18.16	**17.40**
25ppm NAA	16.37	17.05	17.40	17.81	18.02	**17.33**
50ppm NAA	16.34	17.06	17.43	17.87	18.07	**17.35**
75ppm NAA	16.32	17.08	17.47	17.92	18.11	**17.38**
20ppm GA_3 + 25ppm NAA	16.27	17.11	17.52	18.01	18.23	**17.43**
20ppm GA_3 + 50ppm NAA	16.25	17.13	17.55	18.03	18.28	**17.45**
20ppm GA_3 + 75ppm NAA	16.24	17.14	17.57	18.06	18.37	**17.48**
MEANS (D)	**16.31**	**17.09**	**17.47**	**17.93**	**18.14**	
new L.S.D. at 0.05 (T) =	**0.09**					
new L.S.D. at 0.05 (D) =	**0.07**					
new L.S.D. at 0.05 (TXD) =	**0.20**					
2010, season						
Date (D) / Treatment (T)	Days in cold storage					
	0	7	14	21	28	MEANS (T)
Control	16.78	17.45	17.82	18.23	18.37	**17.73**
20ppm GA_3	16.67	17.51	17.97	18.45	18.56	**17.83**
25ppm NAA	16.73	17.48	17.86	18.31	18.42	**17.76**
50ppm NAA	16.70	17.50	17.90	18.38	18.47	**17.79**
75ppm NAA	16.69	17.51	17.93	18.41	18.52	**17.81**
20ppm GA_3 + 25ppm NAA	16.63	17.54	17.98	18.49	18.63	**17.85**
20ppm GA_3 + 50ppm NAA	16.60	17.56	18.02	18.52	18.68	**17.88**
20ppm GA_3 + 75ppm NAA	16.58	17.57	18.05	18.57	18.77	**17.91**
MEANS (D)	**16.67**	**17.52**	**17.94**	**18.42**	**18.55**	
new L.S.D. at 0.05 (T) =	**0.11**					
new L.S.D. at 0.05 (D) =	**0.09**					
new L.S.D. at 0.05 (TXD) =	**0.25**					

Table (12): Effect of different treatments on acidity (%) during cold storage in 2009 and 2010 seasons						
2009, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	0.57	0.56	0.56	0.55	0.53	**0.55**
20ppm GA_3	0.61	0.57	0.51	0.48	0.45	**0.52**
25ppm NAA	0.57	0.55	0.52	0.50	0.49	**0.53**
50ppm NAA	0.58	0.55	0.51	0.50	0.48	**0.52**
75ppm NAA	0.59	0.56	0.51	0.49	0.46	**0.52**
20ppm GA_3 + 25ppm NAA	0.61	0.57	0.50	0.48	0.45	**0.52**
20ppm GA_3 + 50ppm NAA	0.62	0.57	0.49	0.48	0.43	**0.52**
20ppm GA_3 + 75ppm NAA	0.63	0.58	0.48	0.47	0.40	**0.51**
MEANS (D)	**0.60**	**0.56**	**0.51**	**0.49**	**0.46**	
new L.S.D. at 0.05 (T) =	**0.05**					
new L.S.D. at 0.05 (D) =	**0.04**					
new L.S.D. at 0.05 (TXD) =	**0.11**					
2010, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	0.54	0.52	0.49	0.47	0.43	**0.49**
20ppm GA_3	0.57	0.49	0.44	0.37	0.37	**0.45**
25ppm NAA	0.55	0.50	0.46	0.41	0.38	**0.46**
50ppm NAA	0.56	0.49	0.46	0.40	0.38	**0.46**
75ppm NAA	0.57	0.49	0.45	0.38	0.37	**0.45**
20ppm GA_3 + 25ppm NAA	0.57	0.51	0.44	0.37	0.36	**0.45**
20ppm GA_3 + 50ppm NAA	0.58	0.50	0.44	0.36	0.34	**0.44**
20ppm GA_3 + 75ppm NAA	0.58	0.51	0.41	0.34	0.31	**0.43**
MEANS (D)	**0.57**	**0.50**	**0.45**	**0.39**	**0.37**	
new L.S.D. at 0.05 (T) =	**0.07**					
new L.S.D. at 0.05 (D) =	**0.06**					
new L.S.D. at 0.05 (TXD) =	**0.16**					

Table (13): Effect of different treatments on TSS/acid ratio during cold storage in 2009 and 2010 seasons						
2009, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	28.79	30.41	31.00	32.25	33.79	**31.25**
20ppm GA_3	26.70	29.98	34.29	37.42	40.36	**33.75**
25ppm NAA	28.72	31.00	33.46	35.62	36.78	**33.12**
50ppm NAA	28.17	31.02	34.18	35.74	37.65	**33.35**
75ppm NAA	27.66	30.50	34.25	36.57	39.37	**33.67**
20ppm GA_3 + 25ppm NAA	26.67	30.02	35.04	37.52	40.51	**33.95**
20ppm GA_3 + 50ppm NAA	26.21	30.05	35.82	37.56	42.51	**34.43**
20ppm GA_3 + 75ppm NAA	25.78	29.55	36.60	38.43	45.93	**35.26**
MEANS (D)	**27.34**	**30.32**	**34.33**	**36.39**	**39.61**	
new L.S.D. at 0.05 (T) =	**2.17**					
new L.S.D. at 0.05 (D) =	**1.72**					
new L.S.D. at 0.05 (TXD) =	**4.85**					
2010, season						
Date (D) / **Treatment (T)**	**Days in cold storage**					
	0	**7**	**14**	**21**	**28**	**MEANS (T)**
Control	31.07	33.56	36.37	38.79	42.72	**36.50**
20ppm GA_3	29.25	35.73	40.84	49.86	50.16	**41.17**
25ppm NAA	30.42	34.96	38.83	44.66	48.47	**39.47**
50ppm NAA	29.82	35.71	38.91	45.95	48.61	**39.80**
75ppm NAA	29.28	35.73	39.84	48.45	50.05	**40.67**
20ppm GA_3 + 25ppm NAA	29.18	34.39	40.86	49.97	51.75	**41.23**
20ppm GA_3 + 50ppm NAA	28.62	35.12	40.95	51.44	54.94	**42.22**
20ppm GA_3 + 75ppm NAA	28.59	34.45	44.02	54.62	60.55	**44.45**
MEANS (D)	**29.53**	**34.96**	**40.08**	**47.97**	**50.91**	
new L.S.D. at 0.05 (T) =	**1.93**					
new L.S.D. at 0.05 (D) =	**1.53**					
new L.S.D. at 0.05 (TXD) =	**4.32**					

Table (14): Cost and net profit/Feddan for the recommended treatment (spraying with 20 ppm GA_3 + 75 ppm NAA) compared with control				
Per Feddan	2009, season		2010, season	
	20 ppm GA_3 + 75 ppm NAA	control	20 ppm GA_3 + 75 ppm NAA	control
*GA_3 (g)	4	---	4	---
*NAA (g)	15	---	15	---
*Price of GA_3 (g)	36.0	---	36.0	---
*Price of NAA (g)	45.0	---	45.0	---
Labour cost (L.E.)	100.0	---	120.0	---
Cost of cultural practices (L.E.)	2000	2000	2100	2100
Total cost (L.E.)	2181	2000	2301	2100
Increase of the total cost over control (L.E.)	181.0	---	201.0	---
Yield (Kg)	11128.3	9876.7	10542.4	9352.2
Increase of the yield over control (Kg)	1251.6	---	1190.3	---
Kg (L.E.)	2.00	1.90	2.50	2.40
Yield (L.E.)	22256.6	18765.8	26356.1	22445.2
Price of increase in yield over control (L.E.)	3490.9	---	3910.9	---
The net profit (L.E.)	20075.6	16765.8	24055.1	20345.2
The net profit (L.E.) over control (L.E.)	3309.9	---	3709.9	---

*GA_3 (g)	4g X 200 Litre		
*NAA (g)	15g X 200 Litre		
*Price of GA_3 (g)	4g X 9 L.E. = 36 L.E.		
*Price of NAA (g)	15g X 3 L.E. = 45 L.E.		

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12/2/2010

Impact of sources on waste production in activities across supply chain: A new approach

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Abstract: Productivity of construction industry is low especially in waste production. To demonstrate how it can be better than this situation, its waste sources should be identified. Whereas sources of waste are different for any material, construction activities across supply chain that use so many kinds of materials have some different sources of waste. In order to respond to the question, "which kind of sources effect on waste production in activities?" 30 questionnaires were distributed between experts. By following question about impact of five top sources on waste in activities, using binominal test, it is observed that sources of waste for any activity are the same as waste sources of materials used in that. Indeed, a category of sources which influence on waste production of some materials are effective on waste in activities that use them.
[Mohamad Reza Parsanejad, Mansor Momeni, Ali Mohaghar. Impact of sources on waste production in activities across supply chain:A new approach. Nature and Science 2011; 9(1):20-28]. (ISSN: 1545-0740).

Keywords: Waste, source of waste, waste in activities, supply chain, dimensional and weight based materials.

1) Introduction

Based on statistics and municipal reports, about 20 million tones of construction waste are produced in Tehran every year. This rate of production along with population increment has created lots of problems in capital and also in other big cities (Report of material section of construction and housing research center, 2008; Report of Tehran municipal recycle organization, 2008; Omrani et al., 2008; Report of environmental committee of consoling Tehran city, 2008).

Amount of waste in construction industry are high in another countries too. We can see these high amounts in some researches (Ekanayake and Ofori, 2004; McDonald and Smithers ,1998; Chun-Li et al., 1997; Kang ,2000; Katz and Baum ,2010; Formoso et al.,1993; Bossink and Brouwers, 1996).

Because of this negative productivity researchers develop some solutions for management and prevention of construction wastes. Among various methodologies of waste management, a categorization is more popular. It classifies waste management solutions to four categories: minimization, reuse, recycle and disposal (Gavilan and Bernaold, 1994; Begum et al., 2007; Silva and Vithana, 2008) Almost all researchers emphasize that minimization and elimination of waste is the best solution between these solutions (Gavilan and Bernold, 1994; Skoyles and Skoyles, 1987; Begum et al., 2006).

Waste minimization cannot be done unless identifying sources of waste and reducing them at its source. There are many researches about this area but in the work of parsanejad et al., (2010) it seems that materials were categorized based on their sources of waste. So impacts of sources on production of waste were illustrated. For more understanding this impact we should illustrate impact of sources on production of waste in activities. In this article we try to know are any relation between impact of sources in material waste production and impact of sources in material waste in activities? Indeed we try to demonstrate that sources of waste in any activity are those sources that are effective in waste production of materials used in that activity. If this happen the categorization of material in two type based on their sources of waste (weight based materials and dimensional materials), will consolidated.

2) Literature review

2-1) Material waste

Construction material wastes refer to materials from construction sites that are unusable for the purpose of construction and have to be discarded for whatever reason (Yahya and Boussabaine, 2006). Material waste can be seen from three views:

1. Construction waste of a specific material as percentage of total construction waste,
2. Construction waste of a specific material as percentage of its total amount,
3. Cost of construction waste of a specific material as percentage of total waste costs (Bossink and Brouwers, 1996).

There are many studies about kinds of material wastes that have so many overlaps. In these studies composition of waste in case studies has calculated (Begum et al., 2006; Yahya and Boussabaine, 2006; Guzman et al., 2009).

2-2) Waste sources

To investigating impact of sources on material waste in activities we should know what are sources of waste. There are many researches about sources of waste.

At first Gavilan and Bernold (1994) grouped the causes of direct and indirect wastes into six categories, including design, procurement, material handling, operation, residual and others such as theft (Silva and Vithana, 2008) .

Then Bossink and Brouwers (1996) worked more detail about elements of this categorization (Ekanayake and Ofori, 2004). in a recent study parsanejad et al., (2010) gathered 32 sources of waste and prioritized them. Some other studies have found sources of waste for any material in case studies (Formoso et al., 2002; Wang et al., 2008; Serpell and Alarcon, 1998).

2-3) Waste in activities

Since the flow of construction waste must be evaluated according to the type of waste and construction activity, the ideal method would be to isolate the different construction activities and monitor the waste generated in the course of each activity. This would probably yield the most accurate information on the waste associated with each activity. This concept is seen in part in the work of Snook et al. (1995).

Thus the purpose of site observation is twofold:

(1) Evaluation of the composition of the waste,

(2) Estimation of construction stage at the time of observation.

Construction works were divided into three categories according to the waste generated in each one: structural frame, early finishing and late finishing (Fig.1).In general, the structural frame works produce the least waste for all types of construction materials whether it is made of steel, concrete or wood. Construction materials are supplied to the construction site in accurate amounts with little wastage, small amounts of packing materials are used, and most of the waste is recyclable. The early finishing works (e.g. partition walls, plastering, drywalls, floor tiles, and piping) produce larger quantities of mixed waste that requires more extensive separation treatment before recycling. Waste from the late finishing works are the most difficult to treat and are produced in the largest quantities. Waste from this stage is expected to consist of a mixture of all materials found on the construction site, including significant amounts of packing materials. Foundation and underground activities vary from site to site and were not included.

When monitoring the waste accumulated on a construction site, it is reasonable to assume that waste accumulated during the early stages of the work is related to the structural frame, whereas waste accumulated during the final stages of construction is related to the late finishing works only (Fig. 1). On large construction sites, the time overlap between the stages and activities is greater and ‘‘pure” structural frame works or late finishing works can be found only at the very beginning or very end of the project. In such sites, waste produced during the majority of the project duration is a mixture of waste from all three stages (Katz and Baum, 2010).

A case study about waste minimization in British building sector shows also different wastes in different stages of construction. Observations indicate many waste overlaps and amount of waste in any stage of project life cycle. This study also illustrates many wastes happen in structure stage and fitting that can be seen in the Fig 2 (Jones and Greenwood, 2003).

The waste from construction site activities will vary from one site to another depending on the type of project and its design. It is proven that, project and material specifications contribute to a large extent to waste generation. For example, building construction involves several activities that can be broadly grouped as land clearing, road and sewers, substructure work (excavation and foundation work), superstructure (framing), internal carcassing and service installation (wiring, plumbing, insulation, drywall), finishing work (paint, exterior finishing and roofing), energizing phase prior to handling, landscaping and completion of external works. Each of these activities has a high potential to generate waste from materials such as soil, contaminated soil, wood, metal, concrete, plastics, waste solvents, gypsum, wallboard, cardboard, boxes, paint solvents, bricks, masonry, vinyl, stucco, asphalt shingles and tiles, as shown in Fig 3.

In this study, wastes have been gathered in any activity as below:

Site preparation: soil, wood, vegetation; Excavation: soil, contaminated soil; Foundation work: wood, metal, concrete; Farming: wood; Metal work and wiring: metal; Plumping: metal, plastic, waste solvents; Insulation: metal, plastic, rubber; Drywall: gypsum wallboard, cardboard, boxes; Painting: paint, solvents; Exterior finishing: wood, brick, masonry, vinyl, mortar; Roofing finishing: asphalt, cedar shakes, tiles (Yahya and Boussabaine, 2006).

A debatable point in diversity of material waste is that composition of waste is related to the construction technologies. For example in prefabricated concrete elements, the amount of cement waste is very low (Jaillon et al., 2009).

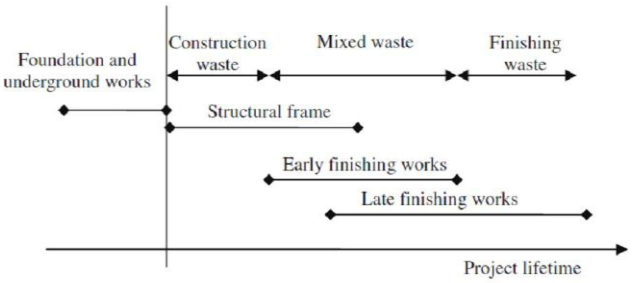

Fig 1: Type of construction works and waste generated during them

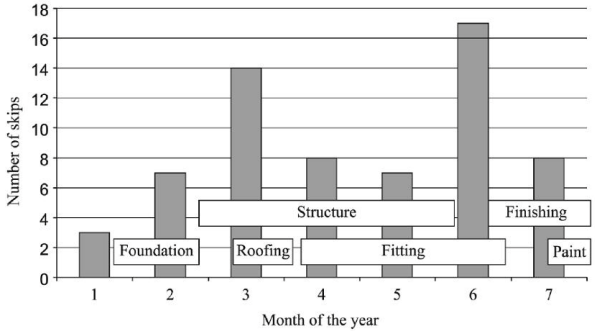

Fig 2: Number of skips in project life cycle

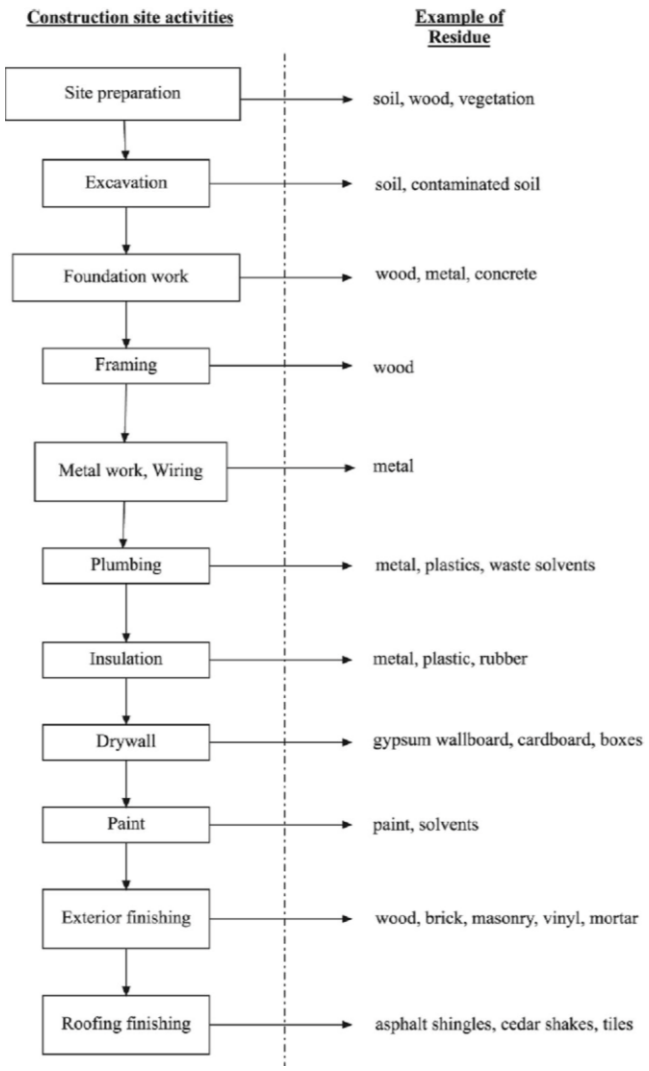

Fig 3: Construction works and material waste generated in that

The impact of new technologies on waste has been investigated in a study in 2007. In this study the amount of waste in buildings with prefabricated and traditional technologies calculated for seven activities. Although wastage levels may vary from different types or natures of project, the wastage levels are believed to be affected by the adoption of conventional in situ and prefabrication construction methods.

A structured survey was conducted to measure the wastage level for the different construction methods. The average wastage level (in per cent) for various construction trades, namely, concreting, rebar fixing, bricklaying, drywall, plastering, screeding and tiling, are measured for the two groups of projects adopting conventional in situ trades and prefabrication denoted as 'A' and 'B'. After measuring the values of (A) and (B), the percentage in waste reduction, (C), is calculated by obtaining the difference between the average wastage level in conventional and prefabrication construction methods (A) and (B) by the ratio of the waste reduction over the average wastage level for the conventional construction method. According to the findings on the average wastage levels for the major construction activities carried out on site, it is noted that the most effective waste reduction trade is plastering, which can have 100% of wastage reduction after adopting prefabrication. It can be explained that plastering can be avoided since the concrete surface of the precast items is smooth and even enough for receiving tile or subsequent finishes. The contractors argued that tiling was directly applied to the concrete surface after formwork striking, while for painting, only a layer of 1–2mm thick skim coat is required instead of 15–20mm plastering. The average wastage level of the conventional construction method is much higher than that of prefabrication in the trades of concreting, rebar fixing, plastering and tiling. This result shows that the wastage levels vary with different trades when prefabricated building components are adopted; therefore, the standardized designs of building can reduce the wastage levels effectively (Tam et al., 2007).

Another classification about wastes in activities is structure waste and finishing waste. Concrete fragments, steel reinforcement, abandoned timber plates and pieces are generated as structure waste during the course of construction. Finishing waste, including a wide range of waste materials, is generated in the finishing stage of the building.

For instances, surplus cement mortar arising from screeding scatters over the floors inside the building. Broken raw materials like mosaic, tiles, ceramics, paints and plastering materials are wasted because of careless use.

Household facilities such as damaged bathtubs, washtubs and window frames are also parts of the finishing wastes (Poon et al., 2001).

3) Methodology

Problem of this research is that are any relation between impact of sources in material waste production, and impact of sources in material waste in activities? So for understanding the problem, we should try to demonstrate that sources of waste in any activity that we use some material across it, are those sources that are effective in waste production of materials used in that activity.

Thus we should calculate impact of sources on waste production in activities. Sources of waste that we use are five sources in the study of parsanejad et al., (2010). These sources are the most important sources between 32 sources which prioritize by questionnaire in that study. The results of that research show that the five sources have the highest rank as below:

1) Traditional construction methods,
2) Lack of design commensurate with materials exist in market,
3) Lack of coordination between supply chain,
4) Lack of proportionate material ordering of purchasing section,
5) Lack of production of materials with variant dimensions.

In another hand there are some categorizations of construction activities. But because of acquaintance of Iranian specialists with categorization of Report of adjutancy of planning and inspectorate of president (2008) we use its work breakdown structure (WBS) for this research. Based on this report building activities were categorized in 4 categories and 17 subcategories as below:

- Foundation: leveling concrete, reinforcing, farming, pouring concrete.
- Structure: structure installation
- Hard working: external wall, internal walls.
- Finishings: mechanical installations, electrical installations, door and window framework, indoor work, insulations, tiling, Staircase, installations, Frontage works, Paining.

Then impact of selected sources on waste in these activities can be surveyed. This impact can be calculated by many methods. In this research binominal test are used. Questionnaire also had five options and the question was amount of impact of source on material waste in activities. Options "very low" and "low" impact had been located in a group, and "mediocre", "high" and "very high" impact in another group. Hypothesis had been designed as below:

$$\begin{cases} H0: p \le 0.60 \\ H1: p > 0.60 \end{cases}$$

H0 shows high level of impact and H1 shows that there is no meaningful impact. The calculations had been done by SPSS 15, and with amount of significant validity of questionnaire had been tested.

All questionnaires had sent to 30 specialists and with analysis of impact of source on material waste in activities, effective waste sources for any activities were obtained. Table 1 showes significants and acceptance of assumptions.

4) Results and discussion

Here impact of five below sources on material waste and material waste in activities were analyzed.

Source number1 (S1): lack of design commensurate with materials exist in market,
Source number2 (S2): traditional construction methods
Source number3 (S3): lack of coordination between supply chain
Source number4 (S4): lack of proportionate material ordering of purchasing section
Source number5 (S5): lack of production of material with variant dimensions.

With precision in results from questionnaire about impact of five waste sources in waste produced in activities, some new results were obtained that support analyses of waste in materials.

- First result: it was observed in those activities that materials using along them are type1, effective sources are those sources that are effective in waste production of material type1, and it happen for material type2 too.
- Second result: in those activities that materials using along them are variant and composition of both type1 and 2, all significants are higher than 0.05 except S1 and S3.

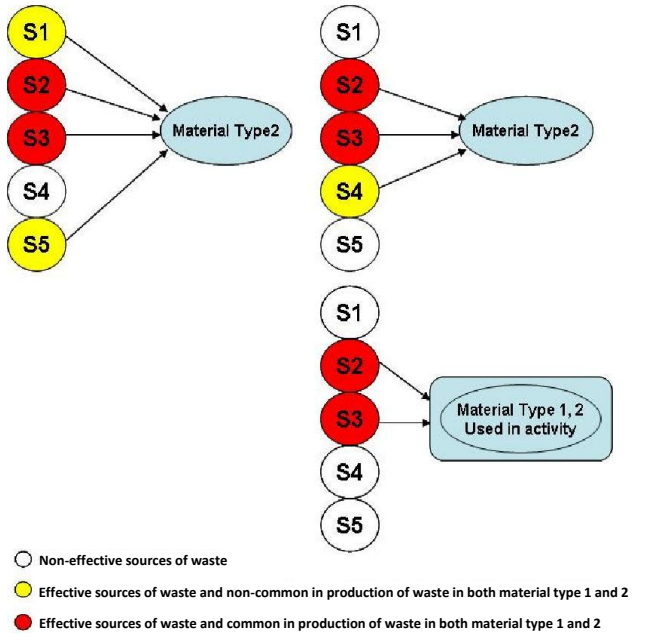

Fig 4: Effectiveness and non-effectiveness of sources on materials waste production in activities.

- Third result: in mechanical installation activity that materials are pipes, waste sources are those that are effective in waste of pipe.
- Forth result: in some activities all five sources are ineffective.
- Fifth result: in framing activity just S2 was effective.

4-1) Result 1:

These results support categorization of material in two types. It shows that sources of waste will related to the type of material. In this section activities like: leveling concrete, pouring concrete, isolation, and painting are among category 1 and reinforcing and door and window framework installations are in second category.

4-2) Result 2:

This section includes activities such as structure installation, external walls, internal walls, indoor work, tiling, Staircase and Frontage works. An introduction is necessary here. Because of these seven activities use both materials type 1 and 2, respondents have not achieved to a consensus unless in S2 and S3. The reason is that these two reasons are common in effective sources of both material type 1and 2.Thus activities in those both two type materials were used S1 and S2 ,are effective and other sources cannot take consensus of respondent because they are not between this sharing. This concept can be seen in Fig 4.

Now with above introduction it can be analyzed result of these seven activities that both two types of materials are used in them.

Structure installation can be from steel or concrete. Concrete structures itself are a composition of materials like reinforcement, cement, sand and water that are both type 1 and 2. Thus respondents that were steel structure in their minds selected some sources and others that concrete structure were in their minds select some others. And thus there is no consensus about sources except in S2 and S3.

In external walls there are so many materials type 1 and 2 such as brick, adobe and block, with composition of cement, sand and water.

Table 1: Questionnaires results about Impact of selected sources on waste production in activities
When Sig >0.05

sources	leveling concrete		Reinforcing		Framing for concreting		pouring concrete		Structure installation		External walls		Internal walls		Mechanical installations		Electrical installations		Door and window framework installations		Indoor works		Insulations		Tiling		Staircase		Installations		Frontage works		Paining	
S1	.002		.000	√	.006		.000		.097		.285		.175		.001	√	.000	–	.003	√	.097		.044		.175		.175		.000	–	.291		.002	
S2	.001	√	.003	√	.000	√	.001	√	.008	√	.001	√	.003	√	.044		.000	–	.021	√	.003	√	.048	√	.008	√	.001	√	.000	–	.003	√	.008	√
S3	.003	√	.008	√	.002		.008	√	.021	√	.003	√	.048	√	.003	√	.000	–	.008	√	.008	√	.008	√	.003	√	.003	√	.002	–	.001	√	.001	√
S4	.008	√	.002		.002		.003	√	.422		.176		.291		.008	√	.002	–	.044		.578		.001	√	.422		.431		.006	–	.097		.003	√
S5	.006		.001	√	.017		.002		.175		.094		.176		.021	√	.000	–	.001	√	.176		.006		.094		.422		.000	–	.094		.044	
Effective sources	*2,3,4*		*1,2,3,5*		*2*		*2,3,4*		*2,3*		*2,3*		*2,3*		*1,3,4,5*		–		*1,2,3,5*		*2,3*		*2,3,4*		*2,3*		*2,3*		–		*2,3*		*2,3,4*	
Non Effective sources	–		–		–		–		*1,4,5*		*1,4,5*		*1,4,5*		–		–		–		*1,4,5*		–		*1,4,5*		*1,4,5*		–		*1,4,5*		–	
Material type	*Type1*		*Type2*		*Non consuming*		*Type1*		*Type1,2*		*Type1 ,2*		*Type1 ,2*		*Type1 ,2*		*low waste*		*Type2*		*Type1 ,2*		*Type1*		*Type1 ,2*		*Type1 ,2*		*low waste*		*Type1 ,2*		*Type1*	

In internal walls those materials using in external walls are used too.

In indoor workings there are two methods, traditional and industrialized. First method is producing Mortar with mixing materials such as gypsum, soil and water in construction site and doing indoor work in traditional method by traditional workers.

Second method is producing gypsum boards in great dimensions in factory and installation of them on walls. It is obvious that first way is traditional with material type1, and second way is industrial with material type2.

In tiling activities tiles and ceramics (material type2) should be conjunct to surfaces with a mortar that is composite of cement, sand and water. Thus both type 1 and 2 materials are used.

In Staircase there are a collection if materials such as ceramic, stone, gypsum, gypsum board, cement, sand, water and so on. Some of them are type1 and others are type2.

In Frontage works there are same events that happen in indoor workings. We mention three methods here:

1. Cementing with materials such as cement, gypsum, soil and water in traditional way.
2. Conjunction of bricks or stones with using material such as cement, sand and water in traditional way.
3. Installing cement boards instead of cementing in industrial way.

In another word about impact of S2 and S3, there are proper consensuses between respondents. Consensus about S2 is because of changing production and construction paradigm. Consensus about S3 is because of generality of it over S1, S4 and S5. However there is no Consensus about these three sources but there are about S3.

4-3) Result 3:

In mechanical installations both material type1 and 2 are used (branch and loop pipes). But, there are no great variant and complexity in comparison with activities in result 2.

Therefore, respondent can reach to consensus in effectiveness of S1, S2 and S4. Because of clear condition and no great variation in using material there are no significant higher than 0.05.

4-4) Result 4:

In some activities such as electrical installation and installations ,all five sources are not effective. The reason is that these activities have not significant waste.

4-5) Result 5:

In framing in foundation just S2 is the source of waste. In traditional methods of framing, bricks were used Today's this method has no tendency between contractors. Some new industrial methods like big steel frames are used in many cases. This material is not consuming and does not have any waste. Thus if contractor apply traditional methods, it will be the single source of waste.

Results 1 to 5 are shown in Table 2 to 6.

5) Conclusions

We know materials inherent properties are methods of usage, important parameters when use, how to supply and how to maintain, measurement units. These properties impact on process of row material conversion to final product and therefore impact on methods that wastes produced in any material in activities.

And also, in weight based materials, that their weight is important in their usage, some sources are effective in their waste that relate to amount of purchasing.

Dimensional materials are those materials which their dimensions in their usage are important and so, some sources are effective in their waste that relate to building design.

Questionnaires Results intensively support the Results of categorization of materials to two categories. Everywhere material used in activities are type1, their waste sources are waste sources of material type1. This happen for material type 2 too. In those activities that materials used in them are composition of type 1 and type2 materials, respondents have Consensus about effectiveness of traditional construction methods and lack of coordination between supply chain, and in another sources there are no consensus.

Another conclusion of these five results is that the categorization of construction materials by their source of waste to two categories (weight based materials and dimensional materials) is true, because it describes waste production very well.

Moreover, this categorization is an appropriate way to recognition of waste production process in construction industry and it helps us to act with any kind of material based on their type and their inherent properties to minimize their waste and then increase the total performance of construction supply chain.

Acknowledgements

We like to thank Mapsa Company and all practitioners that helped us in the research process.

We also gratefully thank the comments from Dr.Ahmad Jafarnejad, Ahmad Ganaatian and Mohamad Mehdi Tohidi for their reformative comments.

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Table 2: Types of construction works that use materials type 1 and 2, and their wastes

No	Activity	Effective sources	Materials used in activity	Types of material used in Activity
1	Structure installation	2,3	Steel, Reinforcement steel/ Cement, sand and water	Type 1,2
2	External walls	2,3	Brick, adobe , block/ Cement, sand and water	Type 1,2
3	Internal walls	2,3	Brick, adobe , block/ Cement, sand and water	Type 1,2
4	Indoor finishing	2,3	Gypsum board/ Gypsum, paint, water	Type 1,2
5	Tiling	2,3	Tile, ceramic/ Cement, sand and water	Type 1,2
6	Staircase	2,3	Stone, ceramic/ Cement, sand and water	Type 1,2
7	Frontage works	2,3	Cement, sand, cement board, stone, brick, sand and water	Type 1,2

Table 3: Types of construction works that use materials type 1 or 2, and their wastes

No	Activity	Effective sources	Materials used in activity	Types of material used in activity
1	leveling concrete	2,3,4	Cement, sand and water	Type1
2	Pouring concrete	2,3,4	Cement, sand and water	Type1
3	Insulations	2,3,4	Liquid and solid insulation	Type1
4	Paining	2,3,4	Paint , toner , water	Type1
5	Reinforcing	1,2,3,5	Reinforcement steel	Type2
6	Door and window framework installations	1,2,3,5	Steel and wood	Type2

Table 4: mechanical installations that use both materials type 1 and 2, and its wastes

No	Activity	Effective sources	Materials used in activity	Types of material used in activity
1	Mechanical installations	1,3,4,5	Looped and branch pipes	Type 1,2

Table 5: Types of construction works that have low wastes generation

No	Activity	Effective sources	Materials used in activity	Types of material used in activity
1	Electrical installations	-	Wire, lighting fixtures	Low waste
2	Installations	-	Cooling and heating installation, bolt and nut, cabinet, UPVC, faucet, plumbing fixture, and other fixtures	Low waste

Table 6: Type of construction works that is not consumable

No	Activity	Effective sources	Materials used in activity	Types of material used in activity
1	Framing	2	Steel and wooden frames	Non-consumable

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12/5/2010

Pasting Properties of Heat-Moisture Treated Starches of White and Yellow Yam (*Dioscorae species*) Cultivars

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Abstract: Starches of white and yellow yam cultivars (Dioscorae species) were extracted, physically modified by means of heat-moisture treatment (HMT) and evaluated for pasting properties, such as gelatinization temperature, paste viscosity, retrogradation and stability by using Rapid Visco-Analyzer (RVA). The modified white yam starch samples exhibited lower values than the native starch sample in terms of viscosity and stability while an opposite trend was obtained in terms of pasting time and gelatinization. Heat-Moisture treated white yam starch at 18% moisture content exhibited the least tendency to retrograde among the starch samples. As heat-moisture treatment increased, there was a noticeable progressive decrease in the values of pasting viscosity and stability for yellow yam starch coupled with a sequential increase in terms of retrogradation, pasting time and gelatinization temperature. An inverse proportionality between the values of retrogradation and paste stability of the starch samples was observed. However, the native yellow yam starch possessed relatively higher paste stability (312 RVU) than the corresponding native white yellow yam (308 RVU). The heat-moisture treated samples seemed to be more applicable in pastries than the native starch samples.

[Oladebeye Abraham Olasupo, Oshodi Aladesanmi Augustine, Oladebeye Aderonke Adenike, Amoo Isiaka Adekunle **Pasting Properties of Heat-Moisture Treated Starches of White and Yellow Yam (*Dioscorae species*) Cultivars**. Nature and Science 2011;9(1):29-33]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: Starch, heat-moisture treatment, pasting properties

1. Introduction

Starch, the food reserve homopolysaccharide of plants (Malcolm, 1990), is a biocompatible, biodegradable, nontoxic polymer (Welsen and Welsen, 2002), which occurs widely in nature and most commonly used with a host of advantages: low density, cost effectiveness, abundant supply and environmental amity. It is widely used in food, paper-making, fine chemicals, packing materials, pharmaceuticals, rubber and plastic industries (Huaiguo et. al., 2006). This is partly because of the wide range of functional properties it can provide in its various natural and modified forms, and partly because of its low cost relative to alternatives (Sanderson, 1981).

A large amount of the commercially available starch is not used in its native form, but rather is chemically or physically modified to improve its functionality for use in modern product formulations. Chemical modification begins by separating the starch molecules from each other by heating, shearing, or mixing it with certain chemical reagents in the presence of water.

Pregelatinization, annealing and heat-moisture treatment are physical methods of modifying starch properties. While pregelatization causes granules disruption, annealing and heat-moisture treatments (HMT) acquire modified properties without rupturing the granules (Glicksman, 1969; Doublier et. al., 1986; Bhattacharya et. al., 1999; Adebowale et. al., 2005). These physical treatments can change certain starch properties using simple and environmentally safe processes. The physical properties of a heat-moisture treated starch depend on the starch origin and treatment conditions used (Adebowale et. al., 2005).

This research work is aimed at studying the effect of heat-moisture treatment on the pasting properties of native starches from selected yam tubers with the view to expanding their industrial applications for both food and non-food products.

2. Materials and Methods

2.1 Sample Preparation

Tubers of white and yellow yam cultivars were purchased at Uchi Market, Auchi. All the chemicals used were analar grade. Starches were extracted from the tubers as described in an earlier research work (Oladebeye et. al., 2009).

Heat-moisture treated starch samples were prepared from the raw starch samples using the standard chemical method described by Franco et. al. (1995). The moisture levels of the starch samples were increased to 18, 21, 24, and 27% by adding the appropriate amount of distilled water. The mixtures were stirred; the sealed samples in glass jars were heated in an air oven at 100°C for 16 h. After cooling, the jars were opened and the starch samples were air dried to a moisture content of 10%.

2.2 Methodology

Rapid Visco-Analyzer (Model 3-D, Newport Scientific) with Thermocline for windows software was used to evaluate the pasting properties such as gelatinization temperature, paste viscosity, paste stability and retrogradation of the starch samples as described by Deffanbaugh and Walker (1989). Test runs were conducted following standard profile 1 which include 1 min of mixing, stirring, and warming up to 50^0C, 3 min and 42 sec of heating at 12^0C/min up to 95^0C, 2.5 min of holding at 95^0C, 3 min and 48 sec of cooling down to 50^0C, at the same rate as the heating (12^0C/min) and 2 min holding at 50^0C, where the process ends after 13 minutes. Starch gelatinization (pasting) curves were recorded on RVA and viscosity was expressed in terms of Rapid Visco Units (RVU) which is equivalent to 10 centipoises.

3. Results and Discussion

Table 1 shows the pasting properties of native and heat-moisture treated starches of white yam (*Dioscorae spp*). Comparing the results obtained, it is observed that heat-moisture treatment could alter the crystalline structure of starch and affect its pasting properties. The heat-moisture treated white yam starches exhibit lower values of viscosity and stability than the native starch of the same origin whereas an opposite trend is observed in terms of their pasting time, gelatinization temperature and retrogradation. These changes in pasting characteristics are probably as a result of interï and intraï molecular hydrogen bonds of amylose chain and amylase-lipid complexes, which occurred during heat-moisture treated. Since the structures of treated starch granules become stronger, they, therefore, become extremely heat- and shear- resistant during pasting time (Hoover and Manuel, 1996). However, with decrease in gelatinization temperature from 75.93 to 71.15^0C, heat-moisture treated white yam starches possess increase in viscosity from 368.08 to 411.92 RVU, accompanied with a drop in viscosity after retrogradation (Figures 1 ï 3). The stability of the starch samples appears to increase as percentage of heat-moisture treatment increases. This may be due to leaching of amylase, resulting from the swelling of the granules.

The pasting time, gelatinization temperature, final viscosity and retrogradation of heat-moisture treated yellow starches exhibit higher values than the corresponding native starch while an opposite trend is obtained in terms of their paste viscosity and stability (Figures 4 ï 6). Unlike in the modified white yam starches, there is lowering of values in terms of paste viscosity and stability as the percentage of modification increases. This may suggest that after heat-moisture treatment, the granules become less shear resistant, indicating high level of amylose units, which oppose the swelling of granules. It is generally observed that the lower the paste stability, the higher the retrogradation for each of the starch samples. This is in line with the earlier report by Oladebeye et. al. (2009) for the pasting properties of red cocoyam and sweet potato.

Table 1: Pasting Properties of White Yam Starch Samples

Sample	Pasting Time (mins)	Gelatinization Temperature (0^oC)	Viscosity (RVU)	Final Viscosity (RVU)	Stability (RVU)	Retrogradation (RVU)
WYS	3.53	68.65	464.50	224.92	308.33	68.75
WYS_{18}	3.93	75.93	368.08	249.25	186.58	67.75
WYS_{24}	3.67	71.15	411.92	215.00	273.92	77.00

WYS = Native starch of white yam
WYS_{18} = Modified starch of white yam at 18% moisture content
WYS_{24} = Modified starch of white yam at 24% moisture content

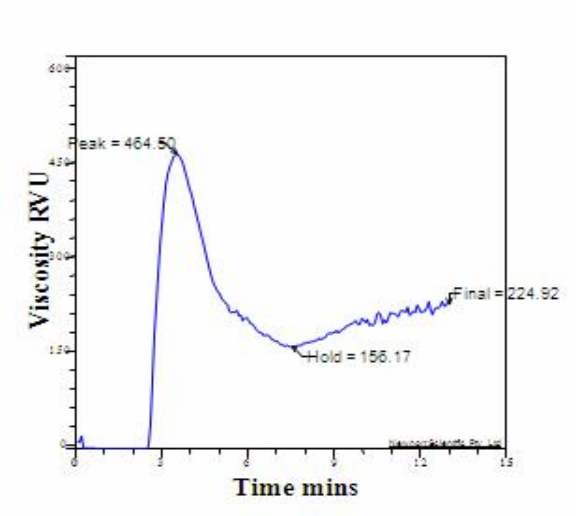

Figure 1: RVA Curve for WYS

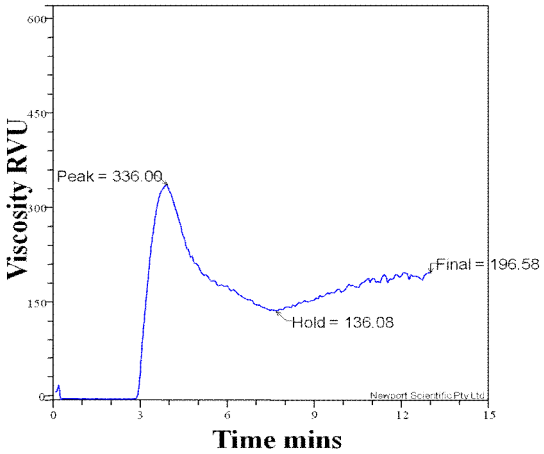

Figure 2: RVA Curve for WYS_{18}

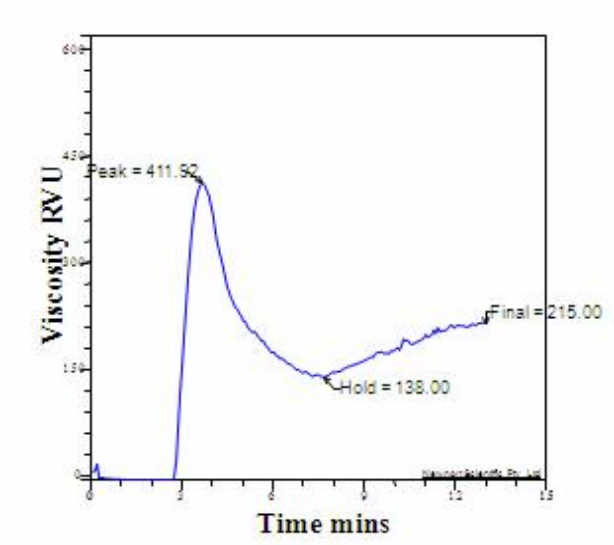

Figure 3: RVA Curve for WYS_{24}

Table 2: Pasting Properties of Yellow Yam Starch Samples

Sample	Pasting Time (mins)	Gelatinization Temperature (0^oC)	Viscosity (RVU)	Final Viscosity (RVU)	Stability (RVU)	Retrogradation (RVU)
YYS	3.67	70.30	496.58	238.75	312.00	54.17
YYS_{18}	4.47	72.55	316.92	253.67	129.67	66.42
YYS_{24}	5.53	75.55	249.50	283.08	41.00	74.53

YYS = Native starch of yellow yam
YYS_{18} = Modified starch of yellow yam at 18% moisture content
YYS_{24} = Modified starch of yellow yam at 24% moisture content

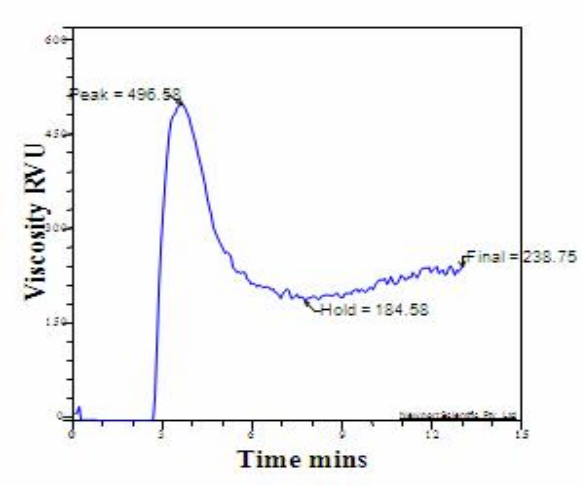

Figure 4.RVA Curve for YYS

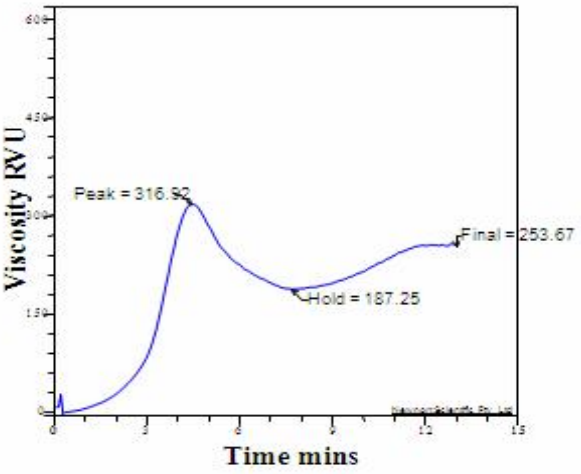

Figure 5. RVA Curve for YYS_{18}

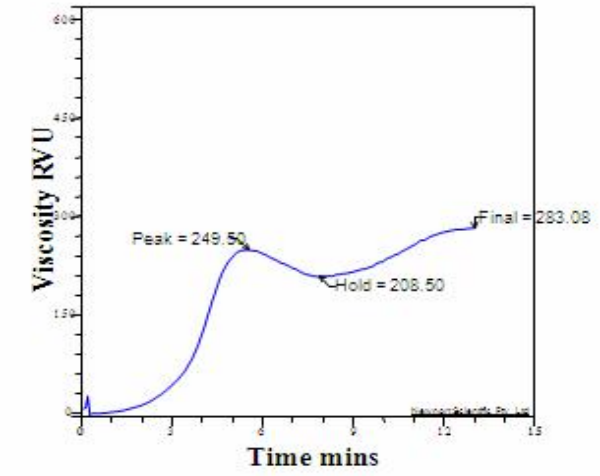

Figure 6. RVA Curve for YYS_{24}

4. Conclusion

This study has shown that heat-moisture treatment is capable of altering the crystalline structure of starch. The properties and functionality of starches can be tailored by carefully modify them. The information obtained from this research work can be found applicable in pastries and other related products.

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12/11/2010

Flavone-5-O-Glycosides from *Cheilanthes dalhousiae* (Hook)

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Abstract: Fern fronds (about 500gm) of *Cheilanthes dalhousiae* Hook. Vouch. Sp. No. 21 was collected from Pindari glacier routes (2200-2800m) of Almora district of Uttarakhand state. It was extracted with acetone-water (1:1, V/V) and extract was concentrated under reduced pressure until H_2O layer (up to 50ml) remained. The H_2O layer was partitioned with CH_2Cl_2, EtOAc and BuOH Successively. The CH_2Cl_2 fraction gave antibacterial tests against *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Escherichia coli* by the standard method of disc-diffusion using DMSO-d_6 solution of CH_2Cl_2 residue impregnated on Whatman No. 3, paper disc (6 nm) and base plates containing 10ml MH agar. Antibacterial activity was expressed as the ratio of the inhibition zone (nm) produced by CH_2Cl_2 extract and the inhibition zone caused by the reference, neomycin (2µg). No antibacterial activity was observed in ethyl-acetate and n-butanol fractions. EtOAc fraction was evaporated to dryness and residue obtained was dissolved in MeOH. The MeOH soluble of EtOAc fraction was fractionated on Whatman No. 3 chromatographic papers using BAW (n- BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. Two blue UV fluorescent flavone-5-O-glycosides: Quercetin-3-methyl ether-5-O-glycoside and Kaempferol-5-O-(6"-O-malonyl)-glycoside were isolated by RPPC from EtOAc fraction of acetone-H_2O (1:1) extract of fern fronds of *Cheilanthes dalhousiae.* The structural elucidation of the compounds was carried out by UV, ^{1}HNMR and MS spectral studies.

[Rachana Mishra and D. L. Verma. **Flavone-5-O-Glycosides from *Cheilanthes dalhousiae* (Hook)**. Nature and Science 2011;9(1):34-38]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Kumaun Himalaya, *Cheilanthes dalhousiae* (Hook), Medicinal plants

Introduction

Cheilanthes dalhousiae Hook, (family Sinopteridaceae), is widely distributed in Kumaun Himalaya from 2000-3000m. Genus *Cheilanthes*, a member of leptosporangiate group of highly advanced ferns, comprises 130 species with cosmopolitan in distribution and its 9 species have been reported from Kumaun Himalaya (Pande, 1990; Pande *et al.*, 1997). In morphological point of view, *Cheilanthes dalhousiae* is characterized by the presence of deltoid-lanceolate and tripinnafied lamina (15x5) and 2, 3 fronds arise from a single rhizome.

Since Vedic period, ferns have been recognized as a medicinal plants in Ayurvedic, Unani and Chinese systems of medicines (Kritikar and Basu, 1935). A number of ferns have been used for curing diseases like cough, bronchitis, asthma, tuberculosis, typhoid and ulcers (Chopra *et al.*, 1958). Therefore various fern species have previously been screened for antimicrobial, antimalarial, antitumoral and anticancer activities (Banerjee and Sen, 1980). Some high altitude species of *Cheilanthes* have been identified as a traditional medicinal ferns (Lal *et al.*, 1944; Chopra *et al.*, 1956; pande *et al.*, 1989).

Literature survey revealed that high altitude species of genus *Cheilanthes* are still awaited for the screening of antibiotic activities and active constituents. Although, flavonoidal constituents have been reported (Erdtman *et al.*, 1966; sunder *et al.*, 1974; Wollenweber *et al.*, 1980; Scheele *et al.*, 1987; Imperato, 1989; Wollenweber and Roitman, 1991; Tandon *et al.*, 1991; Khetwal and Verma, 1983, 1984, 1986, 1990; Khetwal *et al.*, 1985, 1986) from medicinal plants. Present communication reveals the screening of antibacterial activity, isolation and structural elucidation of two flavonoid compounds from *Cheilanthes dalhousiae.*

Material and Methods

Plant Material

Fern fronds of *Cheilanthes dalhosiae* were collected from Pindari glacier routes (2200-2800m.) of Almora district (Uttarakhand state). Its authentification was made by the help of taxonomist of Botany Department, Faculty of science, DSB Campus, Nainital and vouchers specimen No. 21, was deposited in the Botany Department of Kumaun University, SSJ Campus, Almora (India).

Extraction of Plant Material

About 1kg air dried fern fronds were extracted with Acetone: Water (1:1) by cold percolation methods for three days. The aqueous-acetone extract was decanted and concentrated under reduced pressure until only H_2O layer (75ml) remained. It was partitioned with CH_2Cl_2, EtOAc and BuOH successively.

The CH_2Cl_2 fraction was evaporated to dryness in

Rota-evaporator at 30^0C. The residue was adsorbed on cellulose column (Merck grade) and eluted initially with H_2O and then increasing polarities with HOAc. On eluting column with 10% HOAc, three dark purple fluorescent bands were observed on column with UV light (360nm). All the purple fluorescing bands on column were eluted and combined.

The combined fraction was concentrated and residue obtained was used for the characterization of flavonoidal constituents by the help of 2DPC and screening of antibacterial activity. A part of residue was dissolved in MeOH and examined for flavonoidal compounds on 2DPC using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) and 15% HOAC, as a developing solvent. The dried and developed chromatogram was inspected with UV light (360nm). Eight UV fluorescent spots were discernible on PC. Out of eight spots, five purple UV fluorescent spots were identified as a flavonoids on the basis of their colour reaction with NH_3, UV+NA+PES, UV+$AlCl_3$ and UV+$ZrOCl_2$ (Mabry *et al.*, 1970; Homberg and Geiger,1 980, 1983; Markham, 1982, 1989). Using BAW and 15%HOAc as developing solvents on 2DPC, the high motilities of the purple UV fluorescent flavonoidal compounds were observed. On the basis of colour reactions and Rf values in BAW and 15% HOAc solvent systems, these flavonoidal constituents were characterized as a 3-O- methoxylated flavonols (Mabry *et al.*, 1970; Fang *et al.*, 1985a, 1985b, 1986; Markham, 1989; Liu *et al.*, 1997; Mousallami *et al.*, 2002).

The major portion of the residue which comprises five purple UV fluorescent compounds on 2DPC was dissolve in MeOH and methanolic solution was used for the isolation of flavonoidal compounds. On RPPC of the methanolic solution using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent, five purple fluorescent bands were observed on PC with UV light (360nm). Each band was cut and eluted with 70% MeOH. The isolate of each compound was finally purified on Sephadex LH-20 column eluting initially with H_2O and then decreasing polarity with MeOH. Each isolate was examined for antibacterial activities by the standard disc-diffusion method (Rahalison *et al.*, 1991, 1994; Saxsena *et al.*, 1995). From these isolates, five compounds representing structure (A), (B), (C), (D) and (E) were isolated. Using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent the compound (A), Rf 90 and compound (E), Rf 72, were identified as a faster moving and slower moving component respectively on paper chromatogram.

Antibacterial Screening of Each Isolate

Each isolate was screened for antibacterial activities against three different strains of bacteria *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus epidermis* obtained from CMI, London. Bacteria were maintained on Mueller-Hinton (MH) Nutrient Agar (NA) at 4^0C. Molten MH Agar (10ml) was inoculated with a broth culture (1ml containing 10^6-10^8 bacteria, ml) of the respective bacterial strains and poured over base plates containing 10ml MH Agar in sterile 9cm Petri-dishes. Whatman No. 3 chromatographic paper was cut in a disc shape. The residue of each isolate was dissolved in DMSO solution. The paper disc was impregnated with the DMSO solution of sample. The impregnated paper disc was hot air dried. The sample impregnated discs were placed into the seeded top layer of the agar plates. Each plate contained four paper discs with each isolate and a disc with a neomycin control (2mg). Each isolate was tested in quadruplicate. The base plates were incubated at 37^0C for 12hours, where after inhibition zones were recorded. After incubation of the base plates were inspected with visible and UV light. The antibacterial activity of these flavonoidal compounds is being summarized as follows:

Compound (A): The isolate of compound (A) gave zones of inhibition with the *Staphylococcus epidermis, Pseudomonas aeruginosa* and zones of inhibition was not detected with *Bacillus subtitles*.

Compound (B): No any zones of inhibition were observed with *Staphylococcus epidermis, Pseudomonas aeruginosa* and *Bacillus subtitles* strains.

Compound (C): No zones of inhibition were observed with *Staphylococcus epidermis, Pseudomonas aeruginosa* and *Bacillus subtitles.*

Compound (D): It gave zones of inhibition with the bacterial strains *Bacillus subtitles* but no zones of inhibition were detected with the *Staphylococcus epidermis, Pseudomonas aeruginosa.*

Compound (E): Compound (E) did not give any zones of inhibition with these three bacterial strains *Bacillus subtitles, Staphylococcus epidermis* and *Pseudomonas aeruginosa.*

Thus, out of the five purple UV fluorescent compounds isolated from 10% HOAc fractionation of dichloromethane extract on cellulose CC, only two compounds (A) and (D) gave zones of inhibition with the tested bacterial strains.

The EtOAc fraction of aqueous-acetone extract of fern fronds of *Cheilanthes dalhousiae* was evaporated to dryness in rotatory evaporator at 35^0C. The residue was dissolve in MeOH and chromatographed on

Whatman No. 3 strips (10). On RPPC of the fraction using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent, a blue fluorescent band observed on PC with UV light at Rf 60 was eluted with 70% MeOH. The aqueous methanolic elute was concentrated and residue was adsorbed on Sephadex LH-20 column and eluted with H_2O and then decreasing polarity with MeOH. On eluting column with 20% MeOH, two fluorescent blue compounds observed on column with UV light were eluted separately. The compound (I) and (II) were isolated. The structural elucidation of these two compounds is being summarized as follows:

The compound (I) and (II) appeared as a blue fluorescent on PC under UV light and changed to yellow-green with NH_3 vapors, indicating the presence of 4'-hydroxyl group and substituted 5-OH group (Mabry *et al.*, 1970). When a cellulose TLC plate was sprayed with Naturstoffreagent and 5% PEG solution, the compound (I) turned orange and compound (II) turned yellow, indicating presence of orth-di-hydroxyl group in the B-ring of compound (A), 4'-hydroxyl group in B-ring of compound (I) and (II).

Structural determination of compound (I): The compound (I) gave positive Feigl spot test for sugar. It was hydrolyzed with 12% HCl for1hour at 60^0C, gave a dull purple UV fluorescent aglycone in the organic layer and a sugar component was present in the aqueous layer. The aglycone was isolated by paper chromatographic method. The MS of aglycone exhibited a molecular ion at m/z 316 (100%) for $C_{16}H_{12}O_7$ in accord a flavone containing four hydroxyl and one methoxyl group. Flavone appeared as a dark purple fluorescent on paper chromatogram with UV light and changed to lemon yellow with NH_3 vapors indicating the presence of 5- and 4'-hydroxyl groups. When a cellulose TLC plate was sprayed with Naturstoffreagent (NA) reagent, the spot turned orange, indicating the presence of orth-di-hydroxyl group in B-ring. The dark purple fluorescent spot of the compound on PC when sprayed with 2% $ZrOCl_2$, gave a bright yellow colour which disappeared on addition of 2% citric acid and H_2O, indicating 4'-oxygenated flavonol bearing a free hydroxyl at the 5-position and the substituted one at the 3- position (Liu *et al.*, 1997; Mousallami *et al.*, 2002). The dark purple fluorescence of the compound was turned to dull yellow fluorescence, when the alcoholic solution of the compound treated with HI reagent, indicating the OCH_3 group at C-3 position.

The dull yellow UV fluorescent compound, which obtained after the treatment of aglycone with HI reagent was identified as a quercetin by its CoPC using four solvent systems, BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer), 30% HOAc, 50% HOAc and t-BAW (t-BuOH-AcOH-H_2O, 3:1:1). Thus, the aglycone was identified as 3-methoxy-quercetin.

The aqueous hydrolysate was repeatedly evaporated to dryness and residue was dissolved in isopropanol and chrmatographed on Whatman No. 1 PC, using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as a developing solvent. The dried and developed chromatogram was sprayed with benzidine reagent, a brown spot at Rf 21 appeared was identified as a glucose by its CoPC using two solvent systems, BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) and BuOH saturated water. Thus, an acid hydrolysis of compound (I), gave an aglycone, quercetin-3-methyl ether and a sugar, glucose. The compound (I) appeared as a blue fluorescent on PC under UV light while its aglycone gave dark purple fluorescent spot on PC with UV light, indicating the glucose moiety is attached with 5-OH group (Mabry *et al.*, 1970; Markham, 1989).

Thus, on the basis of colour reactions, acid hydrolysis with 12% HCl and HI, the compound was identified as a quercetin-3-methyl ether-5-O-glucoside (Fig. 1).

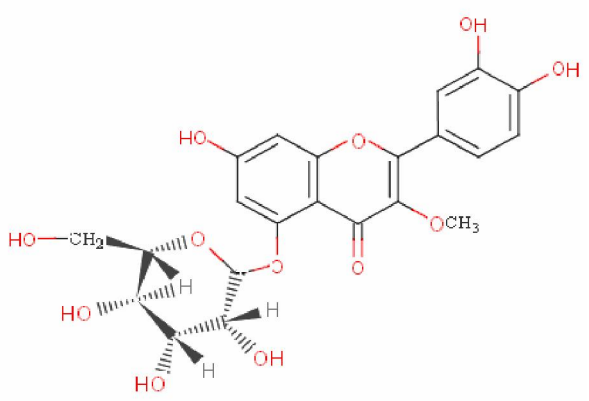

Fig. 1

Further, the compound (I) was identified on the basis of its UV spectral datas in MeOH and shifts obtained with various diagnostic reagents (Table 1) and ^{1}HNMR spectra in DMSO solution (Table 2).

Table 1**:** UV spectra of compound [I] in MeOH (λ_{max}, nm)

Shift Reagent	Shift (λ_{max}, nm)		
	band II	band I	
MeOH	252	351	
NaOH	263	361	402
$AlCl_3$	260	375	
$AlCl_3$+HCl	251sh	350	
NaOAc	268	320	
NaOAc+H_3BO_3	255	373	

Table 2: ^{1}HNMR of compound [I] in DMSO-d_6, 400MHz

Shift (δ)	Multiplicity	Identification
7.53	1H, d, J=2.0Hz	H-2'
7.40	1H, dd, J=2.3Hz and 2.0Hz	H-6'
6.89	1H, d, J=8.0 Hz	H-5'
6.75	1H, d, J=2.0 Hz	H-8
6.63	1H, d, J=2.0 Hz	H-6
4.76	1H, d, J=7.8Hz	glucose anomeric proton
3.2 to 3.7	6H, m	glycosyl proton

Structural Determination of Compound (II): The compound appeared as a dull blue fluorescent spot on PC under UV light and changed to lemon yellow with NH_3 vapours and NA reagent indicating a 4'-hydroxyl group but no ortho-di-hydroxyl group in the B-ring and presence of a substituted 5-OH group in the A-ring (Mabry *et al.*, 1970). Acid hydrolysis of the compound gave a dark purple UV fluorescent aglycone which was identified as kaempferol-3-methyl ether and a sugar, glucose. Alkaline hydrolysis of the compound with 2N NaOH at room temperature for 120 minutes, gave kaempferol-3-methyl ether-5-O-glucoside and malonic acid. Both the constituents were identified by their respective authentic by CoPC using three solvent systems, BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer), t- BAW (t-BuOH-AcOH-H_2O, 3:1:1) and BEW (n-BuOH-EtOH-H_2O, 4:1:2.2, upper layer).

^{1}HNMR of the compound (II) gave two symmetrical doublets each with J=8.0 Hz, at δ 8.08 (2H, d, for H-2' and H-6') and at δ 6.89 (2H, d, for H-3' and H-5') and two down field meta coupled protons each with J=2.0 Hz, at δ 6.85 (1H, d, J = 2.0, +1.8) and δ 6.49 (1H, d, H-6) appeared in aromatic region. In aliphatic region, a singlet appeared at δ 3.15, is identified for methylene protons of malonic acid and anomeric proton singlet appeared at δ 4.77 while remaining protons of sugar appeared as multiplet between δ 3.2 to 3.9. In comparing the ^{1}HNMR of kaempferol-5-O-glycoside with the ^{1}HNMR of compound (II) in sugar region, the down field shift of H-6' and H-6" proton (H-6', 3.9 and H-6", 4.2) of compound (II) clearly indicated that the malonyl group substitutes C-6 OH of glucose sugar. Thus, the compound (II) was identified as kaempferol-3-methyl ether -5-O-β-(6'-malonyl) glycoside (Fig.2).

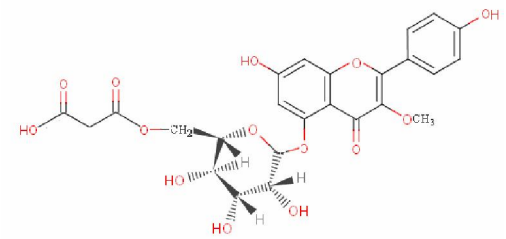

Fig.2

Acknowledgements: We thank to the authority of Central Drug Research Institute (CDRI), Lucknow (U. P.), India for their kind co-operation in the structural analysis of flavonoids by ^{1}HNMR, UV and MS spectral studies.

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4/15/2009

Optimization Of 2, 4 Dichlorophenol Degradable Crude Extracts Produced By *Pseudomonas Aeruginosa* Using Box Behnken Design

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ABSTRACT: *Pseudomonas aeruginosa* was grown on mineral medium containing 2, 4 dichlorophenol as a sole source of carbon and energy. Process optimization was carried out by developing 17 combinations using Box Behnken design to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The highest protein concentration in biomass from 17 combinations obtained from the experiment is 4.99 mg/ml (35 ml of medium, 6 ml of inducer and 6 ml of inoculum). The point prediction from the analysis of variance for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 4.5 ml of inducer and 4 ml of inoculum.

[R. Manikandan, H. Janardhana Prabhu, P. Sivashanmugam, CN Pratheeba[‡] and Pankaj Sah. **Optimization Of 2, 4 Dichlorophenol Degradable Crude Extracts Produced By *Pseudomonas Aeruginosa* Using Box Behnken Design**. Nature and Science 2011;9(1):39-44]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: 2, 4 Dichlorophenol, Crude extract, *Pseudomonas aeruginosa,* Optimization , ANOVA and Box Behnken design

1. INTRODUCTION

Tabak *et. al.,* (1964) described microbial metabolism of aromatic carbon compounds. The purpose of this investigation was to determine the ability of specifically adapted bacteria to degrade phenol and substituted phenols, and to study the relationship between the chemical structure of phenol derivatives and cyclic hydrocarbons and their susceptibility to decomposition by organisms adapted to related aromatic compounds.

Walter Reinke *et. al.,* (1984) isolated 2,4 dichlorophenol-degrading bacterium (, strain WR1306) by continuous enrichment from a mixture of soil and sewage sample & grown in a chemostat on a mineral medium with 2,4 dichlorophenol. Respiration data and enzyme activities in cell extracts as well as the isolation of 3-chlorocatechol from the culture fluid are consistent with the degradation of 2,4 dichlorophenol. Michel Rutgers *et. al.,* (1993) used nutristat to grow pentachlorophenol (PCP)-degrading microorganisms. Rebecca M Goldstein *et. al.,* (1985) explained the reasons for possible failure of inoculation to enhance biodegradation.

Ayami Nakagawa *et. al.,* (2006) found 32% of DCP was degraded within 1h.He is the first one to prove dechlorination pathway by Zygomycetes. Khadar valli *et. al.,* (1991) examined the degradative pathway of 2,4-dichlorophenol by P. chrysosporium. They showed that this pathway involves several cycles of oxidation and subsequent quinone reduction and hydroquinone methylation.

Mohammad Edrissi and Nima Razzaghi asl (2007) discussed the application of RSM method in optimizing complexation of iron with piroxicam. A response surface methodology (RSM) based on a Box-Behnken design was applied for study on ferrous ions binding ability to piroxicam in aqueous solution as a function of three numerical factors (extraction time, pH, piroxicam concentration) and extractant type as a categorical variable each in three levels.

Experimental designs nowadays have been regarded as one of the most favorable techniques in covering a large area of practical statistics and obtain unambiguous results with the least expense. Response surface method (RSM) designs help to quantify the relationships between one or more measured responses and the vital input factors. The most popular response surface

methodologies are Central Composite, Box-Behnken designs.

Box-Behnken design is an efficient and creative three-level composite design for fitting second-order response surfaces. It is an independent quadratic design. The methodology is based on the construction of balance designs which are rotatable and enable each factor level to be tested several times. Each factor or independent variable can be placed at one of three equally spaced values (coded as –1, 0, and +1). In this design the treatment combinations are at the midpoints of edges of the cubical design region and at the center.. Box-Behnken designs provide excellent predictability within the spherical design space and require fewer experiments compared to the full factorial designs or central composite designs. The number of required experiments for Box-Behnken design can be calculated according to $N = k^2 + k + c_p$, where k is the factor number and c_p is the replicate number of the central point.

In the present investigation, crude cell extracts from the enriched strain *P. aeruginosa* on 2,4 dichlorophenol was immobilized on sodium alginate beads and the beads were packed in a glass column to study the degradation. Seventeen sets of combinations of process parameters were developed to produce crude extracts. The experiment was carried out in different concentrations 2,4 dichlorophenol in the immobilized beads which contains crude extracts of *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Maintenance and cultivation of microorganism

The strain *P. aeruginosa* was obtained from NCIM, Pune, India. The strain was sub cultured in nutrient broth. The broth was incubated in the shaker with 175 rpm and at 37ºC overnight. Sterile plates containing nutrient agar of specified composition were streak plated with the overnight cultures. The culture on the plates was used as the source for the entire experiment. The mineral medium with specified composition (Table 1) of chemical substances was prepared to conduct the experiment. The pH of the mineral medium was adjusted to 7.0 by using 2 NH_2SO4 or 2N NaOH solution. 50 ml of the medium was taken in each of 250 ml Erlenmeyer flasks and were sterilized at 1.5 kg/cm^2 (gauge) for 20 min. After cooling to room temperature, the medium was added with 2, 4 dichlorophenol and inoculated in a laminar flow chamber. The flasks were then incubated on a rotary shaker for 48 h at 30ºC and 175 rpm, for full growth of the strain. The sub cultured strains were stored at 5ºC.

Table 1 Composition of Medium

Ingredients	Concentration (g/l)
NH_4NO_3	1.0
$(NH_4)_2SO_4$	0.5
NaCl	0.5
K_2HPO_4	1.5
KH_2PO_4	0.5
$Mgso_4.7H_2O$	0.5
$CaCl_2$	0.01
Double distilled Water	1 l

2.2 Suspension of washed cells and cell extracts

Cells grown on 2,4 dichlorophenol as the sole carbon source, were harvested in the mid-exponential growth phase by centrifugation (8,000 rpm for 10 min at 4°C), washed with sodium phosphate buffer (pH 7.0, 50 mM), and suspended in the same buffer. The cell extracts were prepared by disrupting the cells by ultrasonic disintegration (Labsonic-P of Labsonic-Germany). The resulting cell lysate was centrifuged at 8,000 rpm for 10 min at 4°C, and the supernatant, containing approximately 10 to 20 mg of protein ml^{-1}, was the crude cell extract (containing 2,4 dichlorophenol degrading enzyme). The concentrations of protein content in the crude extracts were measured using UV Visible Spectrophotometer (Hitachi UV 2800).

2.3 Optimization of the process parameters

Process optimization was carried out by conducting 17 experiments (Table 2) to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The parameters, the volume of mineral medium (20,35 and 50 ml), inducer (3 -6 ml) and inoculum (2 - 6 ml) were selected. The mineral medium, Inducer (2, 4 dichlorophenol) and inoculum were processed as mentioned different cultures were obtained by varying the three parameters and processed to obtain its crude extract. The concentration of the crude extract was measured at 280 nm. The data obtained from 17 experiments, were used to find out the optimum point of the process parameters by using Box Behnken Design in Response surface methodology. All the data

were treated with the aid of Design Expert from Stat-Ease.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance

Based on design of experiment, 17 combination were developed (Table 2) and processed to obtain crude extracts as mentioned in this paper. The data obtained from the experiments were used to the analysis of variance (Table 3 and 4). The Model F-value of 6.366E+007 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2, A^2B, A^2C, $AB^2$are significant model.

Table 2 Combination of process variables

Run	A:Medium (ml)	B:Inoculum (ml)	C:Inducer (ml)	Crude extract (mg/ml)
13	35	6	6	4.99
6	35	2	6	4.88
7	35	4	4.5	4.74
8	35	4	4.5	4.74
9	35	4	4.5	4.74
10	35	4	4.5	4.74
11	35	4	4.5	4.74
4	20	6	4.5	3.57
1	20	2	4.5	2.97
5	35	2	3	2.05
2	20	4	3	1.98
3	20	4	6	1.34
15	50	4	3	0.96
17	50	6	4.5	0.56
16	50	4	6	0.31
12	35	6	3	0.23
14	50	2	4.5	0.15

Table 3 Analysis of variance (ANOVA):

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	60.13575	12	5.011312	6.366E+007	< 0.0001	significant
A-Medium(ml)	1.05401	1	1.05401	6.366E+007	< 0.0001	
B-Inoculum(ml)	0.727097	1	0.727097	6.366E+007	< 0.0001	
C-Inducer(ml)	14.42253	1	14.42253	6.366E+007	< 0.0001	
AB	0.008603	1	0.008603	6.366E+007	< 0.0001	
AC	2.72E-06	1	2.72E-06	6.366E+007	< 0.0001	
BC	0.920256	1	0.920256	6.366E+007	< 0.0001	
A^2	24.39735	1	24.39735	6.366E+007	< 0.0001	
B^2	1.120969	1	1.120969	6.366E+007	< 0.0001	
C^2	5.894329	1	5.894329	6.366E+007	< 0.0001	
ABC	0	0				
A^2B	0.925412	1	0.925412	6.366E+007	< 0.0001	
A^2C	9.87168	1	9.87168	6.366E+007	< 0.0001	
AB^2	1.790021	1	1.790021	6.366E+007	< 0.0001	
AC^2	0	0				
B^2C	0	0				
BC^2	0	0				
A^3	0	0				
B^3	0	0				
C^3	0	0				
Pure Error	0	4	0			
Cor Total	60.13575	16				

Table 4 Regression Analysis

Std. Dev.	0	**R-Squared**	1
Mean	2.804	**Adj R-Squared**	1
C.V. %	0	**Pred R-Squared**	N/A
PRESS	N/A	**Adeq Precision**	3.1E-308

Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The application of response surface methodology (Kenneth et.al, 1995, Khuri, A.I.,) yielded the following regression equation, which is an empirical relationship between the logarithmic values of protein yields and test variables in coded unit.

Final equation in terms of coded factors with coefficients values (Table 5):

Y (Crude extract mg/ml) = + 4.74 - (0.51 * A) - (0.43 * B) + (1.90 * C) - (0.046* A * B) –(8.250E-004 * A * C) + (0.48 * B * C) - (2.41 * A^{2}) - (0.52 * B^{2}) - (1.18 * C^{2}) + (0.68 * A^{2} * B) – (2.22 * A^{2} * C) – (0.95 * A * B^{2})

Where Y is response that is the protein concentration is expressed in logarithmic values and A, B, and C are the coded values of the test variable medium, inducer and inoculum respectively.

3.2 Analysis of process variables by response surface plots

The optimum values of the selected variables were obtained by solving their regression equation and analyzing response surface contour plots. Response Surface plots as a function of two factor at a time maintaining all other factors at a fixed level (zero for instance) are more helpful in understanding both the main and interaction effects of the two factors. The plots can be easily obtained by calculating the data from the model. The values were taken by one factor, where the second varies with constant of a given Y - values. The yield values of the different concentrations of the variable can also be predicted from respective response surface plots. Figures 1 to 6 shows the relative effects of the two variables with protein concentration level. The coordinates of the central point within the highest contour levels in each of these figures corresponded to the optimum concentrations of the respective components.

Table 5 Coefficients obtained from regression analysis

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.737	1				
A-Medium(ml)	-0.513	1				2
B-Inoculum(ml)	-0.426	1				2
C-Inducer(ml)	1.899	1				2
AB	-0.046	1				1
AC	-0.001	1				1
BC	0.480	1				1
A^{2}	-2.407	1				1.0058
B^{2}	-0.516	1				1.0058
C^{2}	-1.183	1				1.0058
$A^{2}B$	0.680	1				2
$A^{2}C$	-2.222	1				2
AB^{2}	-0.946	1				2
AC^{2} ALIASED A, AB^{2}						
$B^{2}C$ ALIASED C, $A^{2}C$						
BC^{2} ALIASED B, $A^{2}B$						
A^{3} ALIASED A						
B^{3} ALIASED B						
C^{3} ALIASED C						

Figure 1 and 2 show their contour and response surface plot obtained as a function of volume of inoculum vs. medium with protein concentration, while all other variables are maintained at zero level (coded units). Figure 3 and 4 show their contour and response surface plot obtained as a function of volume of inducer vs. medium with protein concentration. Figure 5 and 6 show their contour and response surface plot obtained as a function of volume of inducer vs. inoculum with protein concentration.

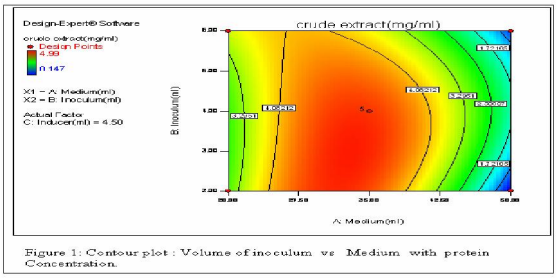

Figure 1: Contour plot : Volume of inoculum vs Medium with protein Concentration.

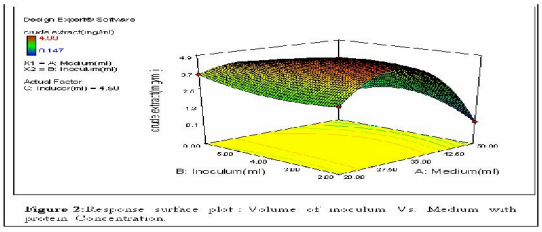

Figure 2: Response surface plot : Volume of inoculum Vs. Medium with protein Concentration.

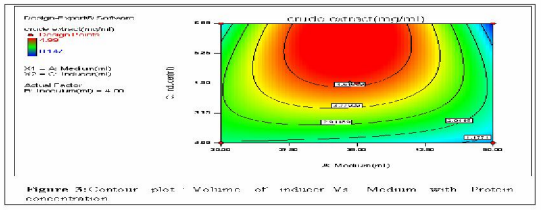

Figure 3: Contour plot : Volume of inducer Vs Medium with Protein concentration

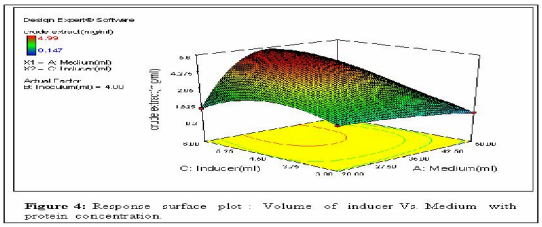

Figure 4: Response surface plot : Volume of inducer Vs Medium with protein concentration.

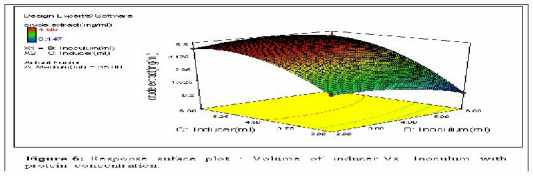

Figure 6: Response surface plot : Volume of inducer Vs Inoculum with protein concentration.

3.3 Optimum Values

The protein production was predominantly influenced by medium and inducer concentration. From the Contour plots the red color shows the region of the desirability for the production of protein. The point prediction from the analysis of variable for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 2 ml of inducer and 6 ml of inoculum.

Table 6 Predicted value from Box -Behnken design

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	Medium(ml)	35	20	50	0	Actual	
B	Inoculum(ml)	2	2	6	0	Actual	
C	Inducer (ml)	6	3	6	0	Actual	
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Crude extract(mg/ml)	4.88	0	4.88	4.88	0	4.88	4.88

PI - Prediction interval

CI - Confidence interval

SE Mean – Standard error of the mean.

SE Pred – Standard error of prediction

4 Conclusion

2,4 Dichlorophenol can induce the synthesis of enzymes in *Pseudomonas aeruginosa* that are able to break down hydrocarbons including 2,4 dichlorophenol. In this work the process conditions were optimized to produce crude extracts. Immobilization of the crude extracts obtained from *Pseudomonas aeruginosa* increases the efficiency of the extract and they have been used to study the degradation of 2,4 dichlorophenol in a packed bed column. Thus it has been concluded that this study will yield good results if extended to large-scale applications.

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6/8/2009

Eco-Toxicological Implications Of Crude Oil Pollution On *Rhizophora Racemosas* (G.F.W. Meyer)

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Abstract: An experiment was conducted in 2008 in Asaba, Delta State, Nigeria to evaluate the eco-toxicological implications of crude oil pollution on *Rhizophora racemosa* seedlings. Five crude oil levels of crude oil (0.0, 12.0, 18.0, 24.0 and 30.0%) per 1.5kg of flood soils served as the treatments. The experiment was laid out in a randomised complete block design with four replications. The results showed that oil pollution at 18.0, 24.0 and 30.0% significantly affected ($P \geq 0.05$) the seedlings of the test plant in terms of plant height, number of leaves, leaf area, collar diameter and root, growth at the 5% probability level when compared with the seedlings grown in the unpolluted soils and those exposed to 12.0% of the oil. Root growth of the seedlings was significantly reduced ($P \geq 0.05$) with increasing oil levels. At 30.0% oil treatment, root hairs were totally absent. The study has established that *R. racemosa* seedlings tolerated all the crude oil concentrations used. No death was recorded throughout the trail period although significant reductions were noticed with increasing oil levels and this may have implications on the growth and establishment of the red mangrove. Conclusively, *R .racemosa* seedlings conserve as a bio-indicator of pollution and can be recommended for use in area of low levels of pollution for environmental clean-up or bioremediation.

[Agbogidi, O.M. **Eco-Toxicological Implications Of Crude Oil Pollution On *Rhizophora Racemosas* (G.F.W. Meyer).** Nature and Science 2011;9(1):45-49]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: Toxic implications, crude oil pollution, *Rhizophora racemosa*, ecosystem

Introduction

Rhizophora racemosa is a wet land tropical tree belongs into the mangrove family Rhizophoracea. *R. racemosa* (Red mangrove) is a medium tall tree, reaching a height of between 30-50m and between 15 and 35cm in girth (Duke *et a*l., 2006). The tree is mostly multi-stemmed rambling to columnar trees with often distinct above the ground prop roots. The flowers are perfect, containing both male and female parts and flowering period is between August and December in the Southern hemisphere and between February and June in the Northern hemisphere (Duke and James, 2006). The leaves are opposite, simple, bright, green; obvate leathery, margins revolute generally with curve surface. Leaf emergence occurs between November and February and leaf fall occurs chiefly between October and February (Saenger, 2002). The fruits when mature are pear like shaped, elongate, waist constricted and smooth brown surface. *R. racemosa* produces viviparous seeds which are hidden in the fruit (Keyejo et al., 2006). The hypocotyls- the growing seedling from the fruit is narrowly cylindrical, elongated, green, and smooth with irregular small brown lenticels (Saenger, 2002). The hypocotyls fall occurs mainly between November and February (De Lacerda, 2002; Bosire *et al*., 2005). The seedlings germinate in the mature fruit while the fruit is attached to the parent plant (Bamidele and Agbogidi, 2006; Bamidele *et al*., 2007).

Rhizophora racemosa is economically important in the following respects:
diversification of wildlife habitat, mulch/organic matter, soil stabilisation, coastal protection, wind break and water quality improvement, timber, fuel, wood production, medicinal uses (the bark and leaves are used for the treatment of angina, boils, fungal infections, diarrhoea, dysentery, fever, malaria, anti-bleeding and leprosy (Bandaranyake, 1999; Koedam, 2000; Aluko *et al.,* 2002; Walters, 2005). *R. racemosa* has been shown to play a vital role in supporting marine food chains; hence De Lacerda (2002) stated that it is used as a stable food and animal fodder.

The importance of wetlands cannot be over emphasised (Bosire *et al*., 2005; Dahdouh- Guedas *et al*., 2005; Bamidele and Agbogidi, 2006; Bamidele *et al.,* 2007). Several mangrove tracts have been destroyed due to susceptibility of some mangroves to oil pollution as a result of leakages of pipelines, flow line, accidents and sabotage in the areas of operations (Agbogidi *et al*., 2005). The spilled oil has a complex mixture of thousands of hydrocarbons and related compounds that are toxic that float as slick buried in the soil or stranded on the shores damage aquatic environment (Azad, 2005; Agbogidi and Bamidele, 2007). The toxic effects of crude oil include acidification of the wetland soil, halt cellular respiration and as such starve the roots of vital oxygen (Merck, 2002). Understanding the critical threshold

level at which adverse effect is felt on *R .racemosa* will enhance the life supporting process of plants, especially the coastal areas, discourage global warming, ameliorate desirable habitats for animals, aid water purification as well as the purification of the atmosphere. It is against this background that eco-toxicological implications of crude oil pollution on *R. racemosa* were embanked on. A study as this will contribute to the struggle against environmental degradation and will help to secure the future of the wetland coastal ecosystems (Nwilo *et al.*, 2007).

Materials and Methods

The study was carried out at the nursery site of the Department of Forestry and Wildlife, Delta State University, Asaba Campus (Latitude: $6^{0}14^{1}$N; Longitude: $6^{0}49^{1}$E) (Asaba Meteorological Station, 2008).

R. racemosa seedlings were collected from Warri, in Warri-West Local Government Area, Delta State and the basic nursery care was given for a period of two weeks. Soil samples used were obtained from flooded site close to Anwai River, Delsu Asaba Campus. The crude oil with specific gravity of 0.889g/cm^3 was obtained from the Nigerian National Petroleum Cooperation (NNPC), Warri, Delta State, Nigeria. 0.0, 12.0, 18.0, 24.0, and 30.0% w/w per 1.5kg of soil constituted the treatments. A seedling was planted per poly pot (30/50cm). The experiment was laid out in a randomized complete design with four replications. The trial ran for 12weeks. The seedlings were sown directly into the poly pots (30/50cm in dimension) the poly pots were watered immediately after planting and thereafter, every other day till the end of the trial. The set up was monitored for 12 weeks after transplanting (WAT) while growth characteristics were measured forth nightly. Parameters measured were plant height (cm), number of leaves, leaf area (cm^2) and collar diameter (cm). Plant height was measured with a meter rule at the distance from the soil level to terminal bud. The number of leavers was determined by counting while the leaf area was determined by tracing the leaves on a graph paper and the total leaf area per seedling was obtained by counting the number of 1cm squares. Collar diameter at 2.5cm above the soil level was measured with vennier callipers. Data obtained were subjected to analysis of variance while the significant means were separated using the Fisher's least significant differences (LSD) as recommended by SAS (2005).

Results and Discussion

Rhizophora racemosa seedlings tolerated all the concentrations (0.0, 12.0, 18.0, 24 and 30.0%) used. No death was recorded in the seedlings even at the highest level of crude oil application to soil. The performance of the seedlings in terms of plant height, number of leaves, leaf area, collar diameter significantly decreased ($P \geq 0.05$) with increasing levels of pollution over time (Tables 1-4 respectively). *R. racemosa* seedlings thrived better at 0.0 and 12.0% levels of pollution. At the highest (30.0%) level of pollution, there was leaf fall and yellowing of leaves from deep green to dark brown. The rate of growth at lower oil levels was also observed to be higher when compared with the seedlings grown in soils with higher levels of crude oil over time (Tables 1-4). Root growth of the seedlings was significantly reduced ($P \geq 0.05$) with increasing oil levels (Tables 5 and 6). At 30.0% oil dose, root hairs were altogether absent (Table 6).

The reductions in the growth characteristics of *R. racemosa* seedlings with increasing oil levels with time may be attributed to the various hydrocarbons and related compounds that are toxic to the biological organisms as well as the acidification of crude oil on wetland soils that halt cellular respiration as such, starve roots of vital oxygen hence the reduced root growth at higher concentrations of oil pollution. This observation agrees with the reports of Azad (2005) and Bamidele and Agbogidi (2006) on *Metamysidopsis insularis* and *Machaerium lunatus* respectively. The better performance in the seedlings of *R. racemosa* sown in the unpolluted soil could be attributed to the uninterrupted translocation of nutrients and water to xylem vessels while stress imposed by the crude oil could have accounted for a reduction in the performance of the seedlings exposed to the oil treatment. The study has demonstrated that crude oil application to soil has a negative effect of reducing the growth characteristics of *R. racemosa* seedlings and this may have implication on the growth and establishment of the red mangrove along the coastal region. At low levels (0.0-12.0%), *R. racemosa* seedlings were significantly unaffected in its growth parameters. The present study has established that *R. racemosa* can serve as a bio-indicator of pollution and can be recommended for use in areas of low levels of pollution for environmental clean-up or bioremediation.

Table 1. Plant height (cm) of *R. racemosa* seedlings as influence by different crude oil levels in soil

Oil in soil	Plant height/weeks after transplanting (WAP)					
% (w/w)	2	4	6	8	10	12
0.00	6.4^{a}	9.4^{a}	11.00^{a}	14.0^{a}	16.0^{a}	18.0^{a}
12.00	5.6^{a}	6.0^{b}	6.1^{b}	7.00^{b}	7.8^{b}	9.4^{b}
18.00	4.5^{ab}	4.9^{bc}	5.6^{bc}	6.3^{b}	6.4^{bc}	6.5^{c}

24.00	4.3[b]	4.5[bc]	5.0[bc]	5.1[bc]	5.2[bc]	5.3[cd]
30.00	2.8[b]	3.1[c]	3.9[c]	4.0[c]	4.1[d]	4.2[d]

Means with different superscripts are significantly different at P≤ 0.05 level of significance using the Fisher's least significant different (LSD).

Table 2. Number of leaves of *R..racemosa* seedlings as influenced by crude oil in soil

Oil in soil % w/w)	Number of leaves/WAP					
	2	4	6	8	10	12
0.00	0.66 [a]	1.33[a]	1.33[a]	2.00 [a]	2.00 [a]	2.66[a]
12.00	0.66 [a]	1.33[a]	1.33[a]	1.33 [ab]	1.33 [ab]	2.00 [ab]
18.00	0.66 [a]	1.33 [a]	1.33 [a]	1.33 [ab]	1.33 [ab]	1.33 [ab]
24.00	0.66 [a]	1.33 [a]	1.33 [a]	1.33 [ab]	1.00 [ab]	1.00[c]
30.00	0.33 [a]	0.66 [a]	0.66 [a]	1.00[b]	0.66[b]	1.66 [c]

Means with different superscripts are significantly different as P≤ 0.05 level of significance using the Fisher's least significant different (LSD)

Table 3. Leaf area of *R. racemosa* seedlings as influenced by crude oil in the soil.

Oil in soil % w/w)	Leaf area/WAP					
	2	4	6	8	10	12
0.00	42.75 [a]	45.03[a]	45.72[a]	52.70 [a]	88.00 [a]	138.00[a]
12.00	30.43 [a]	45.69[ab]	45.15[a]	50.30 [a]	52.70 [b]	85.00 [b]
18.00	15.85 [c]	35.95 [bc]	35.48 [b]	50.20 [a]	49.70 [b]	52.30 [c]
24.00	13.93 [c]	33.50 [c]	35.45 [b]	40.20 [b]	34.98 [c]	36.90[d]
30.00	7.00 [d]	16.59d [a]	34.50 [b]	39.20[b]	32.89[c]	34.60 [d]

Means with different superscripts are significantly different as P≤ 0.05 level of significance using the Fisher's least significant different (LSD)

Table 4. Collar diameter of *Rhizophora racemosa* seedlings as influenced by crude oil in soil.

Oil in soil % w/w)	Collar girth/WAP					
	2	4	6	8	10	12
0.00	4.5 [a]	4.8[a]	4.9[a]	5.0 [a]	50.1 [a]	5.2[a]
12.00	4.5 [a]	4.8[a]	4.9[a]	4.9 [a]	5.0 [a]	5.2[a]
18.00	4.4 [a]	4.6 [a]	4.8 [a]	4.8 [a]	4.8 [ab]	4.9 [ab]
24.00	4.4 [a]	4.6 [a]	4.7[a]	4.8 [a]	4.7 [ab]	4.7[ab]
30.00	4.1 [d]	4.5 [a]	4.6[a]	4.7[b]	4.5[ab]	4.5[ab]

Means with different superscripts are significantly different as P≤ 0.05 level of significance using the Fisher's least significant different (LSD)

Table 5. Root growth (length) of *R. racemosa* seedlings as affected by crude oil in soil

Oil in soil %(w/w)	Root length/WAP					
	2	4	6	8	10	12
0.0	6.7^{a}	7.8^{a}	8.6^{a}	9.4^{a}	10.2^{a}	10.8^{a}
12.0	6.5^{a}	7.4^{a}	8.2^{b}	9.1^{a}	9.8^{a}	10.6^{a}
18.0	6.1^{b}	6.6^{b}	6.8^{b}	7.1^{b}	7.3^{b}	7.4^{b}
24.0	6.0^{b}	6.2^{b}	6.3^{c}	6.2^{c}	6.2^{c}	6.2^{c}
30.0	5.6^{c}	5.4^{c}	5.3^{d}	5.2^{d}	5.2^{d}	5.2^{d}

Means with different superscripts are significantly different as $P \leq 0.05$ level of significance using the Fisher's least significant different (LSD)

Table 6. Root growth (root hairs) of *R. racemosa* seedlings as influenced by crude oil in soil

Oil in soil %(w/w)	Root hairs/WAP					
	2	4	6	8	10	12
0.0	3.4a	5.4a	6.5a	6.8a	7.2a	10.6a
12.0	3.3a	5.3a	6.3a	6.7a	7.0a	10.0a
18.0	3.0b	4.1b	4.6b	4.8b	4.9b	5.0b
24.0	2.6c	2.7c	2.1c	1.4c	0.2c	0.2c
30.0	1.0d	0.6d	0.1d	0.0d	0.0d	0.0d

Means with different superscripts are significantly different as $P \leq 0.05$ level of significance using the Fisher's least significant different (LSD)

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Effect Of Particulate Materials On Lactic Fermentation Of New Local White Variety Cassava ("Bianbasse") Using Both Spontaneous And Starter Culture

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ABSTRACT: Lactic acid bacteria isolated in the fermentation of cassava for 'fufu' were Lactobacillus plantarium, Lactobacillus sp and Leuconostoc mesenterodes. L plantarium was identified as the most predominant lactic acid bacteria and was used as a starter culture for the fermentation of 'fufu' production. The mean value counts during spontaneous fermentation, the total dissolved loads in all the samples, the total reducing sugars of all samples, the microbial loads in all the samples, the contents of crude protein, crude father, ash, crude fidbe, phytic acid and Tannin were determined. The mean value counts during spontaneous fermentation process from zero hour to 72hours were found to increase 0.67 x 1012cfu/ml to 3.56 x 1012 in lactic acid bacteria than total bacteria with an increase from 0.69 x 1012 to 2.94 x 1012cfu/ml and yeasts which increased from0.07 x 1012 to 2.06 x 1012cfu/ml. There was corresponding increase in total dissolved solids of sample from 600mg/l to 2500mg/l, when varying the concentration of particulate materials for 72 hours and from 500mg/l to 1400mg/l when varying the concentration of Osmoregulators. The total reducing sugar for all the samples ranged from 5.8mg/l to 5.7mg/l at zero hour. At 24 hours, it ranged from 3.0mg/l to 5.4mg/l, at 48 hours it ranged from 3.5mg/l to 6.2mg/l and 72 hours, it ranged from 4.8mg/l to 6.4mg/l. Sample A inoculated with starter culture highest counts of Lactic acid bacteria ranged from 3.35 to 5.50 x 109cfu/ml while total bacterial counts ranged from 1.23 to 1.32 x 109cfu/ml. Other samples with supplemented materials had lactic acid bacterial counts ranged from 2.60cfu/ml to 3.92 x 109cfu/ml while bacterial counts ranged from 3.15 to 3.80 x 109cfu/ml. Control had LAB counts ranged from 2.52 to 3.04 x 109cfu/ml while total bacterial counts ranged from 2.48 to 2.80 x 109cfu/ml.

[Adetunde L. A., Onilude A. A., Adetunde I. A. **Effect Of Particulate Materials On Lactic Fermentation Of New Local White Variety Cassava ("Bianbasse") Using Both Spontaneous And Starter Culture**. Nature and Science 2011;9(1):50-56]. (ISSN: 1545-0740). http://www.sciencepub.net.

KEY WORDS: Lactobacillus planetarium,, Lactobacillus sp ,Leuconostoc mesenterodes, fufu, Osmoregulators, Lactic acid bacteria and fermentation.

INTRODUCTION

In Africa, Cassava is very important to the people because fermented cassava products constitute a major part of the daily diets of many homes in Nigeria and most parts of West Africa (Oyewole and Odunfa 1990). Cassava processing methods involve peeling, crushing, milling, slicing, dewatering, decanting sun-drying, smoke-frying, fermenting, heaping, stacking, sieving, cooking, boiling or steaming. Different combinations of these activities result in different products (Nweke, 1996). In 'fufu' production, the peeled cassava roots are retted for a period of 5 days, followed by a process of sieving and dewatering. 'Fufu' is not subjected to any drying before being cooked for consumption.

Cassava tuber in itself considered as an unbalance feed-stock rich in starch but poor in protein and growth factors. Figueroa et al 1997). The low protein contents of cassava has been of major concern in its utilization (Brook et al., 1969). Ngaba and lee (1979) implicated *Lactobacillus plantarium, L buchneri, Leucostoc sp., streptococcus* sp and yeast in cassava fermentation. Lactobacillus sp and Leuconostoc sp dominated the final stage of the fermentation. (Nwankwo et al., 1989). Lactic acid bacteria have been identified as the most useful micro-organism to the society with the possible future benefits and has been found to be beneficial in flavoring foods, inhibiting spoilage bacteria, and pathogens in intestinal health and other health benefits related blood cholesterol levels, immunocompetence and antibiotic production. (Sandine, 1987). The

development of Lactobacillus strains as started cultures for cassava fermentation could offer some advantages and could help in optimizing the processing. This process of fermentation with protein and legume enriching micro-organism improve protein contents of cassava.

Therefore the objective of this work is to study the effect of starter cultures, and varying concentrations of particulate materials on lactic fermentation of cassava in order to evaluating the proximate composition and nutritional analysis of fermented cassava.

MATERIALS AND METHODS

Materials

Cassava tubers of the new local white variety ("Bianbasse") about 12 months old obtained from savanna Agriculture Research Institute (SARI) farm at Nyankpala- Tamale, Ghana, particulate material-such as-prepared soybean husk and soy bean meal, brewer soluble (worts) and mash solid obtained from Ghana Brewery PLC Accra, and alumina obtained form the Department of Chemistry, University for Development Studies were used to study it effects on cassava fermentation using spontaneous and starter culture- *Lactobacillus planetarium.* The Cassava tubers were selected such that no surface attack of pathogen or external wound was observed.

Experimental Procedures

Fermenter were prepared using about 50g of cut cassava tubers which were steeped into 250ml of sterile water to form pulps in 500ml sterile fermenter which was covered. This was fermented spontaneously for 3 days at laboratory temperature of $29^o \pm 2^oc$. The process was monitored on 24 hours basis for 3 days to observe any change in microfloral composition. Thus cassava was fermented by the traditional 'fufu' preparation method.

Culture Media used for Isolation

De Mann Rogosa and Sharpe (MRS) Agar, De Mann Rogosa and Sharpe broth, peptone water, and Plate Count Agar (PCA) were used for the isolation of microorganisms. All media were autoclaved at 121^oC for 15 minutes after melted.

Isolation of Micro-organism in fermenting medium

Lactic acid bacteria were isolated from 5g of fermenting tuber. The samples were homogenized with 50ml of sterile 0.1% peptone water. One-tenth and one-eleventh dilutions were poured on sterile plates and prepared sterile MRS agar cooled to 40^o C was poured on the plates (in duplicate), swirled and left to solidify. These plates were incubated at 30^oC for 2 days under anaerobic condition (BBL Gas pack, H_2 and $C0_2$ anaerobic system Becton Dickorison) Representative colonies were picked randomly from the plates and purified by sub-culturing on fresh gar plates of MRS agar.

Preliminary Identification

Preliminary tests were done according to Sharpe (1979). For lactic acid bacteria, the organisms were tested for biochemical characterization according to the procedure described by Seeley and Van Demark (1972).

Cultivation of starter culture

The selected lactic acid bacteria isolated from cassava fermentation were separately cultivated on MRS broth that had been sterilized at 121^oC for 15 minutes and adjusted to pH 5.5. The culture flasks were incubated an aerobically at 30^oC for 48 hours (using BBL Gas Pack, H_2 and $C0_2$ anaerobic system). At the end of incubation period, the broth was centrifuged at 5000g revolution per minutes for 10 minutes. The supernatant were decanted while the pellets were washed with sterile distilled water and used as the inoculums. 1ml of the inoculums (starter culture) produced a concentration of approximately $10^5 - 10^6$ cfu/ml when grown on MRS agar. This test was done according to (Huang and Lin 1993) procedure and (Burrows et al .,1986) method).

Preparation of Cassava for fermentation

Cassava tubers were cut into small pieces of about 3 – 5cm length. 200g of cut cassava tubers were separately weighed into Eight (8) different fermenter. The cut cassava tubers were sterilized using 0.1% Hg CL in 70% ethanol followed by rinsing with sterile distilled water. One of the fermenter (A) was inoculated with starter culture. Three fermenters labeled A_1, A_2, A_3 were used to determine the effect of particulate materials. These particulate materials, were added in varying concentrations into the fermenter. Fermenter C contained only cassava, and it served as control.

Legends/sample codes

A = 200g Cassava + Lactobacillus plantrum

A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.

A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.

A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml brewer soluble.

C = 200g Cassava only (Uninoculated) control.

Fermentation of Cassava samples using starter culture- Lactobacillus plantarium.
Fermenter A was singly inoculated with 3ml of starter culture, and it fermented for 72 hours at room temperature. Fermenter C was fermented for 72 hours at room temperature.

Effect of varying concentration of particulate materials on Lactic fermentation of cassava spontaneously.
Varying Concentration of particulate materials were added to each (3) three fermenters that contained 200g of sterile cassava tubers in this order. Fermenter A_1 contained 2.5g each of soy-bean husk, soy bean meal, alumina, 2.5g mash solid and 2.5ml brewer soluble.Fermenter A_2 contained 2.5g each of soy bean husk, soybean meal and alumina;1.5g mash solid and 1.5ml brewer soluble. Fermenter A_3 contained 1.5g each of soybean husk, soy bean mieal and alumina; 2.5g mash solid and 2.5ml brewer soluble.

Eavaluation of Total Dissolved Solid.
A method described by frank and Watkins (1950) was used to evaluate the total dissolved solid contents. 50ml of the sample was put in weighed crucible and heated to dryness in water bath. After heating the crucible was cooled in desiccator and reweighed.

Determination of the Concentration of Total Reducing Sugar
The DNSA reagent method of Miller (1959) was used to determine the concentration of total reducing sugar.

Biochemical (Proximate) Analysis of the Fermented Cassava Products in the Fermenters.
A method described by (A. O.A.C 1984) was used to estimiated crude protein, crude fat/ether, crude fibre contents and ash.

Nutritional Analysis of the Fermented Cassava Products in the Fermenters.
A method described by Maga (1982) was used to estimate phytic acid and a method described by Broadhurst and Jones (1978) was used to estimate tannin contents.

RESULTS
The predominant isolate during the spontaneous fermentation of cassava for 72 hours was identified as *Lactobacillus plantarium* and it was selected as starter culture for the fermentation.

Effect of varying concentration of particulate materials on total dissolved solids (mg/L) using spontaneous fermentation and starter culture- Lactobacillus plantarum is shown in Table1. Sample A and C had reduced total dissolved sol, while sample A_1, A_2, and A_3 had highest total dissolved solids. Sample A and C had their total dissolved solids increased from 300mg/L to 600mg/L after 72 hours of fermentation. While sample A_1, A_2 andA_3 had their total dissolved solids ranged form 600mg/L to 2,500mg/L after 72 hours of fermentation. After 72 hours of fermentation, sample A had total dissolved solid of 600mg/l, sample A_I had 2500mg/l, sample A_2 had 1100mg/l, sample A_3 had 1050mg/l and sample C had 600mg/l.

Table 2 showed the effect of varying concentration of particulate materials on total reducing sugar. At zero hour, total reducing sugars increased for all samples, later at 24hours, it reduced for all samples and increased again after 24hours for all the samples, till 72 hours of fermentation. Sample A had highest total reducing sugars of 6.4mg/L at 72hours, while sample C had lowest total reducing sugar of 4.8mg/L at 78hours of fermentation. Other samples had their total reducing sugar contents with approximately 6.2mg/L at 72 hours of fermentation.

Table 3 showed the effect of varying concentration of particulate materials on microbial load (cfu/ml). Samples A and C had increase in total lactic acid bacterial counts throughout the fermentation than total bacterial counts. In sample A, total lactic acid bacteria increased from 3.35×10^9 at 24 h to 5.50×10^9cfu/ml after 72 h of fermentation while that of total bacteria counts reduced from 1.32×10^9 at 24h to 1.23×10^9 cfu/ml after 72h of fermentation. For sample C, total lactic acid bacteria and total bacteria increased from 2.52×10^9 at 24h to 3.04×10^9cfu/ml after 72h and from 2.48×10^9 to 3.80×10^9 cfu/ml after 72h respectively.Other samples A_1,A_2,and A_3 had an increase in their total lactic acid bacteria counts ranging from 2.82×10^9 at 24h to 3.92×10^9cfu/ml after 72h of fermentation while their total bacteria counts ranging from 3.30×10^9 to 3.80×10^9 after 72h of fermentation.

Proximate composition of the entire sample at various 24, 48, 72 hours of fermentation are shown in Table 4, 5 and 6 respectively. Table 4 showed that, at 24 hours sample A had low crude protein, crude fibre, ether extract and phytic acid of 2.63%,2.82%,0.77% and 0.001% respectively compare with other sample Sample C that had lowest crude protein, fibre, ether extract, ash and tannins of 2.46%,1.40%,0.44%,1.52% and0.08% respectively. Sample A_1, A_3, had the highest crude protein ranging from 6.13% to 6.56%;. Sample A_2 had highest crude fibre and ash contents of 4.18% and 2.13% respectively

In table 5 at 48 hours of fermentation, sample A had highest crude protein content of 8.75%, while sample C had the least protein contents of 1.86% Crude protein contents for samples A_1,A_2 and A_3 ranged from 2.19% to 4.63%.Sample A_3 had highest crude fibre of 4.16%.

In table 6, at 72 hours of fermentation, sample A, A_1, had highest crude protein content of 9.19% and 8.38% respectively while Sample C had least crude protein content of 1.33% Sample A_2 and A had highest crude fibre content of 8.30% and 7.90% respectively..

From table 4, 5 and 6, sample A and A_1 had highest crude protein. Sample A and A_1 showed highest crude fibre. Sample A showed lowest phytic acids and Tannins than other samples. Only C had least value in crude protein, fibre, ether, phytic acid, and Tannins.

TABLE 1:- EFFECT OF VARYING CONCENTRATION OF PARTICULATE MATERIALS ON TOTAL DISSOLVED SOLIDS (mg/l) DURING FERMENTATION OF CASSAVA USING BOTH SPONTANEOUS AND STARTER CULTURE -*Lactobacillus plantarium*.

Samples	Fermentation	Time (Hours)	
	24	48	72
A	300mg/l	540mg/l	600mg/l
A_1	200mg/l	2400mg/l	2500mg/l
A_2	700mg/l	960mg/l	1,110mg/l
A_3	6000mg/l	960/mg/l	1050mg/l
C	300mg/l	520mg/l	600mg/l

Legends/sample codes
A = 200g Cassava + Lactobacillus plantrum
A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.
A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.
A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml brewer soluble.
C = 200g Cassava only (Uninoculated) control.

TABLE 2: EFFECTS OF VARYING CONCENTRATION OF PARTICULATE MATERIALS AND SOME OSMOREGULATORS ON TOTAL REDUCING SUGAR (mg/l) USING BOTH SPONTANEOUS AND STARTER CULTURE

Samples	Fermentation Time (Hours)			
	0	24	48	72
A	5.8mg/l	5.4mg/l	6.2mg/l	6.4mg/l
A_1	5.8mg/l	5.2mg/l	6.0mg/l	6.3mg/l
A_2	5.8mg/l	5.0mg/l	6.0mg/l	6.2mg/l
A_3	5.8mg/l	5.2mg/l	6.1mg/l	6.2mg/l
C	5.8mg/l	3.0mg/l	3.5mg/l	4.8mg/l

Legends/sample codes
A = 200g Cassava + Lactobacillus plantrum
A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.
A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.
A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml brewer soluble.
C = 200g Cassava only (Uninoculated) control.

TABLE 3:- EFFECT OF VARYING CONCENTRATION OF PARTICULATE MATERIALS ON MICRO BILA LOADS (cfu/ml) DURING FERMENTATION OF CASSAVA USING BOTH SP0NTANEOUS AND STATER CULTURE – Lactobacillus plantarum

Time	24 Hours		48 Hours		78 Hours	
Samples	Total bacteria count on PCA (cfu/ml)	Lactic Acid Bacteria counts on MRS (cfu/ll)	Total bacteria PCA (cfu/ml)	Lactic bacteria counts on MRS (cfu/ml)	Total bacteria counts PCA	Lactic acid counts on MRS (cfu/ml)
A	1.32 X 10^9	3.35 X 10^9	1.28 X 10^9	4.26 X 10^9	1.32 X 10^9	5.50 X 10^9
A_1	3.82 X 10^9	3.51 X 10^9	3.88 X 10^9	4.26 X 10^9	1.32 X 10^9	5.50 X10^9
A_2	3.31 X 10^9	2.82 X 10^9	3.35 X 10^9	3.06 X 10^9	3.28 X 10^9	3.28 X 10^9
A_3	3.30 X 10^9	2.87 X 10^9	3.23 X 10^9	3.34 X 10^9	3.35 X 10^9	3.40 X 10^9
C	2.48 X 10^9	2.52 X 10^9	2.60 X 10^9	2.89 X 10^9	2.80 X 10^9	3.04 X 10^9

TABLE 4:- EFFECT OF PARTICULATE ON PROMIXATE COMPOSITION FERMENTED CASSSAVA USING BOTH SPONTANEOUS AND STARTED CULTURE- (*L.plantarum*) AT 24HRS FERMENTATION

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
A	2.63	2.84	0.77	1.80	0.001	0.18
A1	6.13	4.16	1.11	1.14	0.015	0.08
A2	3.06	4.18	1.24	2.13	0.001	0.13
A3	6.56	2.94	.83	1.83	0.004	0.12
C	2.46	1.40	0.44	1.52	0.004	0.08

Table 5:- EFFECT OF PARTICULATE MATERIALS AND SOME OSMOREGULATORS ON PROXIMATE COMPOSITION OF FERMENTED CASSAVA USING BOTH SPONTANEOUS AND ATARTER CULTURE (*L. plantarum*) AT 48 HOURS OF FERMENTATION.

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
A	8.75	1.91	0.96	0.57	0.007	0.17
A1	3.50	1.81	1.42	3.95	0.012	0.28
A2	2.19	4.05	0.88	3.82	0.008	0.22
A3	4.63	4.16	1.01	3.78	0.006	0.21
C	1.86	2.60	0.72	1.64	0.006	0.10

Table 6: EFFECT OF PARTICULATE MATERIALS AND SOME OSMOREGULATORS ON PROXIMATE COMPOSITION OF FERMENTED CASSAVA USING BOTH SPONTANEOUS AND ATARTER CULTURE (*L. plantarum*) AT 72 HOURS OF FERMENTATION

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
A	9.19	7.90	0.18	1.95	0.007	0.21
A1	3.38	4.63	0.76	2.10	0.009	0.15
A2	2.63	8.30	1.01	1.93	0.041	0.38
A3	3.06	4.06	1.26	1.91	0.048	0.34
C	1.33	3.44	0.74	1.70	0.004	0.12

DISCUSSION OF RESULTS AND CONCLUSION

Fermented products of cassava constitute a major part of the daily diets of many homes in most part of West Africa countries.The most predominant bacteria in cassava fermentation processes is the Lactic acid bacteria of which *Lactobacillus plantarum* is the predominant amongst lactic acid bacteria.(Ngaba and Lee,1979,Okafor et al.,1984, Oyewole and Odunfa 1990).In this study, *Lactobacillus plantarum* was used as a starter culture in the fermentation of cassava.

Increase in total dissolved solids in sample A_1 and A_2,A_3 in table 1 may be due to the added materials that dissolved in the medium inspite of the ones consumed by the fermenting organisms. Decrease in total dissolved solids in samples A and C may be as a result of non added materials to the medium.

Increase in total reducing sugar content in sample A in table 2 after 24h is a confirmation of starch degrading potential and the added materials. Decrease in total reducing sugar in sample C as compare to other samples may be due to the utilization of available simple sugar for metabolic activities of the fermenting bacteria. Longe(1980) reported similar reduction in the total reducing sugar with in 24h of spontaneous fermentation. However increase in the total reducing sugar contents till the end of 72h of fermentation may be due to the action of other bacteria species which produces amylase necessary for breakdown of starch to sugar which are used for the growth of the lactic acid bacteria. Olatunji(1986) and Ejiofor and Okafor(1981) confirmed the activities of amylase for initial breakdown of cassava starch to simple sugar increase.

Increase in lactic acid bacteria counts recorded in all samples in table 3 may be due to their acid tolerant. Decrease in total bacteria counts in all the samples in table 3 may be due to the high acidity of the fermenting medium created by lactic acid bacteria, which they cannot tolerate. Though varying concentration of particulate materials on the medium provide medium of growth for lactic acid bacteria which enable them to produce more acid that suppress the growth of other bacteria.

Increase in crude protein contents recorded in sample A and other samples may be as a result of the contributing protein content of the added materials and lactic acid bacteria involved or added as a starter culture in the fermentation. Decrease in crude protein contents in sample C may be as a result of non added particulate materials and the starter culture. Increase in crude fibre contents, ether extract and ash contents in all samples till 72h of fermentation, may be due to the added particulate materials while non inclusion of particulate materials in samples A and C reduce their proximate composition.

The use of starter culture therefore can be employed to control the acid content of the fermenting medium to inhibit and discourage undesirable bacterial from the medium, to control fermenting time, improve odour and flavor and nutritional value of cassava fermenting products. Moreover addition of appropriate concentration of particulate materials to the fermenting medium of cassava can increase the growth of lactic acid bacterial and this in addition can improve and better produce acceptable nutritional value of cassava products than naturally fermented cassava products.

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12/12/2009

Floristic structure and phytodiversity along an elevational gradient in Peepalkoti-Joshimath area of Garhwal Himalaya, India

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Abstract: The present study was conducted in temperate Himalayan forests of Joshimath area in Chamoli district of Uttarakhand to understand the effect of altitudinal variation on structure and composition of the vegetation and to record the floristic diversity and economic utilities of the plants in the study area. Three altitudinal zones viz., upper zone (U) = 2000-2200m asl, middle zone (M) = 1800-2000m asl and lower zone (L) = 1600-1800m asl were selected for the study. In the present floristic survey the total of 74 families (72 Angiospermous and 2 Gymnospermous), 149 Genera (145 Angiospermous and 4 Gymnospermous) and 177 species (173 Angiospermous and 4 Gymnospermous) were recorded in the study area. Out of these 177 species identified in the study area 100, 47, 20 and 10 were herbs, shrubs, trees and climbers respectively. Rosaceae was the dominant family recorded with 16 species in the study area followed by the Asteraceae (15), Lamiaceae (11), Fabaceae (11) and Caryophyllaceae (5). In Ethnobotanical survey very useful information was recorded about the economic utility of the plants species present in the study area. Uses recorded were medicinal, fuel, fodder, edible and timber. Tree Species richness (SR) decreased from lower altitude to higher altitude. Species diversity (richness) and dominance (Simpson index) were found to be inversely related to each other. Tree density decreased from lower altitude to upper altitude, whereas TBC showed reverse trend.

[V P Bhatt, Vijay Kant Purohit, **Floristic structure and phytodiversity along an elevational gradient in Peepalkoti-Joshimath area of Garhwal Himalaya, India**. Nature and Science 2011;9(1):57-67]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Phytosociology, floristic composition, diversity indices, economic utility of plants, altitude.

Introduction

The Indian Himalayan region occupies a special place in the mountain ecosystems of the world. These geodynamically young mountains are not only important from the stand point of climate and as a provider of life, giving water to a large part of the Indian subcontinent, but they also harbor a rich variety of flora, fauna, human communities and cultural diversity (Singh, 2006). The biodiversity which few years ago was considered unimportant by ecosystem ecologists, has now been shown to be significantly important for many aspects of ecosystem functioning. Diversity at all organizational levels, ranging from genetic diversity within populations to the diversity of ecosystems in landscapes, contributes to global biodiversity. The biodiversity has long been a source of amazement and scientific curiosity and increasingly a source of concern. Understanding of forest structure is a pre-requisite to describe various ecological processes and also to model the functioning and dynamics of forests (Elourard *et al.*, 1997).

Species diversity has functional consequences, because the number and kinds of species present in any area determine the organismal traits, which influence ecosystem processes. The components of species diversity that determine the expression of traits include the number of species present (species richness), their relative abundance (species evenness), presence of the particular species (species composition), the interactions among species (non-additive effects), and the temporal and spatial variation in these properties. In addition to its effects on current functioning of ecosystems, species diversity influences the resilience and resistance of ecosystems to environmental changes (Chapin *et al.*, 2000).

The altitude and aspect play a key role in determining the temperature regime and atmospheric pressure of any site. Within one altitude the cofactors like topography, aspect, inclination of slope and soil type affect the forest composition (Shank and Noorie, 1950). The micro-environment of different aspects of hill slopes is influenced by the intensity and duration of available sunlight (Yadav and Gupta, 2006). This type of ecological knowledge is fundamental for conservation and sustainable utilization, and may provide important information for the policy makers for drafting management plans of fragile mountain ecosystems. Under the backdrop of the aforesaid facts,

the present study was undertaken in temperate Himalayan forests of Bajoli-Holi area of Chamba district in Himachal Pradesh, 1) to record plant species present in the study area along with their economic uses and 2) to understand the effect of altitude on the structure and composition of the vegetation of natural forests.

Material and Methods

The present study was conducted in temperate Himalayan forests of Joshimath area in Chamoli district of Uttarakhand in year 2008. After the reconnaissance survey three altitudinal zones viz., upper zone (U) = 2200-2000m asl, middle zone (M) = 2000-1800m asl and lower zone (L) = 1800-1600m asl were identified to study the effect of altitudinal variation on structure and composition of the vegetation. The climate of the study area is typical temperate type. The year is represented by three main seasons; the cool and relatively dry winter (December to March); the warm and dry summer (mid-April to June); and a warm and wet period (July to mid-September) called as the monsoon or rainy season. The rainy season accounts for about three-quarters of the annual rainfall. Apart from these main seasons, the transitional periods interconnecting rainy and winter, and winter and summer are referred to as autumn (October to November) and spring (February to March). The mean annual rainfall was recorded as 1500mm and mean annual temperature between 5°C to 28°C.

The composition of the forest along the altitudinal gradient was analysed by using nested quadrate method or centre point quadrat method for trees, shrubs and herbs species as per Kent and Coker (1992). Three vegetation layers, (i.e., trees, shrubs and herbs) were analyzed for species richness, density and diversity. A total of 60 plots (twenty plots in each forest type) measuring 10m X 10m each were sampled. Trees (≥10cm dbh) were analyzed by 10m x 10m sized quadrats, whereas shrubs by 5m x 5m sized quadrats. Further, quadrats of 1x1m size were randomly laid out with in each 10x10m sized quadrat at each site, to study plants in the herb layer. Circumference at breast height (cbh= 1.37m) was taken for the determination of tree basal area and was calculated as πr^2, where r is the radius. Total basal area/cover is the sum of basal area/cover of all species present in the forest. The data were quantitatively analyzed for density, frequency and abundance following Curtis and McIntosh (1950). Species Richness was simply taken as a count of number of species present in that forest type. Basal area (m^2/ha) was used to determine the relative dominance of a tree species. Importance Value Index (IVI) was the sum of relative frequency, relative density and relative dominance (Phillips, 1959). The diversity (H') was determined by using Shannon-Wiener information index (Shannon and Weaver, 1963) as: $H' = - \sum n_i /n \log_2 n_i /n$; where, n_i was the IVI value of a species and n was the sum of total IVI values of all species in that forest type. The Simpson's concentration of dominance (Simpson, 1949) was measured as: $Cd= \sum Pi^2$, where, $\sum Pi = \sum n_i /n$, where, ni and n are same as in Shannon-Wiener diversity index. Simpson's diversity index (Simpson, 1949) was calculated as: D = 1-Cd, where, D = Simpson's diversity and Cd = Simpson's concentration of dominance. Species heterogeneity was calculated as under root of concentration of dominance (Cd).

To study the phytodiversity in the study area, regular field trips were undertaken in different seasons i.e., rainy, winter and summer, to collect the specimens of higher plants (Gymnosperms and Angiosperms). Identification of the specimens was done with the help of the existing Herbariums of Botany Department HNB Garhwal University (GUH), Forest Research Institute (DD) and Botanical Survey of India, Northern Circle (BSD). After identification, the enumeration of plants was done according to Bentham and Hooker's system of classification (1862-1883). The plants were divided into categories of common and uncommon according to their occurrence in the study area. An Ethnobotanical survey was also conducted in the villages nearby the study area to know the economic utility of various plant species encountered.

Results

Forest community structure and composition: Results of forest community structure and composition are given in tables 1 to 3.

Trees: At upper altitude *Cedrus deodara* was the dominant tree species with highest density (170 Ind/ha), TBC (98.82 m^2/ha) and IVI (155.96). At middle altitude *Pinus wallichiana* was the dominant tree species with highest density (180 Ind/ha), TBC (84.41 m^2/ha) and IVI (120.59). At lower altitude *Alnus nepalensis* was the dominant tree species with highest density (340 Ind/ha) and IVI (85.90), whereas highest TBC (3.78 m^2/ha) at this altitude was recorded for *Quercus semecarpifolia*. Tree Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (19) altitude followed by middle (8) and upper (3) altitude. Highest (800 Ind/ha) tree density was recorded at lower zone followed by middle (600 Ind/ha) and lower (330 Ind/ha) altitudinal zone, where as highest (181.5 m^2/ha) TBC was recorded at upper altitude followed by middle (143.05 m^2/ha) and lower (9.63 m^2/ha) altitudes. Tree density decreased from lower altitude to upper altitude, whereas TBC showed reverse trend. Cd was found to be highest (0.4328) on upper altitude followed by middle (0.2561) and lower (0.1958) altitude whereas Simpson's diversity index showed reverse trend with highest (6.80)

value at lower altitude followed by middle (6.74) and upper (2.57) altitude. Value H' was found to be highest (0.67) at upper altitude followed by middle (0.28) and lower (0.15) altitude.

Shrubs: At upper altitude *Rabdosia rugosa* was the dominant shrub species with highest density (520 Ind/ha) and TBC (0.3600 m^2/ha), whereas highest IVI (82.38 m^2/ha) at this altitude was recorded for *Corairia nepalensis.* At middle altitude *Rabdosia rugosa* was the dominant shrub species with highest density (680 Ind/ha), TBC (0.4310 m^2/ha) and IVI (89.98). At lower altitude *Desmodium elegans* was the dominant shrub species with highest density (440 Ind/ha), TBC (0.1300 m^2/ha) and IVI (70.27). Shrub Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (22) altitude followed by middle (10) and upper (7) altitude. Highest (2420 Ind/ha) density was recorded at middle altitude followed by lower (2020 Ind/ha) and upper (1620 Ind/ha) altitudinal zone, where as highest TBC (1.21 m^2/ha) was recorded at middle altitude followed by upper (0.75 m^2/ha) and lower (0.39 m^2/ha) altitudes. Cd was found to be highest (0.1996) on middle altitude followed by upper (0.1896) and lower (0.1138) altitude, whereas H' was found to be highest (0.17) at middle altitude followed by upper (0.14) and lower (0.06) altitude. Simpson's diversity index varied between 15.89 (lower altitude) to 7.81 (upper altitude).

Herbs: At upper altitude *Galium* sp. was the dominant herb species with highest density (15000 Ind/ha), TBC (0.0183 m^2/ha) and IVI (54.36). At middle altitude *Geranium* sp. was the dominant herb species with highest density (28750 Ind/ha), TBC (0.0760 m^2/ha) and IVI (70.52). At lower altitude *Pilea umbrosa* was the dominant herb species with highest density (16250 Ind/ha), TBC (0.0191 m^2/ha) and IVI (44.31). Herb Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (19) altitude followed by middle (16) and upper (7) altitude. Highest (174375 Ind/ha) density was recorded at middle altitude followed by lower (136250 Ind/ha) and upper (112500 Ind/ha) altitudinal zone, where as highest TBC (0.17 m^2/ha) was recorded at middle altitude followed by lower (0.08 m^2/ha) and upper (0.06 m^2/ha) altitudes. Cd was found to be highest (0.0961) on middle altitude followed by upper (0.0777) and lower (0.0711) altitude, whereas H' was found to be highest (0.05) at middle altitude followed by upper (0.03) and lower (0.02) altitude. Simpson's diversity index varied between 21.90 (middle altitude) to 18.93 (lower altitude).

Phytodiversity: In the present floristic survey the total of 74 families (72 Angiospermous and 2 Gymnospermous), 149 Genera (145 Angiospermous and 4 Gymnospermous) and 177 species (173 Angiospermous and 4 Gymnospermous) were recorded in the study area (table 4). Out of these 177 species identified in the study area 100, 47, 20 and 10 were herbs, shrubs, trees and climbers respectively. Rosaceae was the dominant family recorded with 16 species in the study area followed by the Asteraceae (15), Lamiaceae (11), Fabaceae (11) and Caryophyllaceae (5). Families with only one species were Agavaceae, Anacardiaceae, Aquifoliaceae, Araliaceae, Asclepidaceae, Berberidaceae, Betulaceae, Buxaceae, Cannabinaceae, Chenopodiaceae, Coriariaceae, Crassulaceae, Cucurbitaceae, Cuperasaceae, Cuscutaceae, Dioscoreaceae, Dipsacaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Hippocastanaceae, Hydrangeaceae, Juglandaceae, Lythraceae, Malvaceae, Meliaceae, Mimosaceae, Nictaginaceae, Orchidaceae, Oxalidaceae, Philadelphaceae, Phytolaccaceae, Plantaginaceae, Polygalaceae, Primulaceae, Rhamnaceae, Rutaceae, Saxifragaceae, Smilacaceae and Vitaceae. Families with two species were Boraginaceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Onagraceae, Salicaceae, Thymelaeaceae, Ulmaceae, Urticaceae and Violaceae. Families with three species were Acanthaceae, Amaranthaceae, Araceae, Balsaminaceae, Cyperaceae, Moraceae, Oleaceae, Pinaceae, Rubiaceae, Scrophulariaceae and Solanaceae. Families with four species were Apiaceae, Hypericaceae, Poaceae, Polygonaceae and Ranunculaceae. In Ethnobotanical survey of the plant species present in the study area, very useful information was recorded about the economic utility of the plants. Uses recorded were medicinal, fuel, fodder, edible and timber and results are shown in the table 4.

Table 1: Analytical characters for different forest types.

	Density (Ind/ha)			TBC (m^2/ha)			IVI		
Trees	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Aesculus indica	-	40	-	-	3.41	-	-	19.58	-
Alnus nepalensis	30	120	340	0.28	3.76	0.96	25.91	33.15	85.80
Cedrus deodara	170	140	-	98.82	49.32	-	155.96	78.86	-
Celtis australis	-	-	30	-	-	0.35	-	-	15.72
Lyonia ovalifolia	-	40	50	-	1.20	1.26	-	18.03	27.67
Pinus wallichiana	130	180	-	82.40	84.41	-	118.13	120.59	-

Populus ciliata	-	-	40	-	-	0.84	-	-	22.06
Pyrus pashia	-	30	80	-	0.25	1.49	-	10.44	37.97
Quercus semecarpifolia	-	-	190	-	-	3.78	-	-	79.67
Salix alba	-	50	70	-	0.70	0.95	-	19.35	31.12
	330	600	800	181.50	143.05	9.63	300.00	300.00	300.00
Shrubs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Berberis sp	-	120	60	-	0.0040	0.0040	-	10.55	8.26
Buddleja paniculata	-	-	80	-	-	0.0100	-	-	10.80
Corairia nepalensis	340	440	-	0.3300	0.3100	-	82.38	59.61	-
Cotoneaster baccilaris	-	60	-	-	0.0020	-	-	5.28	-
Cotoneaster microphyllus	40	-	60	0.0004	-	0.0100	8.40	-	9.81
Daphne retusa	-	-	80	-	-	0.0050	-	-	11.64
Dapnae sp.	100	140	-	0.0020	0.0050	-	35.85	14.09	-
Desmodium elegans	360	520	440	0.3600	0.4300	0.1300	75.82	72.84	70.27
Deutzia compacta	-	-	160	-	-	0.0200	-	-	23.73
Elaeagnus conferta	-	-	140	-	-	0.0600	-	-	28.82
Lonicera quinquelocularis	-	-	40	-	-	0.0030	-	-	4.88
Princepia utilis	-	60	100	-	0.0020	0.0200	-	7.91	16.50
Rabdosia rugosa	520	680	320	0.0500	0.4310	0.0500	47.55	89.98	39.40
Rhamnus persica	-	-	60	-	-	0.0040	-	-	8.26
Rhamnus sp.	40	60	-	0.0010	0.0040	-	20.25	8.07	-
Rhamnus virgatus	-	-	40	-	-	0.0020	-	-	4.62
Rubus foliolosus	-	-	40	-	-	0.0030	-	-	4.88
Rubus niveus	-	-	80	-	-	0.0050	-	-	11.64
Sorbaria tomentosa	120	200	240	0.0100	0.0200	0.0600	20.50	20.45	35.90
Wikstroemia canescens	100	140	80	0.0010	0.0020	0.0010	9.25	11.21	10.60
	1620	2420	2020	0.7544	1.2090	0.3870	300.00	300.00	300.00
Herbs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Ajuga paviflora	-	-	5000	-	-	0.0008	-	-	9.25
Arisaema sp.	2500	1875	-	0.0004	0.0002	-	5.49	3.44	-
Artemisia capillaris	-	-	2500	-	-	0.0004	-	-	5.40
Bidens pilosa	-	-	5000	-	-	0.0018	-	-	8.93
Chenopodium album	3750	-	-	0.0006	-	-	9.33	-	-
Chenopodium sp.	-	5000	2500	-	0.0016	0.0004	-	9.19	5.40
Circium sp.	-	3750	-	-	0.0011	-	-	6.09	-
Circium verutum	2500	-	-	0.0004	-	-	5.49	-	-
Clinopodium sp.	6250	5000	4375	0.0030	0.0016	0.0008	15.63	8.15	7.26
Conyza japonica	-	-	4375	-	-	0.0011	-	-	7.62
Cynoglossum glochidium	3750	7500	8750	0.0006	0.0046	0.0062	8.14	12.41	20.09
Elsholtzia sp.	5000	8750	-	0.0018	0.0062	-	12.48	12.01	-
Eriophorum comosum	-	-	6250	-	-	0.0029	-	-	11.18
Fragarea sp.	5000	-	-	0.0016	-	-	12.14	-	-
Fragaria nubicola	-	5625	-	-	0.0011	-	-	8.21	-
Galium sp.	15000	17500	10000	0.0183	0.0183	0.0046	54.36	27.54	20.61
Geranium sp.	11250	28750	-	0.0058	0.0760	-	27.42	70.52	-
Hypericum elodeoides	-	3750	-	-	0.0008	-	-	4.88	-
Impatiens sp.	6250	8750	7500	0.0029	0.0062	0.0050	16.65	14.08	17.72
Lactuca sp.	2500	2500	-	0.0004	0.0004	-	5.49	3.92	-
Malva verticilata	-	-	5000	-	-	0.0018	-	-	8.93
Micromeria biflora	-	-	6250	-	-	0.0023	-	-	11.99

Origanum vulgare	8750	11250	9375	0.0050	0.0103	0.0072	23.84	19.18	21.76
Oxalis acetocella	7500	23125	10000	0.0030	0.0220	0.0062	16.74	31.76	22.55
Phytolacca acinosa	5000	5000	-	0.0050	0.0026	-	17.91	9.77	-
Pilea umbrosa	-	-	16250	-	-	0.0191	-	-	44.31
Pimpinella sp.	3750	11250	8750	0.0006	0.0080	0.0046	9.33	17.80	18.15
Plantago sp.	-	2500	-	-	0.0002	-	-	3.80	-
Polygonum sp.	-	-	11250	-	-	0.0109	-	-	29.16
Prunella vulgare	7500	5000	5625	0.0050	0.0018	0.0018	21.33	8.27	12.46
Salvia moocroftiana	-	-	7500	-	-	0.0046	-	-	17.23
Salvia sp.	3750	3750	-	0.0006	0.0006	-	9.33	6.83	-
Stellarea sp.	5000	7500	-	0.0016	0.0029	-	13.34	11.40	-
Thalictrum sp.	-	2500	-	-	0.0002	-	-	3.80	-
Viola sp.	7500	3750	-	0.0023	0.0008	-	15.55	6.95	-
	112500	174375	136250	0.0589	0.1675	0.0825	300.00	300.00	300.00

Abbreviations: U= Upper altitude; M= Middle altitude; L= Lower altitude; TBC= Total Basal Cover; IVI= Importance Value Index.

Table 2: Diversity Indices of different forest types.

	Cd			SDI			H'			Heterogeneity		
Trees	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Aesculus indica	-	0.0043	-	-	0.9957	-	-	0.00	-	-	0.07	-
Alnus nepalensis	0.0075	0.0122	0.0818	0.9925	0.9878	0.9182	0.0021	0.00	0.08	0.09	0.11	0.29
Cedrus deodara	0.2703	0.0691	-	0.7297	0.9309	-	0.4666	0.06	-	0.52	0.26	-
Celtis australis	-	-	0.0027	-	-	0.9973	-	-	0.00	-	-	0.05
Lyonia ovalifolia	-	0.0036	0.0085	-	0.9964	0.9915	-	0.00	0.00	-	0.06	0.09
Pinus wallichiana	0.1550	0.1616	-	0.8450	0.8384	-	0.2027	0.22	-	0.39	0.40	-
Populus ciliata	-	-	0.0054	-	-	0.9946	-	-	0.00	-	-	0.07
Pyrus pashia	-	0.0012	0.0160	-	0.9988	0.9840	-	0.00	0.01	-	0.03	0.13
Quercus semecarpifolia	-	-	0.0705	-	-	0.9295	-	-	0.06	-	-	0.27
Salix alba	-	0.0042	0.0108	-	0.9958	0.9892	-	0.00	0.00	-	0.06	0.10
	0.4328	0.2561	0.1958	2.5672	6.7439	6.8042	0.6715	0.28	0.15	1.00	1.00	1.00
Shrubs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Berberis sp	-	0.0012	0.0008	-	0.9988	0.9992	-	0.00	0.00	-	0.04	0.03
Buddleja paniculata	-	-	0.0013	-	-	0.9987	-	-	0.00	-	-	0.04
Corairia nepalensis	0.0754	0.0395	-	0.9246	0.9605	-	0.0688	0.03	-	0.27	0.20	-
Cotoneaster baccilaris	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02	-
Cotoneaster microphyllus	0.0008	-	0.0011	0.9992	-	0.9989	0.0001	-	0.00	0.03	-	0.03
Daphne retusa	-	-	0.0015	-	-	0.9985	-	-	0.00	-	-	0.04
Dapnae sp.	0.0143	0.0022	-	0.9857	0.9978	-	0.0057	0.00	-	0.12	0.05	-
Desmodium elegans	0.0639	0.0590	0.0549	0.9361	0.9410	0.9451	0.0536	0.05	0.04	0.25	0.24	0.23
Deutzia compacta	-	-	0.0063	-	-	0.9937	-	-	0.00	-	-	0.08
Elaeagnus conferta	-	-	0.0092	-	-	0.9908	-	-	0.00	-	-	0.10
Lonicera quinquelocularis	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Princepia utilis	-	0.0007	0.0030	-	0.9993	0.9970	-	0.00	0.00	-	0.03	0.06
Rabdosia rugosa	0.0251	0.0900	0.0172	0.9749	0.9100	0.9828	0.0132	0.09	0.01	0.16	0.30	0.13
Rhamnus persica	-	-	0.0008	-	-	0.9992	-	-	0.00	-	-	0.03
Rhamnus sp.	0.0046	0.0007	-	0.9954	0.9993	-	0.0010	0.00	-	0.07	0.03	-
Rhamnus virgatus	-	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.02
Rubus foliolosus	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Rubus niveus	-	-	0.0015	-	-	0.9985	-	-	0.00	-	-	0.04
Sorbaria tomentosa	0.0047	0.0046	0.0143	0.9953	0.9954	0.9857	0.0011	0.00	0.01	0.07	0.07	0.12

Wikstroemia canescens	0.0009	0.0014	0.0012	0.9991	0.9986	0.9988	0.0001	0.00	0.00	0.03	0.04	0.04
	0.1896	0.1996	0.1138	7.8104	9.8004	15.8862	0.1435	0.17	0.06	1.00	1.00	1.00
Herbs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Ajuga paviflora	-	-	0.0010	-	-	0.9990	-	-	0.00	-	-	0.03
Arisaema sp.	0.0003	0.0001	-	0.9997	0.9999	-	0.0000	0.00	-	0.02	0.01	-
Artemisia capillaris	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Bidens pilosa	-	-	0.0009	-	-	0.9991	-	-	0.00	-	-	0.03
Chenopodium album	0.0010	-	-	0.9990	-	-	0.0001	-	-	0.03	-	-
Chenopodium sp.	-	0.0009	0.0003	-	0.9991	0.9997	-	0.00	0.00	-	0.03	0.02
Circium sp.	-	0.0004	-	-	0.9996	-	-	0.00	-	-	0.02	-
Circium verutum	0.0003	-	-	0.9997	-	-	0.0000	-	-	0.02	-	-
Clinopodium sp.	0.0027	0.0007	0.0006	0.9973	0.9993	0.9994	0.0005	0.00	0.00	0.05	0.03	0.02
Conyza japonica	-	-	0.0006	-	-	0.9994	-	-	0.00	-	-	0.03
Cynoglossum glochidium	0.0007	0.0017	0.0045	0.9993	0.9983	0.9955	0.0001	0.00	0.00	0.03	0.04	0.07
Elsholtzia sp.	0.0017	0.0016	-	0.9983	0.9984	-	0.0002	0.00	-	0.04	0.04	-
Eriophorum comosum	-	-	0.0014	-	-	0.9986	-	-	0.00	-	-	0.04
Fragarea sp.	0.0016	-	-	0.9984	-	-	0.0002	-	-	0.04	-	-
Fragaria nubicola	-	0.0007	-	-	0.9993	-	-	0.00	-	-	0.03	-
Galium sp.	0.0328	0.0084	0.0047	0.9672	0.9916	0.9953	0.0198	0.00	0.00	0.18	0.09	0.07
Geranium sp.	0.0084	0.0553	-	0.9916	0.9447	-	0.0025	0.04	-	0.09	0.24	-
Hypericum elodeoides	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02	-
Impatiens sp.	0.0031	0.0022	0.0035	0.9969	0.9978	0.9965	0.0006	0.00	0.00	0.06	0.05	0.06
Lactuca sp.	0.0003	0.0002	-	0.9997	0.9998	-	0.0000	0.00		0.02	0.01	-
Malva verticilata	-	-	0.0009	-	-	0.9991	-	-	0.00	-	-	0.03
Micromeria biflora	-	-	0.0016	-	-	0.9984	-	-	0.00	-	-	0.04
Origanum vulgare	0.0063	0.0041	0.0053	0.9937	0.9959	0.9947	0.0017	0.00	0.00	0.08	0.06	0.07
Oxalis acetocella	0.0031	0.0112	0.0056	0.9969	0.9888	0.9944	0.0006	0.00	0.00	0.06	0.11	0.08
Phytolacca acinosa	0.0036	0.0011	-	0.9964	0.9989	-	0.0007	0.00	-	0.06	0.03	-
Pilea umbrosa	-	-	0.0218	-	-	0.9782	-		0.01	-	-	0.15
Pimpinella sp.	0.0010	0.0035	0.0037	0.9990	0.9965	0.9963	0.0001	0.00	0.00	0.03	0.06	0.06
Plantago sp.	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.01	-
Polygonum sp.	-	-	0.0094	-	-	0.9906	-	-	0.00	-	-	0.10
Prunella vulgare	0.0051	0.0008	0.0017	0.9949	0.9992	0.9983	0.0012	0.00	0.00	0.07	0.03	0.04
Salvia moocroftiana	-	-	0.0033	-	-	0.9967	-	-	0.00	-	-	0.06
Salvia sp.	0.0010	0.0005	-	0.9990	0.9995	-	0.0001	0.00	-	0.03	0.02	-
Stellarea sp.	0.0020	0.0014	-	0.9980	0.9986	-	0.0003	0.00	-	0.04	0.04	-
Thalictrum sp.	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.01	-
Viola sp.	0.0027	0.0005	-	0.9973	0.9995	-	0.0005	0.00	-	0.05	0.02	-
	0.0777	0.0961	0.0711	18.9223	21.9039	18.9289	0.0291	0.05	0.02	1.00	0.80	0.00

Abbreviations: U= Upper altitude; M= Middle altitude; L= Lower altitude; Cd= Simpson's Concentration of Dominance; SDI= Simpson's Diversity Index; H'= Shannon-Wiener Diversity Index.

Table 3: Total Diversity Indices of different forest types.

		Density (Ind/ha)	TBC (m^2/ha)	Cd	SWDI	H'	SR
Trees	**Upper**	330	181.50	0.4328	2.57	0.67	3
	Middle	600	143.05	0.2561	6.74	0.28	8
	Lower	800	9.63	0.1958	6.80	0.15	19
Shrubs	**Upper**	1620	0.75	0.1896	7.81	0.14	7
	Middle	2420	1.21	0.1996	9.80	0.17	10
	Lower	2020	0.39	0.1138	15.89	0.06	22
Herbs	**Upper**	112500	0.06	0.0777	18.92	0.03	7
	Middle	174375	0.17	0.0961	21.90	0.05	16
	Lower	136250	0.08	0.0711	18.93	0.02	19

Table 4: Details and uses of the plant species recorded in the Chamoli-Joshimath study area.

Botanical Name	Family	Occurrence	Economic Utility	LF
Abelia triflora	Caprifoliaceae	UC	Fu	S
Abies pindrow	Pinaceae	C	Tm, Me	T
Achyranthes bidentata	Amaranthaceae	UC	-	H
Adenocaulon himalaicum	Asteraceae	C	Me	H
Aesculus indica	Hippocastanaceae	UC	Fo, Me	T
Agave americana	Agavaceae	C	Me	S
Agrimonia pilosa	Rosaceae	C	Me	H
Albizia julibrissin	Mimosaceae	UC	Me	T
Alnus nitida	Betulaceae	C	Fu	T
Alpinia mutica	Poaceae	C	Me	H
Amaranthes viridis	Amaranthaceae	C	Me	H
Ammi majus	Asteraceae	UC	Me	H
Anaphalis contrata	Asteraceae	C	Me	H
A. triplinervis	Asteraceae	C	Me	H
Andropogon controtus	Poaceae	C	Me	H
Androsace sp.	Primulaceae	UC	-	H
Anemone sp.	Rosaceae	UC	Me	H
A. vitifolia	Rosaceae	UC	Me	H
Aquilegia pubiflora	Aquifoliaceae	UC	Me	H
Arghis sp.	Brassicaceae	C	Me	H
Arctium lappa	Asteraceae	C	Me	H
Arenaria sp.	Caryophyllaceae	C	Me	H
Arisaema sp.	Araceae	C	-	H
A. concinnum	Araceae	UC	Me	H
Artemisia capillaris	Asteraceae	C	-	H
A. roxburghiana	Asteraceae	UC	Me	H
Astragalus chlorostachys	Fabaceae	C	Me	S
Barleria cristata	Acanthaceae	UC	Me	S
Berberis angulosa	Berberidaceae	C	-	S
Bergenia ciliata	Saxifragaceae	C	Me	H
Boerhavia diffusa	Nictaginaceae	C	Me	H
Buddleja paniculata	Scrophulariaceae	C	Fu	S
Bupleurum falcatum	Apiaceae	UC	Me	H
Campanula sp.	Campanulaceae	UC	-	H
Canabis sativa	Cannabinaceae	C	Me	S
Carex sp.	Cyperaceae	C	-	H
Cedrus deodara	Pinaceae	UC	Tm, Me	T
Celtis australis	Ulmaceae	C	Ed, Fu	T
Cerastrium sp.	Caryophyllaceae	C	Fo	H
Chenopodium album	Chenopodiaceae	C	Ed	H
Cichorium intybus	Asteraceae	C	-	H
Circium verutum	Asteraceae	UC	Me	H
Clematis connata	Ranunculaceae	C	Me	C
Clinopodium sp.	Lamiaceae	C	Me	H
Corarla nepalensis	Corlariaceae	C	Fu	S
Cotoneaster baccilaris	Rosaceae	C	Fu	S
C. microphyllus	Rosaceae	C	Fu	S
Cupressus torulosa	Cuperasaceae	UC	Tm, Me	T
Cuscuta reflexa	Cuscutaceae	C	Me	H
Cyathula tomentosa	Amaranthaceae	C	Me	H
Cynodon dactylon	Poaceae	C	Me	H
Cynoglossum glochidiatum	Boraginaceae	C	Me	H
C. lanceolatum	Boraginaceae	UC	Me	H
Daphne retusa	Thymelaeaceae	UC	-	S
Datura sp.	Solanaceae	UC	Me	H
Delphinium danudatum	Ranunculaceae	UC	Me	H
Desmodium elegans	Fabaceae	C	Me, Fu	H
D. multiflorum	Fabaceae	C	Fu	H
Deutzia compacta	Hydrangeaceae	C	Fu	S
Dioscorea deltoidea	Dioscoreaceae	C	Me	H
Dipsacus mitis	Dipsacaceae	UC	-	H
Elaeagnus conferta	Elaeagnaceae	C	Ed	S
Elsholtzia sp.	Lamiaceae	C	-	H
E. fruticosa	Lamiaceae	C	Fu	S
E. flava	Lamiaceae	C	Me	S
Epilobium sp.	Onagraceae	UC	Me	H
Erigeron sp.	Asteraceae	UC	-	H
Eriophorum comosum	Cyperaceae	C	Fo	H
Erysimum hieraciifolium	Brassicaceae	UC	Me	H
Euphorbia sp.	Euphorbiaceae	C	Fu	S
Euphrasia himaliana	Scrophulariaceae	C	Me	H
Fagopyrum dibotryis	Polygonaceae	C	Ed	H
F. pallida	Campanulaceae	UC	-	H
Fallopia pterocarpa	Polygonaceae	UC	-	H

Philadelphus tomentosus	Philadelphaceae	UC	Ed, Fu	T
Phyllanthus acinosa	Phyllanthaceae	C	Ed, Me	C
Pimpinella sp.	Apiaceae	UC	Ed	H
Pinus wallichiana	Pinaceae	UC	Tm, Me	T
Plantago himalaica	Plantaginaceae	C	Me	H
Polygala sp.	Polygalaceae	UC	-	H
Populus ciliata	Salicaceae	UC	Fo, Me	T
Potentilla sp.	Rosaceae	UC	Me	H
Princepia utilis	Rosaceae	C	Me, Fu	S
Pteracanthus alatus	Acanthaceae	C	-	H
Pyrus pashia	Rosaceae	C	Ed, Fu	T
Quercus semecarpifolia	Fagaceae	C	Fo, Me	T
Rabdosia rugosa	Lamiaceae	C	Fu	H
Ranunculus sp.	Ranunculaceae	UC	Me	H
Rhamnus persica	Rhamnaceae	UC	-	H
R. virgatus	Rhamnaceae	UC	Fu	H
Rhus javanica	Anacardiaceae	UC	Fo, Me	T
Rosa brunonii	Rosaceae	C	-	S
Rosularia sp.	Crassulaceae	UC	-	H
Rubia cordifolia	Rubiaceae	UC	Me	C
Rubus ellipticus	Rosaceae	C	Ed	S
R. foliolosus	Rosaceae	C	Ed	S
R. prostrata	Rosaceae	C	-	H
Rumex hastatus	Polygonaceae	C	Ed, Me	H
R. nepalensis	Polygonaceae	C	Me	H
Salix alba	Salicaceae	C	Fo	T
Salvia sp.	Lamiaceae	UC	Fu	H
S. mocrotianna	Lamiaceae	C	-	H
Sarcococca saligna	Buxaceae	C	-	S
Saussurea albscens	Asteraceae	C	-	H
Sedum multicaule	Crassulaceae	UC	-	H
Selinum vaginatum	Apiaceae	UC	Me	H
Senecio chrysanthamoides	Asteraceae	UC	-	H
Silene sp.	Caryophyllaceae	C	Fo	H
S. edgeworthii	Caryophyllaceae	C	-	H
Smilax aspra	Smilacaceae	UC	Me	H
Solanum sp.	Solanaceae	C	-	H
S. nigrum	Solanaceae	UC	Me	H
Solena heterophylla	Cucurbitaceae	UC	Ed, Fo	H
Peristrophe paniculata	Acanthaceae	UC	-	H

Sorbaria tomentosa	Rosaceae	C	Fu	S
Spiraea canascens	Rosaceae	C	Fu	S
Spiranthes sinensis	Orchidaceae	UC	Me	H
Stellaria media	Caryophyllaceae	C	-	H
Swertia angustifolia	Gentianaceae	UC	Me	H
Tagetus minuta	Asteraceae	C	-	H
Thalictrum sp.	Ranunculaceae	UC	Me	H
Thymus linearis	Lamiaceae	UC	Me	H
Toona serrata	Meliaceae	UC	Me	T
Trifoleum repens	Fabaceae	C	-	H
Trigonella corniculata	Fabaceae	UC	Me, Ed	H
Typhonium diversifolium	Araceae	UC	-	H
Ulmus villosa	Ulmaceae	UC	Tm, Me	T
Urtica dioica	Urticaceae	C	Me	S
Verbascum thapsus	Scrophulariaceae	UC	Me	H
Vigna sp.	Fabaceae	UC	Fo	C
Vincetoxicum hirundinaria	Asclepidaceae	UC	Me	H
Viola betonicifolia	Violaceae	C	Me	H
V. pilosa	Violaceae	UC	Me	H
Vitis sp.	Vitaceae	C	-	C
Wikstroemia canescens	Thymelaeaceae	UC	-	S
Woodfordia fruticosa	Lythraceae	UC	Me	S
Youngia sp.	Asteraceae	UC	-	H
Zanthoxylum armatum	Rutaceae	UC	Me	S

Abbreviations: C= Climber; C= Common; Ed= Edible; Fo= Fodder; Fu= Fuel; H= Herb; LF= Life Form; Me= Medicinal; S= Shrub; T= Tree; Tm= Timber; UC= Uncommon.

Discussion

The diversity of trees is fundamental to total forest biodiversity, because trees provide resources and habitat for almost all other forest species (Huang *et al*., 2003). At large scales, species diversity generally was found related to climate and productivity (Rahbek, 2005). Franklin *et al.* (1989) proposed that long-term productivity of natural forest ecosystems with high tree species diversity may be greater than that of forests with low diversity as a result of increased ecosystem resilience to disturbance. Slobodkin and Sanders (1969) opined that species richness of any community is a function of severity, variability and predictability of the environment in which it develops. Therefore, diversity tends to increase as the environment becomes more favourable and more predictable (Putman, 1994). Tree species diversity varied greatly from place to place mainly due to variation in biogeography, habitat and disturbance (Sagar *et al*., 2003), which have also been considered as the important factors for structuring the forest communities (Burslem and Whitmore, 1999). Srivastava *et al.* (2005) reported that the community characters differ among aspect, slope and altitude even in the same vegetation type. In our study we found that tree diversity decreased from lower altitude to higher altitude which means in our study area the environment at lower altitude was favourable for increasing tree diversity as compared to higher altitude.

In many other studies, the mean H' values for the other forests of temperate Himalaya varied from 0.4 to 2.8 (Singh *et al.*, 1994), 0.08 to 1.29 (Shivnath *et al.*, 1993) and 1.55 to 1.97 (Mishra *et al*., 2000), whereas in our study it varied between 0.67 to 0.15. Whittaker (1965) and Risser and Rice (1971) have reported the range of values of Cd for certain temperate vegetation from 0.19 to 0.99. The values of concentration of dominance (Cd) of the present study were more or less similar to the earlier reported values for temperate

forests. Mean Cd values of 0.31 to 0.42 (Mishra *et al*., 2000) and 0.07 to 0.25 (Shivnath *et al*., 1993) were reported earlier from other parts of Indian Himalaya. The higher value of Cd in the forest growing on upper altitude was due to lower species richness. According to Baduni and Sharma (1997) the Cd or Simpson's index was strongly affected by the IVI of the first three relatively important species in a community. Species diversity (richness) and dominance (Simpson index) are inversely related to each other (Zobel *et al*., 1976).

The Himalayan region is bestowed with a variety of natural resources which have been exploited by mankind since time immemorial. The link between forest management and the well-being of communities in forested areas has traditionally been defined by forest sector employment opportunities (Sharma and Gairola, 2007). Ethnobotanical studies typically focus on recording the knowledge of traditional societies in remote places (Hodges and Bennett, 2006).Indigenous people have a vast knowledge of, and capacity for, developing innovative practices and products from their environment. Indigenous knowledge grows from close interdependence between knowledge, land, environment and other aspects of culture in indigenous societies, and the oral transmission of knowledge in accordance with well understood cultural principles and rules regarding secrecy and sacredness that govern the management of knowledge (Tripathi *et al*., 2000). In the present study the traditional uses of various plant species by indigenous people have been recorded, which can be utilized in the future for technological advancement, economic prosperity and providing employment opportunity to the local people.

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12/5/2009

Community Participation for Educational Planning and Development

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Abstract: This research set out to explore the roles communities in the development of education. The concept of community participation has been important around the world. In developed countries communities have important role in the processes of educational planning and development. But in third world countries there are some important barriers in face of community participation in education activities. This paper looks at the barriers of community participation in educational activities as well as role of community participation in educational planning. This research draws from my scientific experience in a variety of disciplines namely; anthropology and education.

[**Abrisham Aref. Community Participation for Educational Planning and Development.** Nature and Science. 2011; 9(1): 68-71]. (ISSN: 1545-0740).

Keywords: participation, development, education

1. Introduction

Communities can play a variety of roles in the provision and management of education and learning processes. Community participation can contribute to promoting education (UNICEF 1992). Community participation is a concept that attempts to bring different stakeholders together for problem solving and decision making (Talbot and Verrinder 2005). Community participation is considered necessary to get community support for educational planning and development (Cole 2007). Community participation refers to peoples' engagement in activities within the educational system. It plays an essential and long-standing role in promoting quality of life (Putnam 2000). Community participation in educational development processes can support and uphold local culture, tradition, knowledge and skill, and create pride in community heritage (Lacy et al. 2002).

Community participation is one of the mechanisms to empower people to take part in educational development. It was launched as a key concept of development. Increased participation is a means to achieve development to resolve the educational problems (Aref et al, 2009; Lasker, Weiss, and Miller 2001). This article looks at the barriers and potential of community participation in educational development in Iran.

2. Community Participation

The term "participation" can be interpreted in various ways, depending on the context. Shaeffer (1994) clarifies different degrees or levels of participation, including:

- Ø involvement through the contribution of money, materials, and labor;
- Ø involvement through 'attendance' (e.g. at parents' meetings at school), implying passive acceptance of decisions made by others;
- Ø involvement through consultation on a particular issue;
- Ø participation in the delivery of a service, often as a partner with other actors;
- Ø participation as implementers of delegated powers; and participation "in real decision ing at every stage," including identification of problems, the stay of planning, implementation, and evaluation (Uemura 1999).

Shaeffer stresses that the first four definitions use the word involvement and connote largely passive collaboration, whereas the last three items use the word participation instead, implying a much more active role (Uemura 1999). In other definition Participation is concerned with human development and increases people's sense of control over issues which affect their lives, helps them to learn how to plan and implement and, on

a broader front, prepares them for participation at regional or even national level. In essence, participation is a 'good thing' because it breaks people's isolation and lays the groundwork for them to have not only a more substantial influence on development, but also a greater independence and control over their lives (Oakley 1991; Warburton 1997).

Without community participation, there is obviously no partnership, no development and no program. Hence the lack of community participation in decision making to implement educational development can lead to failure in the community development (Miranda 2007). Meanwhile, some scholars provided a typology of participation, but they do not directly deal with tourism development (Leksakundilok 2006). Table 1 showed six broad categories or levels of participation, which had been formulated.

Table 1: Types of Community Participation for Educational Planning and Development

Types	Characteristics
Empowerment	Local people have control over all development without any influence (Choguill 1996; Dewar 1999).
Partnership	There are some degrees of local influence in development process (Arnstein 1969).
Interaction	People have greater involvement in this level. The rights of local people are recognized and accepted in practice at local level (Pretty 1995).
Consultation	People are consulted in several ways, e.g. being involved in community's meeting or even public hearings. Developers may accept some contribution from the locals that benefits their project (Arnstein 1969).
Informing	People are told about development program, which have been decided already, in the community. The developers run the projects without listening to local people's opinions (Arnstein 1969).
Manipulation	Development is generally developed by some powerful individuals, or government, without any discussion with the people (Arnstein 1969).

Source: Adapted from Leksakundilok (2006) and Aref et al. (2009)

3. Methodology

The research was performed as a qualitative library in which the researcher had to refer to relevant and related sources. I have used a number of articles and official websites of the various Iran known organizations.

4. Barriers of Participation in Education

Understanding barriers of participation is important when a community is getting organized for involvement in educational development planning. This understanding can help community and organizations more effectively impact the educational policy-making process. Further, it is important for government to understand that educational system also face barriers that can hinder its progress in responding and recognizing the priorities of local communities in Iran. Overcoming the barriers to education will serve to facilitate the policy making process. There are several literatures that directly deal with the barriers of communities particularly in third world countries. Especially educational development in Iran has several barriers that cannot develop. Following are the main barriers:

- Inability to analyze the changing socio-cultural dimensions of educational system
- Lack of understanding of the policy process
- Lack of access to information (Steven and Jennifer 2002).

Involving communities in the education planning requires facing and tackling a number of challenges. In general, as Crewe and Harrison

(1998) articulate, participatory approaches tend to overlook complexities and questions of power and conflict within communities. They are designed based on the false assumption that the community, group, or household is homogeneous, or has mutually compatible interests. Differences occur with respect to age, gender, wealth, ethnicity, language, culture, race and so on. Even though marginalized or minority groups may be physically present during discussion, they are not necessarily given a chance to express their views to the same degree as others.

Bushell and Esgles (2007) also states education as a phenomenon of affluent contemporary societies is a particularly difficult concept in communities in developing countries to grasp (Bushell & Eagles, 2007, p. 154). As consequence, community participation may be unacceptable for educational development. Hence building capacity through is necessary for stakeholders involved in educational planning and development.

In attempts to understand factors that prevent communities from being involved in formal education, Shaeffer (1992) found that the degree of community participation is particularly low in socially and economically marginal regions. This is because such regions tend to have the following elements: (a) a lack of appreciation of the overall objectives of education; (b) a mismatch between what parents expect of education and what the school is seen as providing; (c) the belief that education is essentially the task of the State; (d) the length of time required to realize the benefits of better schooling; and (e) ignorance of the structure, functions, and constraints of the school (Uemura 1999).

5. Conclusion

In any effort to promote community participation for educational planning and development, it is necessary to assess the communities' capacity to carry out what they are expected to achieve in a long run. Community participation itself is not a goal in educational development, or a panacea to solve complicated issues contributing to poor educational quality in both developing and developed countries. It is a process that facilitates the realization of improving educational quality and the promotion of democracy within society. In completion this study explored the notion of community participation in processes of educational development. The study also showed the community participation can contribute to educational planning and development through various channels. The following is the ways which communities can contribute to the educational planning and development.

- advocating enrollment and education benefits;
- boosting morale of school teachers;
- raising money for schools;
- constructing, repairing, and improving school facilities;
- recruiting and supporting teachers;
- making decisions about school locations and schedules;
- monitoring and following up on teacher attendance and performance;
- forming village education committees to manage schools;
- actively attending school meetings to learn about children's learning progress
- providing skill instruction and local culture information;
- helping children with studying;
- garnering more resources from and solving problems through the education
- providing security for teachers by preparing adequate housing for them;
- identifying factors contributing to educational problems (Uemura 1999).

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6/02/2010

Growth And Photosynthetic Pigments Of Fodder Beet Plants As Affected By Water Regime And Boron Foliar Fertilization

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ABSTRACT: Pot experiment was conducted in the greenhouse of the National Research Centre, Dokki- Cairo, Egypt during the winter season of 2006/2007 to evaluate the effect of available water depletion before irrigation (AWDBI) and boron foliar spray on growth and photosynthetic pigments of fodder beet plants c.v. Red Forshenger. The experiment contained 3 levels of AWDBI in combination with 2 boric acid treatments in addition to the control treatment *i.e*. 9 treatments in 6 replicates arranged in split plot design. Negative relationship was found between leaf area, and fresh and dry weights of fodder beet plants and AWDBI. The whole fresh weight/plant showed the same response while the dry weight of whole plant with the two drought treatments showed approximately the same values. Top, root and whole plant fresh or dry-weight gave their higher values when plants received 75 ppm boric acid which exceeded than those received 150 ppm boric acid or sprayed by fresh water. However, leaf area and shoot/root ratio increased as the boric acid concentration increased up to 150 ppm. Plant height and number of leaves/plant did not significantly affect by boron spraying. Top/root ratio increased with boron application under different AWDBI. The highest percentages of Chl a, Chl b, carotenoids and total chlorophyll were obtained by spraying 75 ppm boric acid compared to spraying with 150 ppm or control plants. This was true for Chl a / Chl b and total chlorophyll / carotenoids ratio. Positive relations were found among the concentration of N, K, Ca and Zn and drought treatments. Phosphorus, Mg and Na concentrations did not affect. Either Fe or Cu concentration decreased by both drought treatments, however, the concentration of Mn decreased with the 50 days period AWDBI and tended to increase to be more than the control treatment. Increasing the period of available water depletion before irrigation induced positive effect on N and Ca uptake, while, K, Mg, Na, Fe, Mn and Cu uptake showed opposite trend. In the same time the dose 75 ppm boric acid increased both concentration and uptake of macro and micro-nutrients by the plant tops; however the higher dose (150 ppm) led to a reverse effect.

[Hussein, M.M.; Shaaban, M.M., El-Saady, A.M. and El-Sayed, A.A. **Growth And Photosynthetic Pigments Of Fodder Beet Plants As Affected By Water Regime And Boron Foliar Fertilization.** Nature and Science. 2011;9(1):72-79]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Keywords: Fodder beet, Available water depletion, Boron, Growth, Pigments, Mineral status.

1. INTRODUCTION

The increasing demand for animal proteins of the growing population in Egypt is handicapped through the shortage of the carbohydrate components in animal feeds. On the other hand, the horizontal expansion of new reclaimed areas requires the cultivation of crops offering a source for satisfying income to the farmers. Fodder beet can easily fulfill both aims through its high content of carbohydrate which reached about 72% DM and production in some new regions ranged between 25-30 tons/feddan.

Boron plays an important role in carbohydrate metabolism and transportation (Belvins and Lukaszewski, 1998 and Marschner, (1995); but the increase of boron led to toxicity as found by Kato *et al.* (2008). Lewis (1980) assumed that boron controls the metabolic reactions of carbohydrate transport. Boron was also reported to control different reactions in carbohydrate metabolism such as α-amylase, Glucose 6-phosphate dehydrogenase, ß-amylase and reduction of UDPG-synthesis (Goldbach, 1997). The specific B role in carbohydrate metabolism reported to be species dependent (Brown and Hu, 1998). Boron is now known to be mobile in the phloem of all species that utilize polyols (complex sugars) as primary photosynthetic metabolites. In these species a polyol-B-polyol complex is formed in the photosynthetic tissues and is transported in the phloem to currently active sink regions such as vegetative or reproductive meristems. In species that do not produce significant quantities of polyols, B once delivered to the leaf in the transpiration stream cannot reenter the phloem, resulting in essentially complete phloem immobility. Thus, B may cause accumulation of sugars and starch or reduction of sucrose (Agarwala and Chatterjee, 1996).

For many crops, B fertilization is required. Shaaban *et al*. (2004) found that boron foliar application with 25 ppm boron or 25 ppm boron + 50 ppm zinc in the spray solution has significantly increased both fresh and dry weight of cotton plants grown under high calcium carbonate level in the soil. Ziaeyan and Rajaie (2009) stated that Zn and B fertilization significantly increased plant biological yield, grain yield, thousand grain weight, number of

grains per stalk, grain protein content and the concentration of B and Zn in corn tissues grown under high $CaCO_3$ conditions. Climate, particularly high light intensity and low temperature are factors that need to be considered in relation to the occurrence of B deficiency (Shorrocks, 1997). Boron application could help plants to ameliorate water stress of beats as reported by Ahmed, *et al.*, 2009. One of the main problems in the new cultivated areas is the lake of water which affected the growth of different crops grown in these areas. Clover *et al.* (1999); Mohammedian *et al.* (2005) and Hoffmann (2010) found that water deficit has affected reversely the growth and yield of fodder beet. However, the sensitivity of beet to water deficit has been poorly studied. Little is known about physiological traits which can be used to assess the effects of drought. Understanding the physiological responses to water stress and the traits associated with it is therefore strongly desirable to develop mechanistic forecasting systems for fodder beet growth**.** Furthermore, water stress reduces the growth and yield to such an extent that it is likely to depend on stress duration and phenological stages.

The current work was designed to investigate the effect of foliar spray of boron on growth traits, photosynthetic pigments and mineral status of fodder beet plants grown under depletion of different percentage of available soil water before irrigation.

2. MATERIALS AND METHEODS

Pot experiment was conducted in the greenhouse of the National Research Centre at Dokki-Cairo, Egypt during the winter season of 2006/2007 to evaluate the effect of available soil moisture stress and boron foliar spray on growth and photosynthetic pigments of fodder beet plants c.v. Red Forshenger. The experiment included 3 levels of water depletion before irrigation in combination with 2 boric acid treatments in addition to control treatment *i.e*. 9 treatments in 6 replicates arranged in split plot design. Metallic tin pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 kg of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel, (particles about 2-3 cm in diameter) were used to cover the bottom of the pot. Irrigation water was poured through a vertical tube (2.5 cm in diameter), so the movement of water was from the base upward.

Seeds of fodder beet (*Beta vulgaris L.* cv. Red Forshenger) were sown in Dec., 15. The plants were thinned twice: the 1st 20 days after sowing and the 2nd two weeks later to leave three plants/pot. Calcium super phosphate (15.5 % P_2O_5) and potassium sulfate (48.5 % K_2O) in the rate of 2.29 and 1.14 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal portions: the 1st two weeks from sowing and the 2nd two weeks latter. The water regime treatments started 21 days after sowing. Boron treatments in the form of boric acid (17% B) were twice sprayed: the 1st at 21 days after sowing and the 2nd two weeks later. Control plants sprayed with the same amount of fresh water.

Samples from every treatment were taken, cleaned, dried at 70ºC and then ground in a stainless steal mill. The dry matter was digested and the macro and micro-nutrients were determined according to the methods described by Chapman and Pratt (1978). Chlorophyll a, b and carotenoids were determined according to the method of Nush (1980).

The data collected were statistically analyzed as described by Snedecor and Cochran (1990).

3. RESULTS AND DISCUSSION

3.1. Growth

A negative relationship was found between area of leaves, fresh and dry weights of fodder beet plants and AWDBI. The whole fresh weight/plant showed the same response while the dry weight of whole plant with the two drought treatments showed approximately the same values (Table 1). Under drought conditions, beet leaves wilt in response to water deficiency, tend to lie flat on the soil and thus, increase the effective area exposed to the direct sun radiation. As a consequence of the reduction in transpiration rates of such leaves, leaf temperature increases and may result in leaf scorching and death (Clover *et a*l., 1999).

Abdallah and Yassen (2008) showed that extension of irrigation to 21 and 28 days reduced the foliage fresh weight/plant, although foliage dry weight and root diameter were not significantly affected by irrigation augmentation, but the root length/plant was seriously affected and showed a clear reduction. Drought induced reduction in different growth traits through its effect on the physical and chemical properties and/or physiological processes inside the plant tissues. The effect of reduced soil water level included an increase in the solution concentration of non absorbed nutrients and that of exchangeable cations which tend to reduce the concentration of absorbed anions like phosphate (Pariher and Tiwari, 2003).

The decrement in nutrients in top and root at drought treatments might be due to reducing the solubility of mineral in the soil. The films are thin and path length of movement increase; hence movement of cations to root is reduced. High tension exerts a physiological effect on the root, elongation, turgidity and number of root hairs decreased with increasing tension (Abdallah and Yassen, 2008). Monti *et al*. (2006) observed a lower photosynthetic capacity than the potential even when the favorable water conditions were restored. They concluded that this was somewhat related to the

reduction of the root apparatus caused by water stress. Plant vegetative growth was inhibited with reduced water availability.

Leaf water potential, relative water content and canopy transpiration were reduced with increasing soil water stress. Leaf photosynthesis rate was reduced when stomatal resistance exceeded 3.5 $s.cm^{-1}$ (Ismail *et al*., 1994).

Table 1: Growth of fodder beet plants as affected by water regime

AWDBI %	Plant Height (cm)	No of leaves	Leaf area (cm^2/ Plant)	Root (cm)		Fresh weight(g/plant)			Dry weight(g/plant)			Top/root
				L	D	Top	Root	Whole	Top	Root	Whole	
25	44.43	9.57	2011	12.70	2.61	78.4	97.8	176.2	12.38	19.75	32.13	0.627
50	41.23	9.13	1449	11.77	2.53	77.3	77.8	155.1	8.88	10.67	19.55	1.010
100	35.23	9.40	1344	12.03	2.94	70.1	71.0	141.1	8.75	13.65	22.40	0.640
$LSD_{5\%}$	N.S	N.S	567	N.S	0.43	1.1	17.14	32.6	3.32	5.97	6.54	------

L= length, D= diameter, AWDBI= available water depletion before irrigation

Data recorded in Table 2 indicated that top, root and whole plant fresh or dry-weight gave its higher values when plants received 75 ppm boric acid and even more than that received 150 ppm boric acid or that sprayed with fresh water. However, area of leaves and top to root ratio increased as the boric acid concentration increased up to 150 ppm. Plant height and number of leaves/plant did not significantly affected by boron spraying.

Table 2: Growth of fodder beet plants as affected by boron spray

Boric acid ppm	Plant Height (cm)	No of leaves	Leaf area (cm^2/ Plant)	Root (cm)		Fresh weight(g/plant)			Dry weight(g/plant)			Top/root
				L	D	Top	Root	Whole	Top	Root	Whole	
0	40.67	10.13	1173	11.67	2.53	75.0	90. 7	165.7	8.56	14.04	22.60	0.610
75	42.00	8.47	1746	12.70	2.63	84.3	90.0	174.3	12.59	20.02	32.20	0.629
150	34.23	9.50	1885	12.10	2.91	66.6	65.9	132.5	9.17	10.01	2121	0.916
$LSD_{5\%}$	N.S	N.S	638	0.70	N.S	12.28	15.6	41.3	N.S	8.33	8.13	------

L= length, D= diameter

Previously, Crisp *et al*. (1976) noticed that lettuce plants (*Lactuca sativa* L.) grown in a boron deficient nutrient medium developed tip burn. Their leaves showed no overall increased auxin activity compared with those of control plants until they were 66 days old, when boron deficient plants showed a relative increase in the activity of one auxin. Karabal *et al.* (2003) mentioned that compared with controls (no boric acid treatment) boron toxicity resulted in a reduction in root weights and did not cause any significant change in protein contents. Boric acid treatment did not cause significant (P>0.05) changes in proline and H_2O_2 contents of both tissues and cultivars. Wang *et al.* (2006) reported that boron deficiency inhibits growth of the plant apex, which consequently results in a relatively weak apical dominance, and a subsequent sprouting of lateral buds. Boron application to the shoot apex inhibited lateral bud growth and stimulated lateral root formation, presumably by stimulated polar IAA transport. Lopez-Gómez *et al.* (2007) stated that the presence of B produced a decrease in the lipid peroxidation values, suggesting that B additions afforded some protection to the membranes. This means that boron application improved the oxidative defense against stress. Kocábek, *et al.* (2009) found that seedlings grown with 5 mM boric acid were short, stunted and pale. However, at concentrations between 1 and 3 mM, hypocotyls elongation was stimulated in all *Arabidopsis* ecotypes tested relative to plants grown at 0.1 mM H_3BO_3. Cervilla, *et al.* (2009) mentioned that 2 mM B supply inhibited root growth and increased the root B concentration in both tomato cultivars. Kassem *et al.* (2009) observed positive effects on growth of cotton plants when sprayed by 85 and 170 ppm boric acid, but the positive response with 170 ppm treatment was less than the 85 ppm treatment.

It is clear from data presented in Table 3 that plant height, number of green leaves fresh weight of leaves, root diameter and length and dry matter of leaves as well as root dry weight did not show any

significant response to the interactive effects of depletion of available soil moisture before irrigation and boron spraying. However, leaf area, fresh and whole plant fresh and dry weight were significantly responded to this interaction. Pant *et al*. (1998) reported that water stress treatments, regardless of B levels and genotypes. Boron X irrigation interactions indicated the possibility of the influence of water stress on the severity of wheat sterility in South and South-east Asia.

Ben-Gal and Shani (2003) revealed that water application levels were 30, 60, 100, 130 and 160% of potential evapotranspiration. Boron levels in irrigation water were 0.02, 0.37, and 0.74 $M.m^{-3}$. B and drought stresses did not result in a larger effect but rather, one or the other stress causing factor was found to be dominant in plant response. Both irrigation water quantity and boron concentration influenced water use of the plants in the same manner as they influenced the yield.

Top / root ratio increased with boron application under different AWDBI. Abdollahian-Noghabi (1999) declared that due to limited shoot growth in severe drought stress, the ratio of shoot to root dry weight was severely reduced. Under drought stress, on sugar beet as well as fodder beet plants, the ratio of storage root to leaf dry matter of sugar beet decreased indicating a different partitioning of the assimilates (Hoffmann, 2010).

Table 3: Growth of fodder beet as affected by boron foliar spray and water regime

AWDBI %	Boric acid ppm	Plant height (cm)	No of leaves	Leaf area cm^2/ plant	Root (cm)		Fresh weight(g/plant):			Dry weight(g/plant)			Top/ root ratio
					L	D	Top	Root	Whole	Top	Root	Whole	
25	**0**	45.0	10.7	1445	14.0	2.43	92..0	129.0	221.7	11.83	21.50	33.33	0.553
	75	45.0	8.7	2008	12.7	2.53	94.0	119.7	213.7	16.77	27.95	44.72	0.600
	150	34.3	9.3	2579	11.3	2.87	49.3	44.7	94.0	8.55	9.79	18.34	0.873
50	**0**	39.7	8.7	1080	9.3	2.00	77.3	76.7	122.4	6.78	9.01	15.79	0.753
	75	44.3	7.7	1650	12.7	2.30	77.0	66.0	143.0	9.85	13.91	23.46	0.735
	150	39.7	11.0	1618	13.3	3.30	77.7	90.8	168.0	10.02	9.08	15.10	1.764
100	**0**	37.3	11.0	995	11.7	3.17	55.7	66.3	143.6	7.08	11.61	18.69	0.610
	75	39.7	9.0	1580	12.7	3.07	82.0	84.3	166.2	10.24	18.19	28.42	0.563
	150	28.7	8.2	1457	11.7	2.57	72.7	62.3	95.0	8.93	11.15	20.18	0.801
$LSD_{5\%}$		N.S	N.S	1192	N.S	N.S	N.S	44.2	74.7	N.S	N.S	15.08	-----

L= length, D= diameter, AWDBI= available water depletion before irrigation

Photosynthetic pigments

It was observed from data in table 4 that there was no response of Chl a, carotenoids and total chlorophyll concentrations in leaves of fodder beet plants as the increase in depletion of available water before irrigation. The opposite was true for the concentration of Chl b by both drought treatments. Furthermore, Chl a/ Chl b ratio decreased as the AWDBI was increased, but total chlorophyll / carotenoids ratio was increased by 50% depletion of AWDBI and tended to decrease by the irrigation after depletion of 100% of available water. Ardic *et al*. (2009) reported that chlorophyll florescent increased in the drought resistant variety, but decreased in the drought sensitive cowpea variety by boron treatment.

Table 4: Photosynthetic pigments mg.g^{-1}) in leaves of fodder beet as affected by water regime

AWDBI %	Chl a	Chl b	Carot	T.Chl	Chl a/ Chl b	T.Chl/Carot.
25	3.410	1.478	0.885	4.888	2.307	5.523
75	3.285	1.613	0.843	4.898	2.037	5.810
100	3.372	1.905	1.181	5.277	1.770	4.468
$LSD_{5\%}$	N.S	0.15	N.S	N.S	-----	-----

AWDBI: available water depletion before irrigation

Data recorded in table 5 indicated that the highest percentages of Chl a, Chl b, Carotenoids and total chlorophyll values obtained by spraying of 75 ppm boric acid compared to the 150 ppm treatment or control. This was also true for Chl a/Chl b and total chloropyhll / carotenoids ratios. Zhao and Oosterhuis (2000) found that the values of Chl a, Chl b and total chlorophyll of boron deficient plants during the early growth of cotton considerably decreased leaf

photosynthetic rate and carbohydrate transport from leaves to fruits, and depressed plant growth and dry matter accumulation. Mouhtaridou *et al.* (2004) noticed that SPAD units of leaves characterizing chlorophyll contents declined as B concentration of the culture medium increased from 0.1 to 6.0 mM.

Table 5: Photosynthetic pigments (mg.g^{-1})in leaves of fodder beet as affected by boron spray

Boric acid (ppm)	Chl a	Chl b	Carot	T.Chl	Chl a/ Chl b	T.Chl / Caro
0	3.158	1.646	0.962	4.804	1.918	4.992
75	4.308	1.961	1.157	6.269	2.197	5.418
150	2.601	1.389	0.790	3.990	1.873	5.051
$LSD_{5\%}$	N.S	0.49	N.S	0.471	-----	------

Boron deficiency during the early growth of cotton increased leaf chlorophyll content, decreased leaf stomatal conductance and net photosynthetic rate, and reduced non-structural carbohydrate export from the leaf to the fruit (Zhao and Oosterhuis, 2003). Mazhar *et al.* (2006) found that chlorophyll and carotenoids content increased as B concentration increased up to 20 ppm as compared to the untreated *Taxodium destincum L.* plants.

The interaction between water regime and boron fertilization appeared to not affect both chlorophyll a and crotenoids (Table 6). However, the concentration of 100 ppm at the first water regime (25 AWDBI) appeared to negatively affect the concentration of carotenoids followed by the concentration 75 ppm with the two other AWDBI treatments. This means that boron toxicity appeared with less concentration as water is more deficient. Plants exposed to B toxicity found to exhibit increases of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, resulting in oxidative stress and membrane peroxidation (Ardic *et al.* 2009, Cervilla *et al*., 2007, 2009).

Mineral composition

A positive relation was found between the concentration of N, K, Ca and Zn concentration and drought treatments. Phosphorus, Mg and Na concentrations did not significantly affect. Either Fe or Cu concentration decreased by both drought treatments, however, the concentration of Mn decreased with the treatment 50 AWDBI (Table 7).This may be due to nutrient accumulation as the metabolism was depressed with water deficit.

Table 6: Photosynthetic pigments (mg.g^{-1}) in leaves of fodder beet as affected by boron spray and water regime

AWDBI %	Boric acid (ppm)	Chl a	Chl b	Carot	T.Chl	Chl a /Chl b	T.Chl /Carot
25	0	3.295	1.630	0.860	4.925	2.021	5.727
	75	4.071	1.710	1.351	5.781	2.381	4.281
	150	2.860	1.095	0.444	3.955	2.612	8.908
50	0	2.917	1.600	0.810	4.517	1.823	5.577
	75	4.619	1.858	0.872	6.477	2.486	7.428
	150	2.319	1.381	0.848	3.700	1.679	4.363
100	0	3.261	1.707	1.217	4.968	1.910	4.082
	75	4.233	2.316	1.249	6.549	1.828	5.243
	150	2.623	1.691	1.078	4.314	1.551	3.955
$LSD_{5\%}$		N.S	0.84	N.S	0.763	----	----

AWDBI = available water depletion before irrigation

Table 7: Effect of drought on mineral concentration of macro and micro-nutrients in fodder beet tops

AWDBI %	**Macronutrients (%)**						**Micronutrients (ppm)**			
	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
25	3.91	0.31	3.18	0.87	0.39	4.08	665	85.5	54.6	7.18
50	4.55	0.29	3.83	0.83	0.74	4.10	644	65.7	90.9	5.98
100	4.99	0.30	3.90	0.80	0.60	3.98	576	92.8	95.7	6.25

Data in Table (8) showed that increasing the depletion of available water percentage before irrigation induced positive effect on N and Ca uptake, while, K, Mg, Na, Fe, Mn and Cu uptake showed the opposite trend. Mazhar *et al*. (2006) found that N, P, K, B, Cu, Ca, Fe, Zn and Mn increased significantly in shoots by water level decreased from 40 to 100% of water holding capacity.

Table 8: Effect of drought on macro and micro-nutrients uptake by tops of fodder beet plants

AWDBI %	Macronutrients (mg/plant)						Micronutrients (mg/plant)			
	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
25	484.0 a	38.3a	393.6b	107.7b	48.2a	505.1b	8.23b	1.07c	0.675a	0.088b
50	404.0 a	25.7a	340.1a	73.7a	65.7b	364.0a	5.71a	0.58a	0.807a	0.053a
100	436.6 b	26.2a	341.2a	70.0a	52.5a	348.0a	5.04a	0.81b	0.837a	0.054a
$LSD_{5\%}$	41.8	NS	40.1	11.4	11.2	48.3	1.43	0.12	NS	0.02

A marked increase was detected in N and Na concentration by increasing the concentration of boron in the sprayed solution (Table 9). Meanwhile, P, Fe and Mn showed the highest response by spraying 75 ppm. On the contrary, B treatment lowered the concentration of K, Zn and Cu ppm. Zude *et al.* (1997) found that boron foliar application increases the concentrations of Ca, K and Mg in the leaves of apple. Shaaban *et al.* (2004) found that boron foliar application led to significant increases in both concentrations and uptake of calcium, potassium, iron, manganese, zinc and copper in cotton shoots especially plants grown under high calcium carbonate levels in the soil. They found also that a special nutrient balance between boron and other nutrients in the shoot tissues led to a good plant growth. Hanafy-Ahmed *et al.* (2008) reported that boron foliar application increased uptake and concentration of nutrients in wheat leaves.

Table 9: Effect of boron foliar spray on macro and micro-nutrients concentration in fodder beet tops

Boric acid (ppm)	Macronutrients (%)						Micronutrients (ppm)			
	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
0	4.21	0.26	3.75	0.82	0.36	3.85	595	63.8	92.5	6.63
75	4.39	0.39	3.68	0.85	0.93	3.94	718	103.6	72.5	6.70
150	4.85	0.26	3.48	0.83	0.43	4.38	573	81.1	76.2	6.08

Increasing the concentration of B in the foliar sprayed solution increased the uptake of all determined nutrients (Table 10). However, the uptake declined by increasing the boron dose to 150 ppm. Mouhtaridou, *et al.* (2004) noticed that by increasing B concentration of the culture medium from 0.1 to 6.0 mM, contents of B, P, Ca, and Mg in explants increased, whereas, K, Fe, Mn, and Zn contents decreased. Adiloglu and Adiloglu (2006) emphasized that nitrogen, P and K concentrations in maize leaves increased with B application.

Table 10: Effect of boron spray on macro and micro-nutrients uptake by the tops of fodder beet plants

Boric acid (ppm)	Macronutrients (mg/plant)						Micronutrients (mg/plant)			
	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
0	360.3a	22.2a	321.0a	70.2a	30.8a	329.5a	5.09a	0.55a	0.79a	0.057a
75	552.7c	49.1b	463.3b	107.0b	117 b	496.0b	9.04b	1.30b	0.91a	0.084a
150	444.7b	23.8a	319.1a	76.1a	39.4a	401.5a	5.25a	0.75a	0.70a	0.055a
$LSD_{5\%}$	33.2	7.12	47.3	10.0	16.8	43.1	2.35	0.39	NS	NS

Obermeyer *et al.* (1996) suggested that boron stimulates ATP hydrolysis, H^+ transport activity and control membrane voltage charging. A recent study stated that at least three B-binding membrane glycoproteins were detected in the B-deficient plant tissues indicating that B and certain membrane glycoproteins are involved in membrane processes associated with nutrient uptake and cell growth (Redondo-Nieto *et al.* 2007). Limited research work has been done on the interactive effects of B and water deficit. Pant *et al.* (1998) and Mazhar *et al.* (2006) concluded that B application can be used to reduce the harmful effect of water stress up to 40 % of water holding capacity. Smith, *et al.* (2010) observed the increase of boron in the shoot tissues while water stress increased in broccoli plant. Nevertheless, Apostol and Zwiazek (2004) stated that in the plants treated with B for 10 and 6 weeks, stomatal conductance was reduced with a concomitant reduction in a steady-state root water flow; meanwhile, tissue concentrations of essential elements including K, P, Ca, Mg, and S were not altered by B-treatments.

Conclusions:

From the present work it could be concluded that:

1- A negative relationship appeared between leaf area, and fresh and dry weights of fodder beet plants and AWDBI.
2- Top, root and whole plant fresh or dry-weight gave their higher values when plants received 75 ppm boric acid, while leaf area and top/root ratio increased as the boric acid concentration increased up to 150 ppm. Moreover, plant height and number of leaves/plant did not affect.
3- The highest percentages of Chl.a, Chl.b, carotenoids and total chlorophyll were obtained by spraying 75 ppm boric acid
4- A positive relation was found between the concentration of N, K, Ca, Zn and drought treatments. However, P, Mg and Na concentrations did not affected. Either Fe or Cu concentrations decreased by drought treatments, however, the concentration of Mn decreased with the 50 days AWDBI.
5- Increasing AWDBI induced positive effect on N and Ca uptake, while, K, Mg, Na, Fe, Mn and Cu uptake showed the opposite trend.
6- A reasonable dose of boric acid (75 ppm) could increase both concentration and uptake of macro and micro-nutrients by the plant tops, however the higher dose (150 ppm) led to a reverse effect.

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12/9/2010

Evaluation Of Garhwal Springs Water For Drinking Purpose By Using Water Quality Index

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Abstract: A very few studies have been carried out on natural springs of Garhwal Himalayas which is the main source of potable water in Garhwal Himalayas. This paper based on water quality status of these springs, for this purpose parameters like alkalinity, acidity, DO, BOD, free CO_2, nitrate, chlorides, hardness, pH and coliform number were studied. The study elucidates that the water quality of selected natural water springs is suitable for drinking purpose.

[Avnish Chauhan, Suman Chauhan, Amit Pal Singh, Neha Chamoli and Krishna Kumar Pande. **Evaluation Of Garhwal Springs Water For Drinking Purpose By Using Water Quality Index.** Nature and Science. 2011;9(1):80-84]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Keywords: - Spring water, DO, BOD, Alkalinity, Hardness, coliform, Garhwal

Introduction:

According to Times of India that one of every 10 diseases and 6% of all deaths globally are caused by unsafe water and improper water and improper hygiene. In India 1.03 crore people die annually of which, nearly 7.5%-7.8 lakh deaths are related to water, sanitation, and hygiene. In India 15% of disease burden could be prevented by improving water sanitation and hygiene. The fraction of total deaths attributed to unsafe water, inadequate sanitation or insufficient hygiene is more than 20 % in children up to 14 years of age in developing countries. According to UNICEF and WHO report that 1.1 billion peoples do not access to clean water and 2.7 billion people do not have access to basic sanitation (Cohn et al., 1999; Stewart et al., 1988). Continuous assessment of physical, chemical and biological parameters of water is an essential part of current water quality control programmes (Chauhan et al., 2010).

Water quality index (WQI) is well-known method as well as one of the most effective tools to expressing water quality that offers a simple, stable, reproducible unit of measure and communicate information of water quality to the concerned citizens and policy makers. It, thus, becomes an important parameter for the assessment and management of surface water (Chauhan and Singh, 2010).

Springs

Natural springs are major source of potable water in most of villages in Garhwal Himalaya. In natural springs water directly emerges from earth. In hills middle and upper dense vegetation of broad leaved trees viz. *Quercus leucotrichophora* (Banj Oak), *Q. floribunda* (Moru Oak), Rhododendron arboretum etc., absorbs rain water during monsoon. This water is slowly released over the year by these broad leaved plants. Released water percolates in land and forms numerous channels which flows over the year and called as natural springs. The main objective of this research is to obtain a water quality status of springs which is main sources of potable water in Garhwal Himalaya.

Material and Methods

The physico-chemical and biological parameters of the water and sediments were recorded monthly at different stations followed APHA (1998). Total five sites were selected for the study as followings:

Site-1, River Alaknanda

Water samples were collected from the left bank of river near Srikot town just below the Indane LPG store which is at about 450 m upstream from pedestrian bridge which connects Srinagar to Chauras.

Site-2, Kothar Dhara

This sprig lies in Srinagar .Water emerges after passing through dense vegetation at sampling site.

Site-3, Sweeth Bridge

This spring lies adjacent to Government Medical College. Water emerges directly from earth. Spring is surrounded by cemented pool which remains covered by slabs. Water is directly distributed by pipe line from pool. Collection site 15m distant away from pool.

Site-4, Bhola Mahadev

This spring is situated at Srikot. Spring is surrounded by cemented pool. Sample is directly collected from pool.

Site-5, Barkot Spring

The sampling site lies 3.0 km. away from Chauras campus in north. Water was flowing in cemented channels. Water of this spring largely used for irrigation and drinking purpose at Barkot village and Chauras village.

The present study was undertaken during April to May, 2008 for evaluation of water for drinking purpose by using CCME water quality index 1.0. Note a zero (0) value signifies very poor water quality, whereas a value close to 100 signifies excellent water quality.

Conceptual Framework of CCMEWater Quality Index

The CCME WQI was originally developed as the Canadian Water Quality Index (CWQI). It comprises of three factors and is well documented (CCME, 2001).

Factor 1: *F*1 (Scope)

Scope assesses the extent of water quality guideline non-compliance over the time period of interest, which means the number of parameters whose objective limits are not met. It has been adopted directly from the British Columbia Water Quality Index:

$$F_1 = \left\{ \frac{\text{Number of failed variables}}{\text{Total number of variables}} \right\} \times 100$$

Where, the variables indicate those water quality parameters whose objective values (threshold limits) are specified and observed values at the sampling sites are available for the index calculation.

Factor 2 : F_2 (Frequency)

The frequency (i.e. how many occasions the tested or observed value was off the acceptable limits) with which the objectives are not met, which represents the percentage of individual tests that do not meet the objectives ("failed tests"):

$$F_2 = \left\{ \frac{\text{Number of failed tests}}{\text{Total number of variables}} \right\} \times 100$$

The formulation of this factor is drawn directly from the British Columbia Water Quality Index.

Factor 3 : *F*3 (Amplitude)

The amount by which the objectives are not met (amplitude) that represents the amount by which the failed test values do not meet their objectives, and is calculated in three steps.

The number of times by which an individual concentration is greater than (or less than, when the objective is a minimum) the objective is termed an "excursion" and is expressed as follows. When the test value must not exceed the objective:

$$\text{excursion}\,i = \left\{ \frac{\textit{Failed Test Value}_i}{\text{Objective } j} \right\} - 1$$

For the cases in which the test value must not fall below the objective:

$$\text{excursion}i = \left\{ \frac{\text{Objective } j}{\text{Failed Test Value}i} \right\} - 1$$

The collective amount, by which the individual tests are out of compliance, is calculated summing the excursions of individual tests from their objectives and then dividing the sum by the total number of tests. This variable, referred to as the normalized sum of excursions (*nse*) is calculated as:

$$\text{nse} = \left\{ \frac{\sum_{i=1}^{n} \text{excursion}i}{\text{number of tests}} \right\}$$

F_3 is then calculated by an asymptotic function that scales the normalized sum of the excursions from objectives (nse) to yield a value between 0 and 100.

$$F_3 = \left\{ \frac{\text{nse}}{0.01\text{nse} + 0.01} \right\}$$

The CWQI is finally calculated as:

$$\text{CWQI} = 100 - \left\{ \frac{\sqrt{F_1^2 + F_2^2 + F_3^2}}{1.732} \right\}$$

The assignment of CCME WQI values to different categories is somewhat subjective process and also demands expert judgment and public's expectations of water quality. The water quality is ranked in the following 5 categories:

1. Excellent: (CCME WQI values 95–100)
2. Good: (CCME WQI values 80–94)
3. Fair: (CCME WQI values 60–79)
4. Marginal: (CCME WQI values 45–59).
5. Poor: (CCME WQI values 0–44)

Results

Table-1 represents results of various physico-chemical and biological parameters for selected natural springs in Garhwal Himalayas.

The alkalinity of water samples was recorded as 58.00±2.32, 190.00±4.43, 170.00±6.34, 180.00±5.59 and 250.00±7.67 mg/L at site 1, 2, 3, 4 and 5, respectively, whereas the permissible limit is 120 mg/L as per BIS. Acidity of spring was determined as 5.00±0.09, 85.00±2.83, 57.00±1.70, 115.00±3.63 and 80.00±2.76 mg/L at site 1, 2, 3, 4 and 5, respectively, whereas the permissible limit is 120 mg/L as per WHO standard. D.O. was recorded as 11.20±1.69, 2.00±0.69, 2.80±0.39, 1.20±0.27 and 6.00±0.43 mg/L at site 1, 2, 3, 4 and 5, respectively. The acceptable limit of D.O. varies from 5.00 mg/L. BOD of water sample was recorded as4.40±2.37, 0.80±0.17, 1.40±0.29, 0.40±0.13 and 1.20±0.26 mg/L at site 1, 2, 3, 4 and 5, respectively. The acceptable limits of BOD are 5.00 mg/L as per ICMR standard, above this limit water is considered to be not fit for drinking purpose. Nitrate of water sample was recorded as 0.80±0.17, 0.50±0.09, 0.30±0.07 and 0.29 mg/L at site 1, 2, 4 and 5, respectively. Chlorides of water samples were recorded as 5.68±0.66, 35.50±2.19, 15.62±1.17, 36.92±1.98 and 12.78±1.12 mg/L at site 1, 2, 3, 4 and 5, respectively. Free CO_2 recorded as 8.80±0.71, 48.40±2.39, 35.20±2.18, 74.80±3.93 and 39.60±2.34 mg/L at site 1, 2, 3, 4 and 5, respectively. Hardness of water samples were recorded as 60.00±2.02,

188.00±3.88, 147.00±8.86, 242.00±12.13 and 254.00±6.96 mg/L at site1, 2, 3, 4 and 5, respectively. pH was recorded as 7.05±0.66, 6.98±0.43, 7.33±0.14, 7.40±0.19 and 7.40±0.29 at site 1, 2, 3, 4 and 5, respectively.

Discussion:

Chauhan et al., (2010) have studied the physico-chemical parameters of River Ganga at Rishikesh, Uttarakhad, India. Okram et al., (2003) also studied the physico-chemical parameters of Waithou Lake in Manipur state of India on monthly basis. It is well know that groundwater is rich in carbonic acid and dissolved oxygen usually possesses a high solubilizing potential towards soil or rocks that contain appreciable amount of mineral calcite, gypsum and dolomite and consequently hardness level may increased. That's why the values of conductivity, TDS and DO were observed beyond the limit of drinking purpose (Singh et al., 2007). BOD is an indicator of organic pollution. Unpolluted natural waters have a BOD of 5 mg/L or less (Schulze et al. 2001). The world average for nitrate in unpolluted freshwaters as reported by Reid (1961) is 0.30 mg/l.

Table: 1, Showing physico-chemical and biological properties of selected water parameters.

Sampling sites / **Parameters**	**River Alknanda** (Site-1)	**Kothar-dhara** (Site-2)	**Sweeth Bridge** (Site-3)	**Bhola Mahadev** (Site-4)	**Barkot** (Site-4)	**Permissible limit (BIS/ICMR/ WHO)**
Alkalinity (mg/L)	58.00 ±2.32	190.00 ±4.43	170.00 ±6.34	180.00 ±5.59	250.00 ±7.67	120 mg/L
Acidity (mg/L)	5.00 ±0.09	85.00 ±2.83	57.00 ±1.70	115.00 ±3.63	80.00 ±2.76	120 mg/L (WHO)
D.O. (mg/L)	11.20 ±1.69	2.00 ±0.69	2.80 ±0.39	1.20 ±0.27	6.00 ±0.43	5.00 mg/L
B.O.D (mg/L)	4.40 ±2.37	0.80 ±0.17	1.40 ±0.29	0.40 ±0.13	1.20 ±0.26	5.00 mg/L (ICMR)
Nitrate (mg/L)	0.80 ±0.17	0.50 ±0.09	0.30 ±0.07	NT	0.29 ±0.04	45 mg/L
Chlorides (mg/L)	5.68 ±0.66	35.50 ±2.19	15.62 ±1.17	36.92 ±1.98	12.78 ±1.12	250 mg/L
CO_2 (mg/L)	8.80 ±0.71	48.40 ±2.39	35.20 ±2.18	74.80 ±3.93	39.60 ±2.34	10.00 mg/L
T. Hardness (mg/L)	60.00 ±2.02	188.00 ±3.88	147.00 ±8.86	242.00 ±12.13	254.00 ±6.96	300 mg/L
pH	7.05 ±0.66	6.98 ±0.43	7.33 ±0.14	7.40 ±0.19	7.40 ±0.29	6.5-8.5
Overall CWQI Value-72.18						
CWQI category- Fair (CCME WQI values 60–79)						

Whereas, NT=Not Traceable

Conclusion

From present investigations we concluded that the quality of most of the water samples under study was suitable for drinking purpose but it is strongly recommended that these should be protected from any polluted activity while it is main source of potable water in Garhwal Himalayas and Uttarakhand

Government should take appropriate steps in this regard as soon as possible. Moreover plantation of native trees such as *Quercus leucotrichophora* (Banj Oak), *Q. floribunda* (Moru Oak), *Rhododendron arboretum* etc. should be done with effective manner so that availability can maintain. We also recommend to Uttarakhand Government to take suitable steps to aware people of this reason at school level, college and university levels so that people can realize the importance of these precious springs which meet the thrust of local people in this areas.

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12/11/2010

Physiological Studies on the Effect of Inoculation with Arbuscular Mycorrhizae (AM) Fungi on Superior Grape Rootings under Salt Stress Conditions

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Abstract: This study was carried out to disclose the effect of soil inoculation with arbuscular mycorrhizal fungi under different water salinity levels (1000, 2000 and 3000 ppm) in an attempt to improve vegetative growth parameters, nutritional acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings through two successive seasons (2008 & 2009).The results indicated that increasing levels of water salinity, particularly in case of high salinity concentration (3000 ppm) decreased survival percentage and vegetative growth parameters (i.e. shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2), total leaf area/plant (cm^2), coefficient of wood ripening, shoot and root biomass, total biomass and root/shoot ratio). Leaf total chlorophyll, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur content and shoot total carbohydrate content decreased with increasing salinity concentration. On the contrary, leaf proline amino acid, sodium, and chloride content increased with increasing levels of salinity. Concerning the microbial and enzyme activity in the rhizosphere of Superior grape rootings, it was noticed that populations of total microbial count, spore numbers of AM fungi, the percentage of infection of AM fungi, dehydrogenase enzyme activity in the rhizosphere were also decreased with increasing levels of water salinity. Superior grape rootings strategy for salt stress tolerance could be achieved by AM fungi colonization. AM fungi inoculation benefits the plants by avoiding the undesirable effects of saline water and improving of survival percentage, vegetative growth parameters, nutrient acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings under low to medium level salt concentrations (1000-2000 ppm). However, AM fungi inoculation didn't protect the plants at the highest salt concentration (3000 ppm) used in this experiment.

[Abd El-Wahab, M.A.; El-Helw, H. A. and Tolba, H. I. **Physiological Studies on the Effect of Inoculation with Arbuscular Mycorrhizae (AM) Fungi on Superior Grape Rootings under Salt Stress Conditions.** Nature and Science. 2011;9 (1):85-100]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Keywords: Inoculation, Arbuscular Mycorrhizae, Superior Grape.

1. Introduction

Plantation of the grape cultivars in Egypt has been progressively developed in the last few years. However, a great acreage is located at the new reclaimed soils which have many problems including salinity. The concentration and composition of dissolved constituents in water determine its quality for irrigation (Miller *et al* 1990).

Salinity is an environmental stress that results in negative effects on plant survival and considered as the most important biotic factor limiting plant growth and yield by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects (Shannon *et al*., 1994). High salinity causes both hyperosmotic and hyperionic stress effects and the consequence of these can be plant demise (Niu *et al*., 1995; Yeo 1998 and Glenn *et al*., 1999). The most harmful effects is the increase in osmotic stress due to high salt concentration in soil solution and consequently the decrease in the soil-water potential (Saad El-Dien *et al*., 1992), reduction in assimilates partitioning to roots (Gaser, 1992) and imbalance in overall concentrations of the ions due to ion toxic effect on physiological processes (Valia & Potiel, 1997), such as growth inhibitors (Tat, 1977), nucleic acid metabolism (Salem, 1981), photosynthesis (Prior *et al.*, 1992), respiration rate (Walker, 1994) and change of enzyme activity (Lio, 1996).

One of the natural and technological ways which has been among the most studied subjects for the last decades to reduce the salinity damages in agricultural crops was the inoculation with Arbuscular mycorrhizae fungi.

AM fungi can benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stress, increasing resistance to pests and tolerance to environmental stresses (e.g., drought, salinity), and improving soil properties (Bethlenfalvay *et al*., 1988; Bethlenfalvay and Linderman, 1992; Copeman *et al*., 1996; Cordier *et al*., 1996 and Al-Karaki, 2000).

Mycorrhizal fungi also play a vital role in alleviating the effects of salinity (Nasim, 2005). By improved nutrient acquisition, AM fungi compensate

for the nutritional imbalances imposed by salinisation. AM fungi also play a positive role in protecting plants from pH extremes. Many studies have demonstrated that inoculation with AM fungi improved growth of plants under a variety of salinity stress conditions (Ruiz-Lozano *et al*., 1996; Al-Karaki *et al*., 2001 and Feng *et al*., 2002). To some extent, these fungi have been considered as bio-ameliorators of saline soils (Azcón-Aguilar and Barea, 1997; Singh *et al*., 1997 and Rao, 1998).

The goal of this study is to disclose the effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels (1000, 2000 and 3000 ppm) in an attempt to improve vegetative growth parameters, nutritional acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings.

2. Material and Methods

This study was conducted during 2008 and 2009 seasons in the shade house of the Horticultural Research Institute, Giza, Egypt. Uniform and healthy 240 own rooted one-year-old Superior rootings were chosen. The rootings were planted through the first week of March in polyethylene bags filled with 5 kg of a medium containing clean sand carefully washed with tap water several times to remove any soluble salts. All bags had bottom holes to allow excess water drainage. Field capacity and wilting point of the sand medium were: 6.5% and 2.3%, respectively, while the electric conductivity (E.C.) of irrigation tap water was 0.85 m mhos/cm (544 ppm). The plants were irrigated with saline water treatments twice a week to keep moisture content of the planting medium about 70% of the field capacity throughout the period of the experiment from the first of May till the end of October. Leaching of accumulated salts was done every 15 days by tap water up to the end of the experiment.

The applied treatments were as follows:

1) Irrigation with tap water at 544 ppm salinity (control)
2) Inoculation with arbuscular mycorrhizal fungi (AM)
3) Irrigation with saline water at 1000 ppm
4) Irrigation with saline water at 1000 ppm + AM
5) Irrigation with saline water at 2000 ppm
6) Irrigation with saline water at 2000 ppm + AM
7) Irrigation with saline water at 3000 ppm
8) Irrigation with saline water at 3000 ppm + AM

Salinity in the irrigation water was Strogonov stock solution chloride consisting of: 78 gm NaCl, 10 gm $MgSO_4$, 9 gm $CaCO_3$, 2 gm $MgCl_2$, 1 gm $CaSO_4$ mixture dissolved in one litre (Strogonov, 1964) to yield a balance of cations and anions with a value of SAR reaching 6.0 and for preparing 1000, 2000 and 3000 ppm concentrations.

Mycorrhizal spores were originally extracted from the Egyptian soil. Spores of AM-mycorrhizae including Genera Glomus, Gigaspora and Acaulospora were added before planting. Extraction and counting of identified mycorrhizal spores were carried out according to the method described by (Massoud, 1999). Fifty grams per bag of mixed spores (250 spores/gram) of AM fungi genera were prepared and mixed with soil, then the rootings were planted (Massoud 2005).

All treatments were fertilized with a nutrient solution (Hoagland and Arnon, 1950) at half weekly intervals till the end of the growing season.

Each treatment was comprised of 30 plants distributed in 3 replicates (10 plants/ replicate) in completely randomized design.

The following parameters were determined.

1. Morphological studies:

- Survival percentage

Number of survived plants was counted in the end of experimental season.

- Vegetative growth parameters

Shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2) of the apical 5th and 6th leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA. were recorded in both seasons. Total leaf area/plant (cm^2) was determined by multiplying total number of leaves per plant by average leaf area. Coefficient of wood ripening was calculated by dividing length of the ripened part of the shoot by total length of the shoot according to Bouard (1966).

- Plant biomass

Shoot biomass (g dry weight), root biomass (g dry weight), total biomass (g dry weight) and root/shoot ratio were recorded.

2. Chemical studies:

- Leaf total chlorophyll content (SPAD). This was measured by using nondestructive Minolta chlorophyll meter SPAD 502 (Wood *et al.*, 1992).
- Leaf proline content (mg/g) was colorimetrically estimated on fresh weight basis according to the method of Batels *et al.* (1973).
- Shoot total carbohydrate content (%) (Smith *et al*., 1956).
- Leaf mineral content: N (%) (Pregl, 1945), P (%) (Snell and Snell 1967), K (%) (Jackson, 1967) and Ca, Mg, S, Cl, and Na percentages were estimated according to Evenhuis (1978).

3. Microbiological studies:-

Samples were taken for carrying out the following determinations:

- Total microbial count (-x10^5 colony forming unit (cfu)/g soil) according to (Esher and Jensen 1972).
- Number of AM (spore/g soil) according to (Massoud, 2005).
- AM infection (%) according to (Massoud, 2005).
- Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) according to Salman (1967).

Statistical analysis:

The completely randomized design was adopted for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Chocran (1980). Averages were compared using the new L.S.D. values at 5% level.

3. Results and Discussion

1. Morphological studies:

- Survival percentage

As shown in Table (1), it is obvious that increasing salt concentration gradually decreased survival percentage. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of survival percentage compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly improved in survival percentage as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results show that survival percentage of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite significant values of survival percentage were medium salinity (1000-2000 ppm). The highest obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

These results are in harmony with Kilany *et al*., (2006) who found that water stress due to salinity by raising salt concentration in the irrigation water effectively depressed the percentage of survival.

However, Arbuscular mycorrhizal (AM) fungi improve survival percentage of tomato plants for long-term under salt stress (Copeman *et al*., 1996).

- Vegetative growth parameters

Data presented in (Table 1) show the effect of irrigation with saline water and soil inoculation with Aarbuscular mycorrhizae fungi on the vegetative growth parameters (i.e. shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2), total leaf area/plant (cm^2) and coefficient of wood ripening) of Superior grape rootings during 2008 and 2009 seasons.

All of the studied vegetative growth parameters were significantly decreased with increasing levels of salinity, particularly in case of high salinity concentration (3000 ppm) compared to control which recorded the highest values for these parameters regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that it caused significant increases in all studied vegetative growth parameters as compared with non-AM plants which took an adverse trend in both seasons.

A significant interaction was observed between saline water and inoculation with AM fungi, it is clear from the results that vegetative growth parameters of non-AM plants significantly decreased with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was detected for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of growth parameters were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) were shown to have that the lowest values in both seasons of the study.

- Plant biomass

The results concerning dry biomass production in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 2).

In the salinity treatments; there was a decline in plant biomass with increasing salinity level. Shoot biomass, root biomass, total biomass and root/shoot ratio recorded the lowest values in plants grown under the highest salinity concentration (3000 ppm) as compared to control regardless of AM fungi inoculation status.

Concerning the effect of inoculation with AM fungi, it is obvious that the shoot biomass, root biomass, total biomass and root/shoot ratio was higher in AM plants than those of non-AM plants grown under both saline and non-saline conditions in both seasons.

Table (1): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on survival (%) and some vegetative growth characteristics of Superior grape rootings (2008 and 2009 seasons)

		Survival (%)		Average shoot length (cm)		Average shoot diameter (cm)		No. of leaves/shoot		Average leaf area/shoot (cm^2)		Total leaf area/plant (cm^2)		Coefficient of wood ripening	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	94.0	97.6	74.8	78.7	0.82	0.85	26.7	28.3	121.8	126.7	3259.5	3592.6	0.83	0.89
	(A2) 1000 ppm	83.0	87.1	70.2	73.6	0.80	0.83	25.7	27.1	117.7	122.1	3025.6	3309.0	0.81	0.86
	(A3) 2000 ppm	57.0	56.6	60.8	65.0	0.76	0.78	23.4	24.5	109.0	114.7	2552.6	2811.1	0.74	0.80
	(A4) 3000 ppm	43.2	45.2	49.4	50.4	0.61	0.59	16.6	17.7	93.9	99.1	1562.6	1758.8	0.70	0.74
new L.S.D. (A) =		**9.0**	**6.5**	**6.0**	**6.6**	**0.04**	**0.04**	**2.5**	**2.3**	**7.2**	**6.9**	**483.8**	**478.2**	**0.04**	**0.03**
(B) : Soil inoculation	(B1) non-AM	65.8	69.1	61.6	64.4	0.72	0.73	22.2	23.6	108.0	112.9	2427.4	2694.9	0.75	0.80
	(B2) AM	72.8	74.1	66.0	69.5	0.77	0.79	24.1	25.3	113.2	118.4	2772.8	3040.8	0.79	0.84
new L.S.D. (B) =		**6.4**	**4.6**	**4.3**	**4.7**	**0.03**	**0.03**	**1.8**	**1.6**	**5.1**	**4.9**	**340.7**	**336.8**	**0.03**	**0.02**
(AXB) : Interaction	A1 B1	92.6	96.5	72.1	75.7	0.80	0.82	25.6	27.0	117.5	122.5	3012.8	3311.1	0.81	0.86
	B2	95.4	98.6	77.4	81.7	0.84	0.87	27.8	29.6	126.0	130.8	3506.2	3874.1	0.86	0.91
	A2 B1	79.7	84.4	67.8	70.8	0.78	0.81	24.7	25.9	113.7	119.3	2805.5	3088.5	0.78	0.83
	B2	86.2	89.9	72.5	76.4	0.81	0.85	26.7	28.3	121.6	124.9	3245.7	3529.4	0.83	0.89
	A3 B1	52.5	53.7	58.7	62.8	0.74	0.76	22.5	24.1	107.0	112.4	2410.8	2708.8	0.73	0.79
	B2	61.5	59.5	62.8	67.2	0.77	0.79	24.3	24.9	110.9	117.0	2694.4	2913.3	0.76	0.81
	A4 B1	38.4	41.9	47.6	48.3	0.57	0.54	15.8	17.2	93.7	97.2	1480.5	1671.2	0.68	0.72
	B2	47.9	48.6	51.1	52.5	0.65	0.63	17.5	18.3	94.1	100.9	1644.8	1846.5	0.71	0.76
new L.S.D. (AXB) =		**12.7**	**9.1**	**8.5**	**9.3**	**0.06**	**0.05**	**3.5**	**3.2**	**10.1**	**9.7**	**681.4**	**673.5**	**0.06**	**0.04**

Table (2): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on shoot biomass, root biomass, total biomass and root/shoot ratio of Superior grape rootings (2008 and 2009 seasons)

		Shoot biomass (g dry weight)		Root biomass (g dry weight)		Total biomass (g dry weight)		Root/shoot ratio	
		2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	12.36	12.52	19.36	20.15	31.72	32.67	1.57	1.61
	(A2) 1000 ppm	12.08	12.23	18.60	19.54	30.68	31.76	1.54	1.60
	(A3) 2000 ppm	11.56	11.65	17.11	17.68	28.67	29.33	1.48	1.52
	(A4) 3000 ppm	10.94	11.04	15.69	16.14	26.62	27.18	1.43	1.46
new L.S.D. (A) =		**0.36**	**0.33**	**1.68**	**1.60**	**2.07**	**1.94**	**0.06**	**0.05**
(B) : Soil inoculation	(B1) non-AM	11.58	11.68	17.09	17.80	28.66	29.48	1.47	1.52
	(B2) AM	11.89	12.03	18.29	18.95	30.18	30.98	1.54	1.57
new L.S.D. (B) =		**0.26**	**0.24**	**1.19**	**1.13**	**1.46**	**1.37**	**0.05**	**0.04**
(AXB) : Interaction	A1 B1	12.19	12.23	18.53	19.43	30.72	31.66	1.52	1.59
	B2	12.52	12.81	20.19	20.87	32.71	33.68	1.61	1.63
	A2 B1	11.89	11.98	18.03	18.93	29.92	30.91	1.52	1.58
	B2	12.26	12.47	19.17	20.14	31.43	32.61	1.56	1.62
	A3 B1	11.44	11.57	16.39	16.93	27.83	28.50	1.43	1.46
	B2	11.68	11.73	17.83	18.42	29.51	30.15	1.53	1.57
	A4 B1	10.78	10.95	15.39	15.91	26.17	26.86	1.43	1.45
	B2	11.09	11.12	15.98	16.37	27.07	27.49	1.44	1.47
new L.S.D. (AXB) =		**0.51**	**0.47**	**2.37**	**2.26**	**2.91**	**2.73**	**0.09**	**0.07**

The interaction effect was shown to be significant. It is apparent from the results that AMF inoculation has benefited the plants under low to medium level salt concentrations (1000-2000 ppm). However, AMF didn't protect the plants at the highest salt concentration. The highest significant values of plant biomass were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

The reduction observed on growth parameters at increasing salinity levels can, in some instances, be attributed to salinity-induced adverse change in leaf water relations reducing photosynthesis, dehydration of proteins and protoplasm to a lower extent (Nieves *et al*., 1991 and Tozlu *et al*. 2000) and this may also be because of osmotic effect of salt on root and toxic effect of accumulated ions on the plant tissues (Lea-Cox and Syvertsen 1993 and Storey 1995).

Several mechanisms for the explanation of AM role have been proposed: AM plants have an improved ability for growth and tolerance to salt stress. Ruiz-Lozano *et al*. (1996) concluded that the mechanisms underlying AM plant growth improvement under saline conditions were based on physiological processes (increased carbon dioxide exchange rate, transpiration, stomatal conductance and water use efficiency) rather than on nutrient uptake (N or P). In addition, Feng *et al*., (2002) showed that arbuscular mycorrhizal fungus improved the resistance capacity to osmotic stress by increasing soluble sugar and electrolyte concentrations in plants roots.

Many studies have indicated that AM fungi contribute to plant growth via enhancement of mineral nutrient uptake (Bethlenfalvay *et al*., 1988; Marschner and Dell, 1994 and Ruiz-Lozano and Azcon 2000).

2. Chemical studies:

- Leaf total chlorophyll content

It's clear from data of Table (3) that increasing salt concentration gradually decreased chlorophyll content. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of leaf total chlorophyll compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that it significantly increased the leaf total chlorophyll content as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi; the results clearly show that leaf total chlorophyll content of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was shown for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of leaf total chlorophyll content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

The adverse effects of water salinity on total chlorophyll content in the leaves can be attributed to its negative action on interrupting and reducing the availability of water and nutrients particularly magnesium, destroying the building and conductance tissue and decreasing the biosynthesis of pigments and photosynthesis (Nijjer, 1985). In this concern, Gaser (1992) stated that irrigation with saline water greatly affected plant photosynthesis process, via inhibiting pigment formation. Also, Murkute *et al.*, (2006) recorded that chlorophyll decreased under stress due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments.

However, the previous increase of total chlorophyll content in the leaves in mycorrhizal plants could be ascribed due to the cytokinin-like substances secreted by fungi, which enhance the chloroplast development (Marks and Kozlowski 1973). In addition, the increase in total chlorophyll content in the leaves in mycorrhizal plants could be attributed to the ability of AM to secrete the cytokinen like substances (Nawar *et al*., 1988).

- Leaf proline content

The data in Table (3) showed that the irrigation with saline water significantly increased the proline content in the leaves. The capacity of the plant to accumulate proline under saline conditions is positively correlated with salt concentration in the irrigation water. Leaf proline content recorded the highest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

As regards to the effect of inoculation with AM fungi, it is clear that the soil inoculation with AM fungi had no effect on leaf proline content under both salinty and non-salinity conditions in both seasons.

The interaction effect in this respect was significant. However, the lowest significant values of leaf proline content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded that the highest values in both seasons of the study.

Table (3): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on leaf content of total chlorophyll and proline and shoot content of total carbohydrate of Superior grape rootings (2008 and 2009 seasons)

		Leaf total chlorophyll content (SPAD)		Leaf proline content (mg/g F.W.)		Shoot total carbohydrate content (%)	
		2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	37.2	28.3	0.07	0.09	22.4	23.8
	(A2) 1000 ppm	36.5	26.7	0.08	0.10	21.7	22.9
	(A3) 2000 ppm	34.4	25.1	0.11	0.12	20.4	21.7
	(A4) 3000 ppm	30.9	23.3	0.13	0.14	19.6	20.7
new L.S.D. (A) =		**1.3**	**1.2**	**0.04**	**0.02**	**0.6**	**0.5**
(B) : Soil inoculation	(B1) non-AM	34.1	24.9	0.10	0.12	20.7	22.0
	(B2) AM	35.4	26.7	0.09	0.11	21.3	22.6
new L.S.D. (B) =		**0.9**	**0.9**	**N.S**	**N.S**	**0.5**	**0.4**
(AXB) : Interaction	A1 B1	37.0	26.3	0.08	0.09	21.8	23.1
	B2	37.4	30.3	0.05	0.08	23.0	24.4
	A2 B1	35.8	25.7	0.09	0.10	21.4	22.6
	B2	37.2	27.6	0.07	0.09	22.0	23.2
	A3 B1	33.2	24.6	0.11	0.12	20.2	21.6
	B2	35.5	25.6	0.10	0.12	20.5	21.8
	A4 B1	30.2	23.1	0.13	0.15	19.4	20.5
	B2	31.6	23.4	0.12	0.13	19.8	20.8
new L.S.D. (AXB) =		**1.8**	**1.7**	**0.05**	**0.03**	**0.9**	**0.7**

Increasing proline content in the leaves with increasing water salinity might be attributed to the increase of hydrolytic enzymes caused by chloride salts and salinity (Klyskov and Rakova, 1964). Furthermore, leaf proline content has been used as an evaluation parameter for selecting salinity and drought resistant varieties (Batels *et al.*, 1973). In addition, plants build up proline in the tissues to maintain osmotic balance with the soil solution (Salisbury and Ross, 1992). In this connection, El-Said *et al*. (1995) and Abbas (1999) suggested that proline functions as a source of solute for intera-cellular osmotic adjustments under saline condition.

- Shoot total carbohydrate content

As shown in Table (3), it is obvious that increasing salt concentration gradually decreased shoot content of total carbohydrates. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of shoot content of total carbohydrates compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly improved in shoot content of total carbohydrates as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results show that total carbohydrate content in the shoots of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was found with AM plants under low and medium salinity (1000-2000 ppm). The highest significant values of shoot carbohydrate content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

These results are in harmony with Kilany *et al*., (2006) who found that water stress due to salinity by raising salt concentration in the irrigation water effectively depressed the synthesis of carbohydrates.

However, Arbuscular mycorrhizal (AM) fungi improve physiological processes, like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Rosendahl and Rosendahl 1991).

- Leaf mineral content

The results concerning leaf mineral content in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 4).

The data showed that the irrigation with increased salinity level up to 3000 ppm significantly decreased the nitrogen, phosphorus, potassium, calcium, magnesium and sulfur content in the leaves as compared with non- salted ones. On the contrary, sodium, and chloride content in the leaves recorded the highest values in plants grown under the highest salinity concentration (3000 ppm) as compared to the control regardless of AM fungi inoculation.

As regards the effect of inoculation with AM fungi, it is clear that this resulted in an increase in leaf N, P, K, Ca, Mg and S content as compared with the untreated plants. On the contrary, the addition of AM fungi reduced leaf Na, and Cl content under both salinity and non-salinity conditions in both seasons.

The interaction effect in this connection was found to be significant. It is clear from the results that AM fungi inoculation increased leaf N, P, K, Ca, Mg and S content and decreased leaf Na, and Cl content under non-salinity conditions, while non-AM plants grown under high saline conditions (3000 ppm) took the opposite trend., it caused an obvious reduction in leaf N, P, K, Ca, Mg and S content while it was responsible for enhancing leaf Na, and Cl content in both seasons of the study.

The reduction occurring in N, P, K, Ca, Mg and S content of the leaves under salt stress might be attributed to the increase in osmotic pressure, thereby reducing the water and nutrients uptake. These results were confirmed by Gaser (1999), Hassan *et al*. (1999), Sivritepe (2000) and Stevens and Walker (2002).

Some mechanisms have been suggested to explain the role of AM inoculation: AM can improve salt tolerance through inducing osmotic adjustment (Duke et al., 1986), AM capability of dissolving weakly soluble soil minerals by releasing acids (Leyval, and Berthelin, 1989), improve and balance nutrition in plants could also increase salt tolerance (Marschner, 1995), reduce the negative effects of Na and Cl by maintaining membrane integrity (Mancuso and Rinaldelli, 1996 and Rinaldelli and Mancuso, 1996) that would facilitate compartmentalization within vacuoles, and selective ion intake. In this respect, Cantrell and Linderman (2001) suggested that improved mineral nutrient absorption by AM fungi in plants grown under saline conditions might reduce the negative effects of Na and Cl and retain them in roots without being translocated to the shoots by maintaining vacuolar membrane integrity and retaining in intracellular AM fungal hyphae or was compartmentalized in the root cell vacuoles which prevented these ions from interfering in the metabolic pathways of growth.

Table (4): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on leaf mineral content of Superior grape rootings (2008 and 2009 seasons)

		N (%)		P (%)		K (%)		Ca (%)		Mg (%)		S (%)		Cl (%)		Na (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	2.16	2.22	0.36	0.39	1.46	1.54	2.63	2.75	0.63	0.69	0.32	0.35	1.08	1.02	0.38	0.43
	(A2) 1000 ppm	2.07	2.16	0.34	0.37	1.42	1.51	2.56	2.66	0.60	0.66	0.29	0.32	1.15	1.10	0.42	0.47
	(A3) 2000 ppm	1.97	2.00	0.28	0.33	1.35	1.43	2.44	2.51	0.51	0.59	0.22	0.25	1.26	1.21	0.53	0.57
	(A4) 3000 ppm	1.88	1.92	0.25	0.27	1.28	1.37	2.21	2.30	0.44	0.51	0.16	0.20	1.54	1.44	0.63	0.65
new L.S.D. (A) =		**0.13**	**0.11**	**0.03**	**0.01**	**0.06**	**0.04**	**0.06**	**0.05**	**0.06**	**0.04**	**0.04**	**0.03**	**0.12**	**0.09**	**0.07**	**0.06**
(B) : Soil inoculation	(B1) non-AM	1.97	2.02	0.29	0.32	1.35	1.44	2.43	2.51	0.52	0.58	0.23	0.25	1.31	1.23	0.52	0.56
	(B2) AM	2.07	2.13	0.32	0.36	1.40	1.49	2.49	2.60	0.57	0.63	0.27	0.30	1.21	1.15	0.46	0.50
new L.S.D. (B) =		**0.10**	**0.08**	**0.02**	**0.01**	**0.04**	**0.03**	**0.05**	**0.04**	**0.04**	**0.03**	**0.03**	**0.02**	**0.09**	**0.07**	**0.05**	**0.04**
(AXB) : Interaction	A1 B1	2.06	2.14	0.34	0.37	1.43	1.50	2.58	2.70	0.60	0.66	0.29	0.31	1.13	1.07	0.42	0.46
	B2	2.25	2.31	0.38	0.41	1.49	1.57	2.67	2.79	0.65	0.71	0.34	0.38	1.03	0.97	0.34	0.39
	A2 B1	2.01	2.08	0.32	0.35	1.40	1.48	2.50	2.57	0.57	0.62	0.26	0.29	1.21	1.15	0.46	0.51
	B2	2.14	2.23	0.35	0.39	1.45	1.54	2.62	2.75	0.62	0.69	0.31	0.34	1.09	1.05	0.37	0.43
	A3 B1	1.95	1.97	0.27	0.31	1.33	1.41	2.43	2.48	0.49	0.57	0.20	0.23	1.28	1.23	0.55	0.58
	B2	1.99	2.03	0.29	0.34	1.36	1.45	2.45	2.53	0.53	0.60	0.24	0.26	1.24	1.19	0.51	0.55
	A4 B1	1.84	1.89	0.23	0.25	1.26	1.35	2.19	2.27	0.42	0.48	0.15	0.18	1.61	1.48	0.64	0.67
	B2	1.91	1.94	0.26	0.29	1.30	1.39	2.23	2.33	0.46	0.53	0.17	0.21	1.46	1.39	0.61	0.63
new L.S.D. (AXB) =		**0.19**	**0.16**	**0.04**	**0.02**	**0.08**	**0.05**	**0.09**	**0.07**	**0.08**	**0.05**	**0.06**	**0.04**	**0.17**	**0.13**	**0.10**	**0.08**

In addition, Zhu, (2003) recorded that improved plant nutrition by AM fungi allows cells to more effectively regulate and separate flowing ions which its pump in the plasma membrane and tonoplast of root cells.

The results are in agreement with those obtained by Duponnois *et al*., (2005) and Al-Karaki, (2006) who explained that the higher mineral nutrient acquisition in AM compared to non-AM plants likely occurred because of increased availabilities or transport (absorption and/or translocation) by AM fungi hyphae.

3. Microbiological studies:-

Data concerning the effect of saline water and soil inoculation with Aarbuscular mycorrhizae fungi on microbial and enzyme activity in the rhizosphere of Superior grape rootings during 2008 and 2009 seasons are shown in Table (5) and Figure (1, 2, 3 and 4).

- Total microbial count

It's clear from data of Table (5) and Figure (1) that increasing salt concentration gradually decreased total microbial count. Irrigation with high saline water (at 3000 ppm) significantly recorded the lowest values of total microbial count compared to the other treatments while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that, it was found that soil inoculation with AM significantly increased in total microbial count as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results revealed that total microbial count of non-AM plants significantly decreased with increasing salinity level, particularly in case of the high salinity concentration (3000 ppm), while the opposite trend was shown for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of total microbial count were obtained from the AM plants under non-salinity conditions recording (117 & 134 $x10^5$cfu/g soil) for both seasons respectively, and resulting in an increase over control by (1.40 & 1.39) fold for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

The results are in agreement with those obtained by (Godeas *et al*., 1999) who explained that the increase in populations of rhizospheric microorganisms in the roots of most plants are influenced by a combined inoculation of microorganism and AM fungi.

- Number of AM

The results concerning number of AM spores / soil in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 5) and Figure (2).

In the salinity treatments; there was a decline in number of AM spores in soil with increased salinity level. Number of AM spores in soil recorded the lowest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

Concerning the effect of inoculation with AM fungi, it is clear that the number of AM spores in soil was higher in AM plants than those of non-AM plants grown under both salinity and non-salinity conditions in both seasons.

The interaction effect was significant. It can be shown from the results that AMF inoculation benefits the plants under low to medium levels of salt concentrations (1000-2000 ppm). However, AMF didn't protect the plants at the highest salt concentration. The highest significant values of number of AM spores in soil were obtained from the AM plants under non-salinity conditions recording (760 & 893 spores/g soil) for both seasons respectively, and resulting in an increase over control by (20.54 & 15.95) fold for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

These findings are in line with those obtained by (Turk *et al*., 2006) who pointed out that AM-mycorrhizae colonize plant roots and mainly in the surrounding soil extending the roots depletion zone around the root system.

- AM infection

Data concerning the effect of saline water and soil inoculation with Aarbuscular mycorrhizae fungi on percentage of AM infection of Superior grape rootings during 2008 and 2009 seasons are shown in Table (5) and Figure (3).

It's obvious that increasing salt concentration gradually decreased percentage of AM infection. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of total microbial count compared to the other treatments, while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly increased the percentage of AM infection as compared with non-AM in both seasons.

Table (5): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on microbial and enzyme activity in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

		Total microbial count (-x10^5cfu/g soil)		Number of AM (spore/g soil)		AM infection (%)		Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day)	
		2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	101	115	399	475	41.3	48.8	72	80
	(A2) 1000 ppm	88	99	328	433	33.3	44.9	63	71
	(A3) 2000 ppm	45	54	292	377	29.6	39.7	32	40
	(A4) 3000 ppm	25	30	214	333	21.7	34.6	18	23
new L.S.D. (A) =		**31**	**26**	**129**	**124**	**13.7**	**10.7**	**23**	**21**
(B) : Soil inoculation	(B1) non-AM	52	60	22	37	2.6	5.9	37	44
	(B2) AM	78	89	594	772	60.3	78.0	55	63
new L.S.D. (B) =		**22**	**19**	**91**	**87**	**9.7**	**7.6**	**17**	**15**
(AXB) : Interaction	A1 B1	84	97	37	56	5.2	8.3	60	68
	B2	117	134	760	893	77.4	89.2	84	93
	A2 B1	67	74	23	47	2.3	6.2	48	56
	B2	109	123	632	819	64.3	83.5	78	85
	A3 B1	39	46	17	28	1.7	5.8	28	34
	B2	52	61	567	726	57.4	73.6	37	45
	A4 B1	19	23	11	16	1.2	3.4	14	17
	B2	32	37	417	649	42.2	65.8	23	28
new L.S.D. (AXB) =		**43**	**37**	**181**	**174**	**19.3**	**15.1**	**33**	**29**

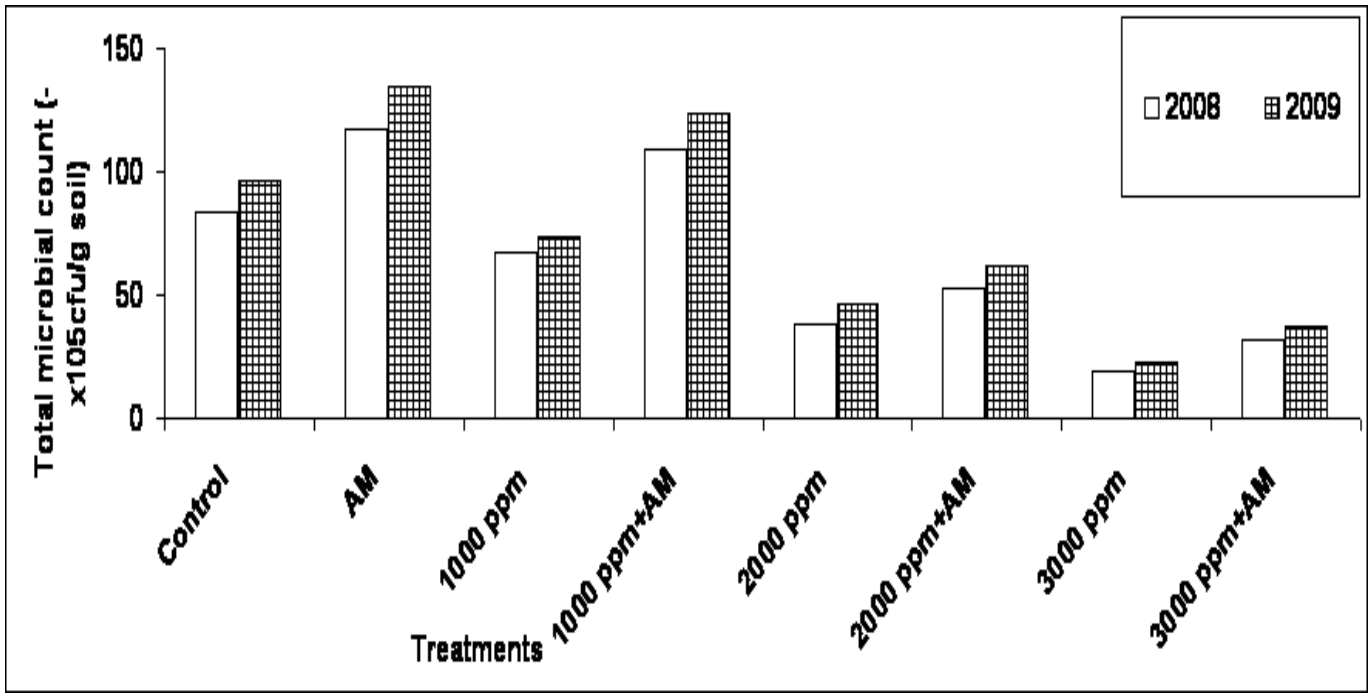

Fig (1): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on total microbial count (-x105cfu/g soil) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

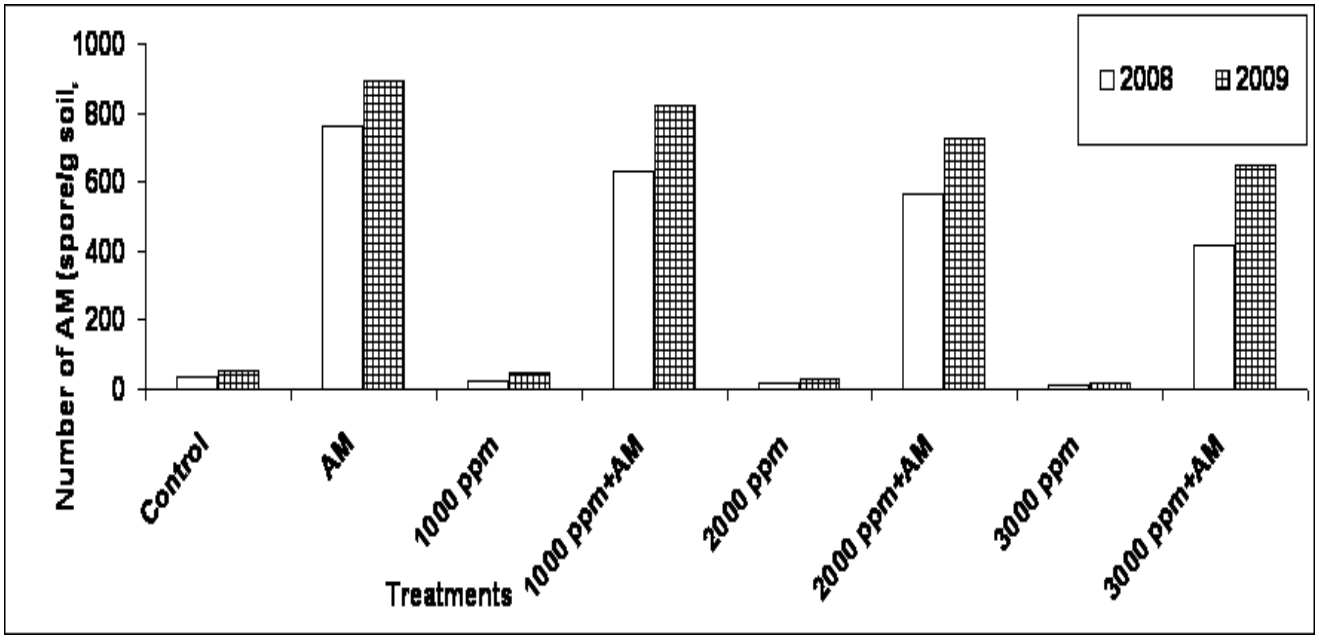

Fig (2): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on number of AM (spore/g soil) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

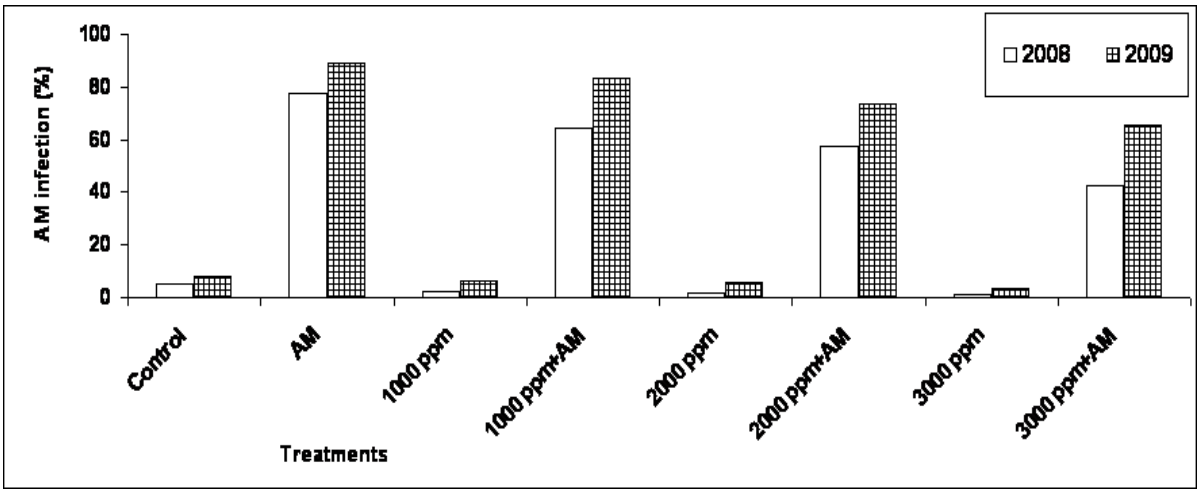

Fig (3): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on AM infection (%) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

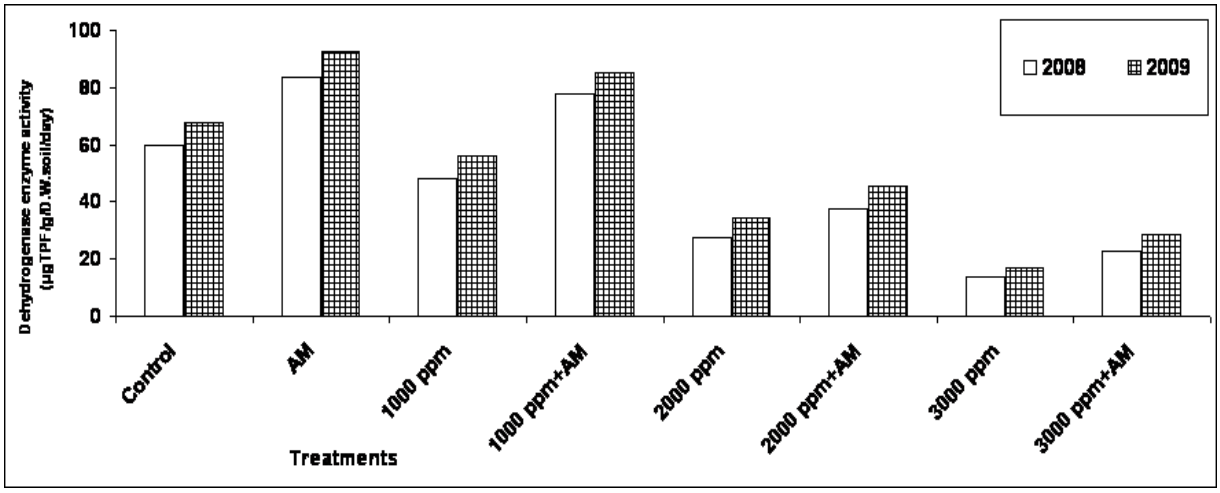

Fig (4): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

A significant interaction was observed between saline water and soil inoculation with AM fungi, it is clear from the results that percentage of AM infection of non-AM plants significantly declined with increasing salinity level, particularly in case of the high salinity concentration (3000 ppm), while the opposite trend was found for AM plants under low and medium salinity (1000-2000 ppm). The highest significant values of percentage of AM infection were obtained from the AM plants under non-salinity conditions for both seasons, while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

Previous researches have shown that salinity may reduce mycorrhizal colonization by inhibiting the germination of spores (Hirrel, 1981), finding of adverse conditions for sporulation and development of spores under unfavorable rhizosphere conditions (Duke *et al*. 1986), reducing the number of arbuscules (Pfeiffer and Bloss, 1988) and inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen *et al*., 1998).

- Dehydrogenase enzyme activity

Data shown in Table (5) and Figure (4) revealed the existence of dehydrogenase enzyme activity among treatments giving an indication of microbial activity in the soil inoculated with arbuscular mycorrhiza (AM) at different concentrations of salinity.

In the salinity treatments; there was a decline in activity of dehydrogenase enzyme with increasing salinity level. Number of AM spores / soil recorded the lowest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

As regards the effect of inoculation with AM fungi, it is clear that the activity of dehydrogenase enzyme was higher in AM plants than that of non-AM plants grown under both salinity and non-salinity conditions in both seasons.

The interaction effect was found to be significant. The highest significant values of activity of dehydrogenase enzyme were obtained from the AM plants under non-salinity conditions recording (84 & 93 µgTPF/g/D.W.soil/day) for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values recording (14 & 17 µgTPF/g/D.W.soil/day) for both seasons of the study.

The increase in dehydrogenase enzyme activity was attributed to the intense activity of microflora as a mixture of biomass than each individual one. The highest increase in microbial respiration was recorded with the mixture of microorganism in the soil (Massoud, 2005).

In conclusion, it seems that Superior grape rootings strategy for salt stress tolerance could be achieved by AM fungi colonization. AM fungi inoculation benefits the plants by avoiding the undesirable effects of saline water and improving of survival percentage, vegetative growth parameters, nutrient acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings under low to medium levels of salt concentrations (1000-2000 ppm). However, AM fungi inoculation didn't protect the plants at highest salt concentration (3000 ppm) used in the study.

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12/2/2010

Cytologenetical Study of some Wild Plants from Taif, Saudi Arabia

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Abstract: Saudi Arabia is the largest country of the Arabian Peninsula which has a diverse higher plant flora in its varied landscapes with more than 2243 plant species which has a valuable economic importance due to its usage as pharmaceuticals, nutritional, fire wood suppliers as well as its use in popular remedy. Due to the scant of wild plant species studies of Arabian in literatures, the present study aim to report the chromosome numbers of 8 taxa belonging to 4 families of angiosperms collected from Taif province, Saudi Arabia flora. These taxa are: *Solanum villosum* Mill., *Datura stramonium* L., *Aerva javanica* (Burm.f.) Juss. Ex Shult, *Calotropus procera* (Aiton) W.T. Aiton, *Acacia tortilis* subspecies tortilis (Forssk.) Hayne, *Acacia oerfota* (Forssk.) Schweinf, and *Acacia gerrardii* Benth.

[Soliman, M.S.A., El-Tarras. A. and El-Awady, M. A. **Cytologenetical Study of some Wild Plants from Taif, Saudi Arabia.** Nature and Science. 2011;9(1):101-104]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Key words: Chromosome number, *Solanum*, *Datura*, *Aerva*, *Calotropus*, *Acacia*.

1. Introduction

Saudi Arabia, the largest country of the Arabian Peninsula, has a diversified higher plant flora in its varied landscapes, with about 2243 species in 837 genera and 142 families, Collenette (1998 & 1999). Taif, as a town situated on eastern slope of Makka region in Saudi Arabia above Sarawat mountains by1700-2500m altitude and attitude of 20-22º horizontal and 40-42º longitudinal.

Aspects of the plant diversity of Saudi Arabia have been documented by Mandaville (1990), Chaudhary (1999) and Chaudhary & Al-Jowaid (1999).

Although chromosome numbers of some species found in Saudi Arabia are known from other parts of their distribution area, there are many which have never been reported previously.

Only few species growing in Saudi Arabia were cytologically investigated so far (Al-Turki 1992, Badr & Gassim 1992 and Al-Turki *et al*. 2000). The present study therefore aims at a completion of our knowledge of the cytology of the flowering plants in the Kingdom, providing a basis for nature conservation and other applied programs as well as for taxonomic and genetic studies.

In this study, chromosome numbers of 7 taxa belonging to 4 families of flowering plants are provided. These taxa are: *Solanum villosum* Mill. (Family: Solanaceae), *Datura stramonium* L. (Family: Solanaceae), *Aerva javanica* (Burm.f.) Juss. Ex Shult. (Family: Amaranthaceae), *Calotropus procera* (Aiton) W.T. Aiton (Family: Asclepiadaceae), *Acacia tortilis* subspecies tortilis (Forssk.) Hayne (Family: Leguminosae), *Acacia oerfota* (Forssk.) Schweinf. (Family: Leguminosae), and *Acacia gerrardii* Benth. (Family: Leguminosae).

2. Material and methods

Plants and seeds of the taxa investigated were collected from Taif province of the Kingdom of Saudi Arabia.

The plants were identified by staff members of the Herbarium of Taif University, Taif, using the works of Migahid (1978), Miller & Cope (1996), Collenette (1999) and Chaudhary (1999). Voucher specimens of the studied taxa were deposited in the Scientific Research Center, Taif University, Saudi Arabia.

Chromosome counts were made from metaphase plates of mitotic division in squashed root tips of seedling germinated in 9.0 cm plastic Petri-dishes on two layers of Whatman No.1 filter paper moistened with distilled water. They were incubated at 25°C. The cytological preparations were made according to the technique described by Darlington & La-Cour (1976); root-tips were excised, pretreated with 0.05% Colchicine for 4 h in iced-water, fixed in freshly prepared acetic- alcohol solution (3:1) and after a brief wash, hydrolysed in 1N HCl for 10-12 min at 60°C, root tips were stained by Feulgen's reagent for 60 mins. An average of 10 cells for each taxa has been used for chromosome counting. Best observations, were photographed using the 100× oil immersion objective photomicroscope.

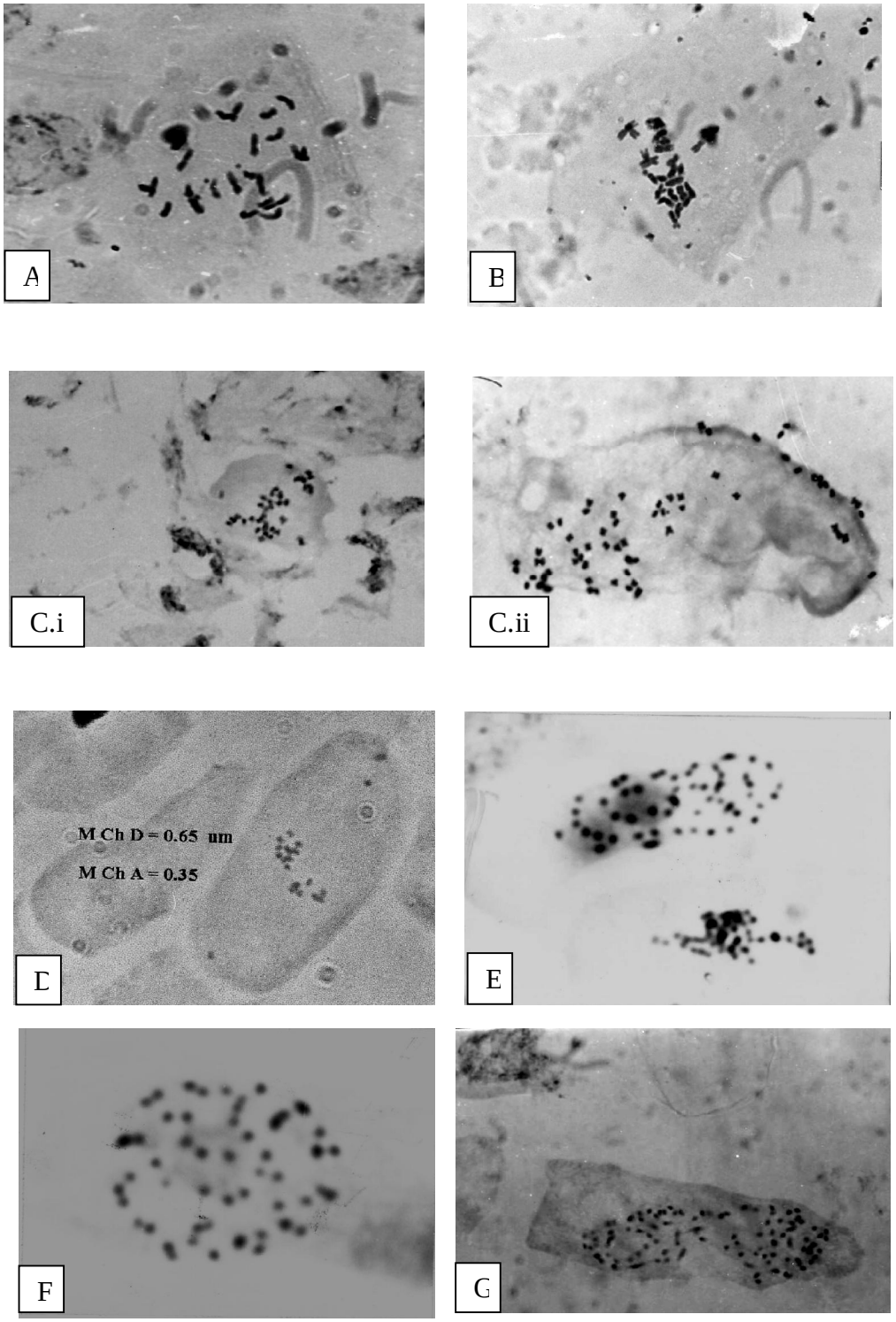

Figure (1): Mitotic cell division of *Solanum villosum* (A), *Datura stramonium* (B), *Aerva javanica* (C), *Calotropus procera* (D), *Acacia tortilis* (E), *Acacia oerfota* (F) and *Acacia gerrardii* (G).

3. Results and Evaluations

The chromosome numbers determined for 7 taxa belonging to 4 families are the first reports from Saudi Arabian populations.

Solanum villosum Mill. (Family: Solanaceae) was found to have 2n=24 chromosomes, Fig. (1-A). The same chromosome number has been recorded for *Solanum nigrum* by Sultana and Alam (2007).

The population of *Datura stramonium* L. (Family: Solanaceae), Fig. (1-B) with a diploid set of chromosomes (2n = 24); there were no chromosomes with satellites and there were 1-2 micro-chromosomes in the metaphases. Spurnà et al (1981), has the same result of chromosome number. He mentioned that *D. stramonium* L. included biotypes with a chromosome number of 21-25; in the metaphases, there were chromosomes with satellites and the metaphases contained 1-3 micro-chromosomes.

Two chromosomal cellular counts of *Aerva javanica* (Burm.f.) Juss. Ex Shult. (Family: Amaranthaceae) chromosomal numbers has been counted, Fig. (1-C-i & C-ii). These are 2n= 32 and 2n= 64. Greizerstein and Poggio (1992) proposed that the species with 2n= 32 are polyploids (basic chromosome number x=8) and that x= 16 is a derived basic number.

Calotropus procera (Aiton) W.T. Aiton (Family: Asclepiadaceae) have the chromosome number 2n=22, (Fig. 1-D). The present result confirms that recorded by Raghavan (1957), where *Calotropus procera* and *Calotropus gigantean* had 2n= 22 chromosomes, while Bramwell *et. al*. (1972) mention that *Calotropus* R. Br. have 2n=26.

The chromosomal cellular counts of *Acacia tortilis* subspecies *tortilis* (Forssk.) Hayne (Family: Leguminosae), Fig. (1-E), was found in populations of *A. tortilis* subspecies *tortilis*, 2n = 52. Ouarda *et al*. (2009) has the same result. The same chromosome number was found in *Acacia oerfota* (Forssk.) Schweinf. (Family: Leguminosae), (Fig. 1. F), while *Acacia gerrardii* Benth. (Family: Leguminosae) has 2n= 104.

The previous chromosome numbers in Acacia species showed the same chromosome number for populations of A. tortilis (Forssk.) Hyne across their wide geographical range have one cytotype with a chromosome number 2n=52 (Oballa and Olng'otie 1993).

More chromosomal detailed analysis for the Arabian Peninsula wild plant flora is needed especially in case of great number of plant flora species and scant cytogenetically analysis.

Acknowledgements

We gratefully acknowledge the support of Scientific Research Funding Programme, Taif University, Taif, Kingdom of Saudi Arabia, for funding the project (No. 487/431/2).

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1/5/2010

Using ISO 5130and ISO 362 for determination of both stationary and pass–by vehicles noise and discuss the difference between them.

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Abstract: The traffic noise is considered as one of the most important public annoyances. Using ISO 362 measurements of vehicles pass- by noise are needed to predict any change in traffic sound levels. Also, ISO 5130 is used for determination the noise emitted by stationary road vehicles. The difference between the two cases, namely, stationary and pass-by, depends on different parameters (tires-road surface – etc). From the measurements carried out on vehicles, using the two mentioned methods, the parameters values could be evaluated.
[Abd-elfattah A. Mahmoud. **Using ISO 5130and ISO 362 for determination of both stationary and pass–by vehicles noise and discuss the difference between them.** Nature and Science. 2011;9(1):105-110]. (ISSN: 1545-0740). http://www.sciencepub.net/nature.

Keywords: Using ISO 5130and ISO 362 for determination of both stationary and pass–by vehicles noise and discuss the difference between them

1- Introduction

The parameters, which have an influence on vehicle noise emission, are well known: vehicle type, speed, driving behaviour (acceleration), road surface, road gradient. A complete prediction method should provide an emission value for each combination of these parameters [1]. A vehicle's acoustic signal consists of a combination of various noise signals generated by the engine, the tires, the exhaust system, aerodynamic effects, and mechanical effects (e.g., axle rotation, brake pads, and suspension). It has a spatial distribution because the noise sources are at different locations on the vehicle. The mixture weighting of these spectral components at any given location is dependent on the vehicle's speed, whether the vehicle is accelerating, decelerating, turning, and whether the vehicle is in good mechanical condition [2]. In general, one can approximate a well maintained vehicle's signal as consisting of four noise components:

a) *Engine Noise:* The noise from an internal combustion engine, the engine noise is largely due to the turbulent air flow in the air intake (or intercooler), the engine cooling systems, and the alternator fans, The strongest tone in the engine noise is called the engine fire rate. Car manufacturers try to suppress the engine noise as much as possible for the passengers' comfort inside the vehicle cabin, also the manufacturers try to suppress the noise levels outside the car. To achieve this, the interior of the engine compartment is usually treated with material for acoustical attenuation . Hence, in some cases, the engine noise might be stronger on the side and at the very front of the car than other directions, because sound propagation through the axle, the front grill, and the bottom of the engine block cannot be filtered effectively.

b) *Tire Noise:* The term tire noise is defined as the noise emitted from a rolling tire as a result of its interaction with the road surface. The tire noise is the main source of a vehicle's total noise at speeds higher than 50km/h. It consists of two components: Vibrational noise and air noise. The vibrational component is caused by the contact between the tire threads and the pavement texture. Its spectrum is most dominant between 100 − 1000Hz. The air noise is generated by the air being sucked-in or forced out of the rubber blocks of a tire and is dominant in the frequency ranges between 1000 and 3000Hz.

c) *Exhaust Noise:* The exhaust system consists of the exhaust manifold, catalytic converter, resonator, exhaust pipe, muffler, and the tail pipe. The system goes from the engine compartment to the back of the car generating the exhaust noise. Due to the system's spatial distribution, this noise is less prominent in the front of a vehicle. Unlike the engine block noise, the exhaust system noise increases significantly with the engine load. The exhaust noise is also affected by engine turbo/super chargers and after-coolers. Manufacturers use a combination of reactive and absorptive

silencers to keep the exhaust noise level down. The exhaust noise has broadband characteristics

with most of its power concentrated around low frequencies.

d) *Air Turbulence Noise:* Vehicle induced turbulence can become an important factor in the overall perceived loudness of a vehicle as the vehicle speed increases. This noise is due to air flow

generated by the boundary layer of the vehicle. The turbulence noise depends on the aerodynamics of the vehicle as well as the ambient wind speed and its orientation.

A new method to determine sound power levels (PWLs) [4], used for modeling outdoor sound simulations, are obtained from sounds that are emitted by various types of vehicles and cause road traffic noise. Several PWL determination methods have been suggested based on the SPLs obtained from a receiver. These methods require environmental correction and consideration of the influence of the SPL measurement surface where the noise, caused by stationary machines or vehicles, is applied.

The effects of vehicles and pavement surface types on noise have been investigated [5], at the Korea Highway Corporation's Test Road along the southbound side of the Jungbu Inland Expressway, South Korea. The study was conducted in 2005 and 2006 through field measurements were carried out at nine surface sections of asphalt concrete and Portland cement concrete pavements using eleven vehicles. For the road noise analysis, the sound power levels PWLs of combined noise (e.g., tire/pavement interaction noise and power-train noise together) and tire/pavement interaction noise using various vehicles were calculated based on the novel close proximity and pass-by methods. Then, the characteristics of the PWLs were evaluated according to surface type, vehicle type, and vehicle speed. The results show that the PWLs of vehicles are diversely affected by vehicle speed and the condition of the road surface. The nine pavement surface types evaluated in this study were compared according to vehicle type and speed. Comparisons were made using the regression equation that is based on several noise prediction models.

2- Test Methods and Measurement

2-1 Procedures to determine the vehicle's L_{Amax}, from the pass-by method (6,7)

The vehicle's L_{Amax} was determined by using the sound level meter, A-weighted maximum noise level obtained from the pass-by method. The L_{Amax} can be simply measured when the distance between a moving vehicle and a microphone measuring SPLs is the closest during a pass-by.

2-1-1 Measurement Site

The data was collected on 6-October highway, on a sunny day. The test track site pavement is drain asphalt, in the portion of the area between the vehicle path and the microphone location (Figure 1). The test track and the surface of the site were dry.

2-1-2. Measurements of meteorological conditions and background noise

Meteorological conditions (temperature, humidity, pressure and the wind speed at the height of microphone did not exceed 5 m/s during the sound measurement intervals) were measured to ascertain the influence of weather or other environmental conditions on noise emission. It is observed that the A-weighted back ground noise was more than 15 dBA below the emission produced by the vehicle under test. As the back ground noise was much below the noise emissions from the vehicle, we can say that the noise recording from the microphone when the vehicle was in the test region is due to the noise from the vehicle only.

It is to be noted that the porous asphalt has noise absorbing characteristics. The large size aggregates increase lightly the noise due to tire road contact; whereas the noise absorbing characteristics (due to porosity and touristy) of the drain asphalt decrease largely the noise

Microphones, located at a height of 1.2 meters and 7.5 meters away from the centre line of the running track, were used to record the noise level during the study period.

2-2 Procedures to determine the vehicle's A-weighted sound pressure levels, from the stationary method (8)

The vehicle's A-weighted sound pressure levels was determined by using the sound level meter, A-weighted maximum noise level obtained from the stationary vehicle method. The height of the microphone above the ground is 0.2m and pointed towards the outlet orifice and located at a distance of 0.5 from the outlet orifice. This international standard specifies a test method for the determination of noise emitted by stationary road vehicles in use, the noise being measured in proximity to the exhaust.

2-2-1 Measurement Site

Any open space may be considered as a suitable test site if it consists of a flat area made of asphalt or hard material having a high acoustical reflectivity. The stationary test were carried out at way, that is no edges of the test site nearer than 3 m from the extremities of the vehicle and there is no any obstacle closer than 3m to the microphone during test with the exception of the observer and driver, no person whose presence influences the meter readings are present during the test.

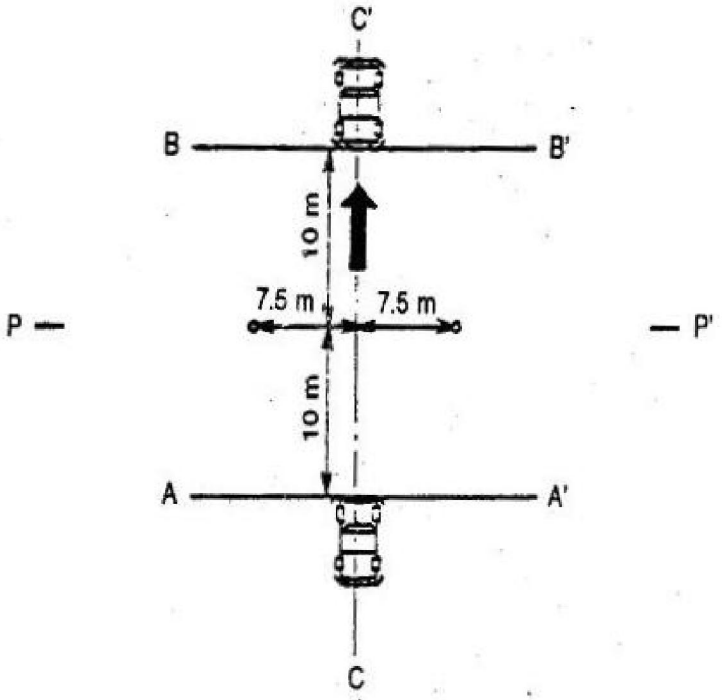

Figure 1. Measurement site and its test track for the pass-by method

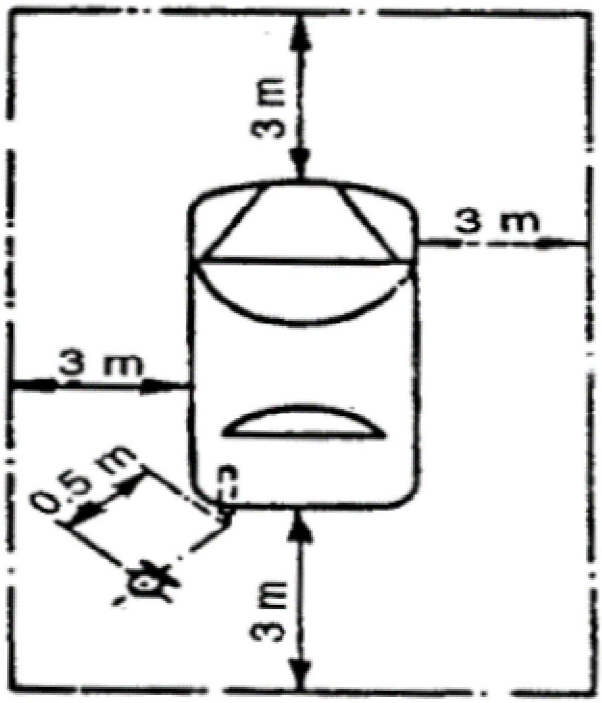

Figure 2. Measurement site and test for the stationary method

3-Measurement and evaluation of the A-weighted sound pressure levels of each vehicle

The results obtained for the sound pressure level of 5 different vehicles represents in figure 3, different vehicles passing with constant speed 50km/hour. The sound pressure level of 5 vehicles were recorded, the tire/road noise and mechanical noise are involved in the measurements when the vehicle is passing. Figure 4, shows the one-third octave band spectrum for vehicles approaching at a constant speed 50km/hour. The relationship between sound pressure level and vehicles speeds illustrated in fig. 5. The noise emitted from vehicles increases as vehicle speed increased. This means that, vehicles speeds only have a limited influence on generated sound pressure level, it is about 10dB per doubling speeds. Also fig 5; illustrates the effect of vehicle acceleration on its noise emission (Lamax), the evaluation of tire/road and motor-noise for each vehicle are involved in measurements, and the noise levels emitted for various engine speeds. The total noise levels are overestimated when the vehicle is running. Figure 6, illustrates the relation between the overall LAmax of pass-by and overall LAmax of stationary, the results appears that, the sound pressure level (SPLs) of stationary are larger than those of LAmax of pass-by. In statistical analyses for stationary case, the standard deviations in overall values are less than 1 dBA but for pass-by case it is about 2.4dBA.

The difference in values obtained from subtracting LAmax of pass-by from LAmax of stationary, the difference in values were calculated based on the measured data. The international standard 5130, specifies a test method for the determination of noise emitted by stationary road vehicles in use, the noise being measured in proximity to the exhaust. However, the purpose of 362, is the measurement of noise emitted by accelerating road vehicles, and the purpose of this study is to evaluate the difference in noise emissions between the two cases. the two methods are slightly different. The main differences are that: the 5130 standard places a single microphone at 0.5m from the outlet orifice (the noise being measured in proximity to the exhaust) at a height of 0.2 m above the ground . For 362 standard, two microphones, located at a height of 1.2 meters and 7.5 meters away from the centre line of the running track.

4- Conclusion

Measurements were made using the 5 vehicles .A comparison of the results is shown in Figure 6, based on the curves of measurements of stationary and pass-by vehicles, the difference between the sound pressure levels in stationary and pass-by methods, the trend lines of vehicles were nearly the same on test vehicles. This means that characteristic of the difference in sound levels between the two methods was very consistent with the behaviour of two curves, it ranged from 30-33 dBA. Average results at stationary case is a 30-33 dBA to the exhaust noise, higher than the level for the accelerating road vehicles.

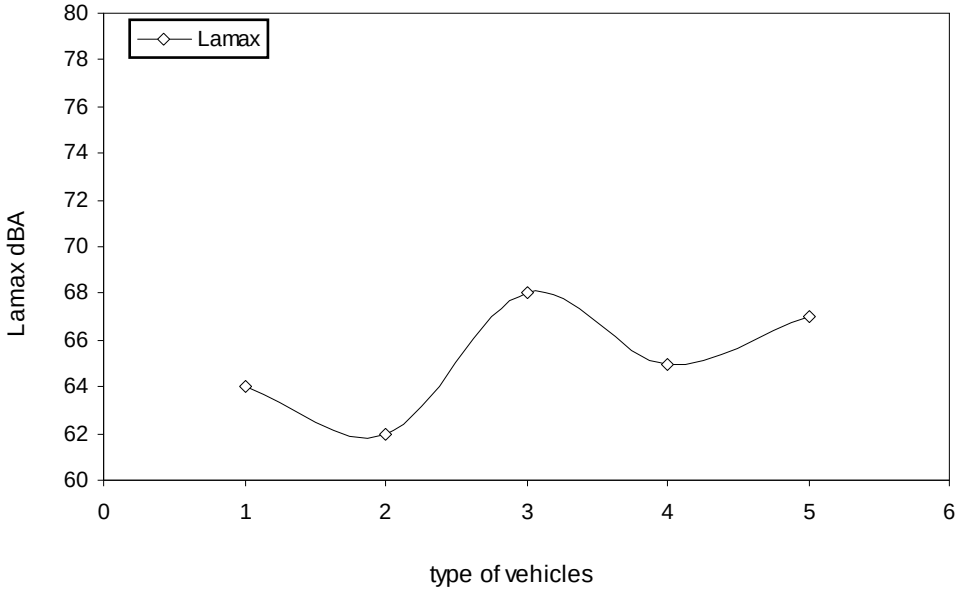

Figure 3. Effect of vehicle type on its LAmax of pass-by noise emission at 50Km/hpur speed

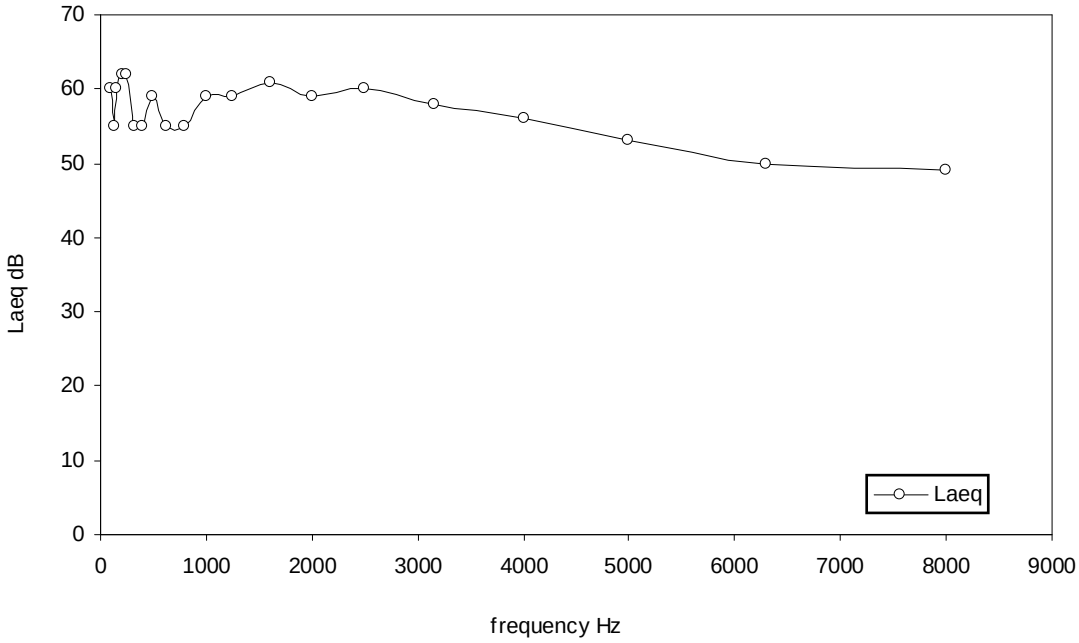

Figure 4. shown the noise emission spectrum of vehicle

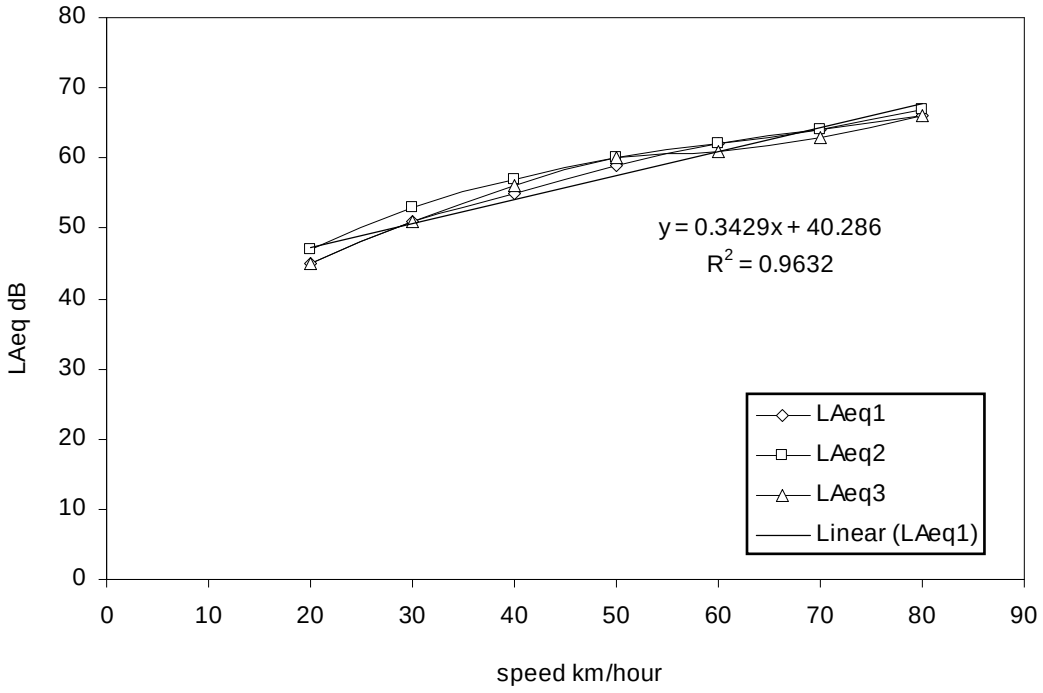

Figure 5. Effect of vehicle acceleration on its noise emission Lamax

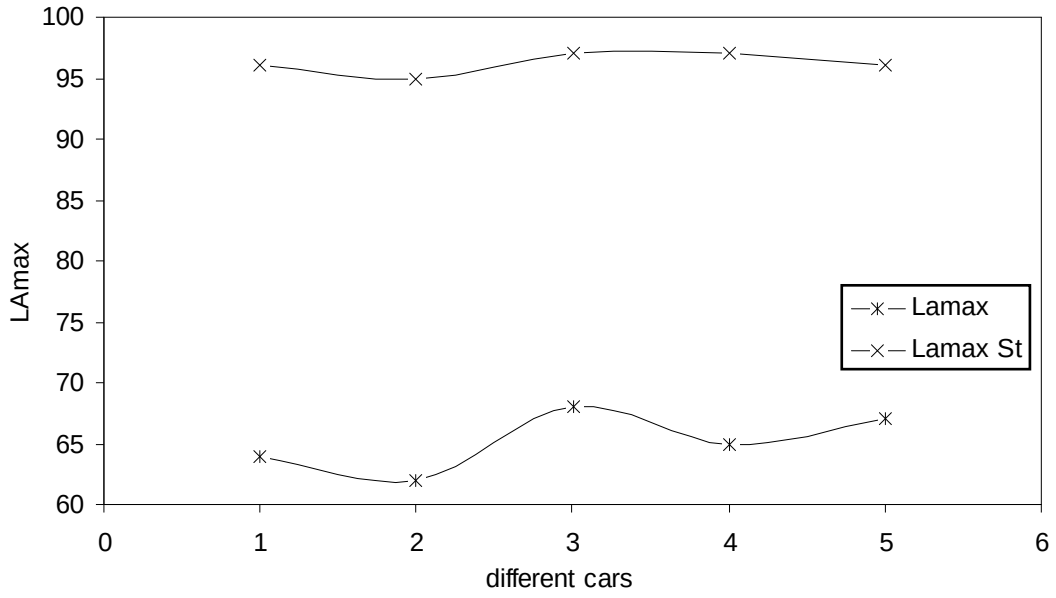

Figure 6. Relation between the overall LAmax of pass-by and overall LAmax of stationary,

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12/2/2010

Nature and Science

ISSN 1545-0740

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Telephone: (347) 321-7172
E-mail: editor@sciencepub.net; sciencepub@gmail.com; naturesciencej@gmail.com
Websites: http://www.sciencepub.net

ISSN 1545-0740