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Cover Page, Introduction, Contents, Call for Papers, All in one file

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Evaluation of Citric Acid and Potassium Sorbate as Preservatives on the Safety and Shelf-Life of Smoked Catfish

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ABSTRACT

Forty-four sample of catfish (*Clarias gariepinus*) were obtained from a fish pond in NIFFR divided into 11 portions of 4 each where 5 portions was treated with 1-5% Potassium sorbate respectively, the next 5 portions was treated with 1-5% citric acid (both are antimicrobial agents) prior to smoking and the last portion was not treated (it serve as control). All treated smoked samples were dominated with *Bacillus coagulans and Klebsiella ozanae* but negative for *E. coli and Streptococcus sp.* Unlike the 3% citric acid concentration, 3% potassium sorbate reduced the staphylococcus *count* to 0 throughout the 8th week of storage. Generally microbial counts were lower in the potassium sorbate treatment. All treated sample had higher protein and amino acid content than the control at the end of 8th week of storage with the highest in. Potassium sorbate. Potassium sorbate proved to be more efficient in controlling microbial quality and extending shelf life of smoked catfish.
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Key words: Potassium sorbate, Citric acid, Catfish, Quality and Safety.

INTRODUCTION

Fish is becoming increasingly important in the diet of the Nigerian as there is an increase awareness that regular red meat intake in adult above 40 years of age is not healthy. Fish constitutes 40% of animal protein intake in Nigeria at present (Olatunde, 1989). This is because fish are a cheap source of animal protein with little or no religious rejection of it, which gives it an advantage over pork or beef. Fish are a very perishable commodity, more than cattle, sheep, and poultry, and get spoiled very easily even in temperate climates. So unless it is disposed of quickly after capture, it must be preserved in some way. World fish production was estimated at 100 million tons in 1989, 15% of which was cured in one or another way. One third of the cured fish was smoked and about 20% of the smoked fish goes into international trade (Ward, 1995). Increasing consumer awareness of the nutritional value of seafood especially smoked fish has stimulated a strong demand from consumers (Pigott and Tucker, 1990). To satisfy the consumer demand, it is necessary to produce good quality and safe smoked fish. Smoked fish and shellfish products can be a source of microbial hazards. Human infections may be caused by bacteria endogenous to fish. Bacterial pathogens, which may be transferred from fish to human beings include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastroenteritis), *Clostridium botulinum* type E (botulism), *Edwardsiella tarda* (diarrhea), *Leptospira interrogans* (leptospirosis), *Mycobacterium fortuitom marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *Salmonella sp.*(food poisoning), and *vibrio parahaemolyticus* (food poisoning) (Austin and Austin, 1989).

Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and chemical preservatives like sorbates and citric acid. Sorbates are the most effective preservatives against a wide spectrum of food spoilage microorganisms; they include sorbic acid and potassium sorbate. They are among the safest, most efficient and versatile preservatives used in the food industry today. Sorbates are tasteless and odourless. Because they are non-toxic, they are used in a wide variety of foods, including cheese, yogurt, sour cream, bread, cakes, baking mixes, icing, beverages, margarine, fermented vegetables, fruit products, salad dressing, smoked and salted fish and mayonnaise. The antimicrobial activity of sorbates against molds, bacteria and fungi has been reported by researchers Sofos and Busta, 1993; Sofos, 2000). Also citric acid is vitamin C's close cousin and it is a natural additive. It works to help keep bacteria and mold from growing on foods. It is found in citrus fruits, such as lemons and limes. However, most of the citric acid manufacturers' use isn't derived from citrus fruits. It is artificially made by a mold called *aspergillus niger*. The mold produces citric acid as

long as it has a supply of sucrose (sugar). citric acid is also found naturally in the human body, so it causes no side effects. This ingredient is used extensively in soft drinks as a preservative and to enhance flavour (US FDA, 1978).

Considering the preservatives effects of sorbates and citric acid this study was therefore carried out to determine the microbial, organoleptic and nutritional quality changes of smoked catfish preserved with these antimicrobial agents at different concentration during storage at room temperature.

MATERIAL AND METHODS

Fresh catfish (*Clarias gariepinus)* were obtained from a private Fish pond in National Institute for Freshwater Fisheries Research (NIFFR) Housing Estate, New Bussa, Niger State in November, 2007. The fish samples measuring 17-28cm in length and weighing 180-250g were transferred within 30 minutes to the laboratory in a sterile polythene bags and then killed by severing the spinal cord with a sterile scalpel and aseptically eviscerated, washed and rinsed in sterile water. The fish samples were randomly chosen and divided into 11 groups of 4 fish for each of the catfish subjected to treatments. The treatments were as follows; (1) control (untreated samples); (2, 3, 4, 5 and 6) are treated with 1, 2, 3, 4 and 5% potassium sorbate and 7, 8, 9, 10 and 11 are treated with 1, 2, 3, 4 and 5% citric acid for 5 minutes, A sample from each group were separated from each treatment and smoked. Smoking was done according to the methods described by Omojowo and Ibitoye (2005). After smoking, the fish were allowed to cool down and were stored in different boxes. This was done to mimic commercial practices. The samples were drawn after two, four, six and eight weeks of storage; then subjected to analysis.

Microbiological Analysis

A 25g representative sample (excluding the head and tail) of each fish sample was obtained aseptically to prepare serial dilution using 0.1% peptone water as diluents. Total bacteria counts and coliform counts were determined according to the method of Sneath *et. al*. (1986). *Faecal streptococci* and *E. coli* in samples were determined employing the methods described by speak (1984). *Staphylococcus aureus* counts in samples were determined by employing the method of Bennett (1984). Moisture contents, fat and Crude protein were estimated as per AOAC (1980). All samples were done in duplicates. Sensory evaluation was carried out according to the method of Afolabi *et. al*. (1984). Statistical analysis was according to SAS, Institute, Inc, (1992) at $P < 0.05$.

RESULTS AND DISCUSSION

Total Viable count (TVC), Coliform, Staphylococci and Fungi count in log CFU/g of fresh and smoked Catfish samples are shown in Tables 1 and 2. TVC of the fresh the control catfish was 6.60 log CFU/g but after the sample were subjected to treatments with 1-5% Citric acid and 1-5% Potassium sorbate the TVC, Coliform, Staphylococcus and fungi count were reduced however, the reduction was higher in the treatment with Potassium sorbate also as the concentration is increases.

Smoking sharply reduced the total viable count (Table 1 and 2) in all samples, but the sample treated with 5% Potassium sorbate showed the greatest reduction and maintained a low level throughout 8 weeks of storage, especially on day 0 with 2.13 log CFU/g as shown in Table 2 while after 8-week storage the TVC was 4.60 log CFU/g. The TVC of the control samples were the highest throughout the period of storage where the sample were completely covered by mold after the 6th week of storage; therefore, no further microbial analysis was conducted. The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the lowest microbial load and maximum shelf stability. Similar to TVC, the coliform count (of the smoked samples treated with 5% Potassium sorbate had the highest reduction of 0.93 log CFU/g on day 0 and remain the lowest of the treatments throughout the period of storage. Significant increases in coliform population of all samples occurred after 4 weeks of storage. Coliform count of all treated samples was less than 3.0 log CFU/g throughout the 8-week storage. In the control samples, the Coliform population was 5.17 log CFU/g on the 6th week while the sample was completely covered by mold on the 8th week of storage.

This result was similar to that reported by Virginia, (2002) where the coliform in the control sample showed 2.6 log CFU/g on the 4th week and the sample was completely covered by mold on the 6th week of storage. The high coliform count recorded in this report may be due to contamination from the animal manure used in fertilizing the ponds at one time or the other. Furthermore, the smoked sample treated with 3-5% potassium sorbate had no staphylococcus count throughout the period of storage while only 4 and 5% citric acid was able to reduce the staphylococcus count to 0 and remained 0 until the end of 8th week storage. Generally, potassium sorbate showed the lowest count throughout the 8th week of storage.

Table 1: Microbial Load of Catfish Treated With Citric Acid (Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
Day 0 – A	**TVC**	6.60 ± 0.4^{a}	5.32 ± 0.2^{b}	5.24 ± 0.3^{b}	5.24 ± 0.6^{b}	5.25 ± 0.4^{b}	5.16 ± 0.5^{b}
Day 0 – B	**TVC**	4.59 ± 1.2^{a}	3.98 ± 0.4^{b}	3.91 ± 0.1^{bc}	3.79 ± 0.3^{c}	3.34 ± 0.1^{d}	3.10 ± 0.3^{e}
2nd wk	**TVC**	6.04 ± 0.3^{a}	4.41 ± 0.7^{b}	4.36 ± 0.7^{bc}	4.24 ± 0.2^{cd}	4.09 ± 0.8^{d}	3.87 ± 0.5^{e}
4th „	**TVC**	6.52 ± 0.8^{a}	5.10 ± 0.5^{b}	5.16 ± 0.9^{b}	5.12 ± 0.3^{b}	5.08 ± 0.4^{b}	4.63 ± 0.7^{c}
6th „	**TVC**	7.35 ± 0.2^{a}	6.03 ± 0.6^{b}	5.88 ± 0.2^{bc}	5.84 ± 0.9^{c}	5.41 ± 0.3^{d}	4.96 ± 0.3^{e}
8th „	**TVC**	Mouldy	6.90 ± 1.0^{a}	6.71 ± 0.8^{b}	6.67 ± 0.2^{b}	6.48 ± 0.5^{c}	6.26 ± 0.9^{d}
Day 0 – A	**Coliform**	4.60 ± 0.9^{a}	4.06 ± 0.9^{b}	4.00 ± 0.6^{bc}	3.88 ± 0.2^{cd}	3.80 ± 0.02^{d}	3.74 ± 0.4^{d}
Day 0 – B	**Coliform**	3.54 ± 1.0^{a}	1.75 ± 0.1^{b}	1.60 ± 0.5^{b}	1.38 ± 0.4^{c}	1.25 ± 0.5^{cd}	1.10 ± 0.3^{d}
2nd wk	**Coliform**	4.10 ± 0.1^{a}	1.91 ± 0.7^{b}	1.80 ± 0.5^{b}	1.48 ± 0.3^{c}	1.34 ± 0.7^{cd}	1.28 ± 0.5^{d}
4th „	**Coliform**	4.43 ± 0.4^{a}	2.10 ± 0.4^{b}	2.06 ± 1.3^{b}	1.67 ± 0.7^{c}	1.76 ± 0.8^{c}	1.63 ± 0.1^{c}
6th „	**Coliform**	5.17 ± 1.0^{a}	2.60 ± 0.6^{b}	2.42 ± 0.6^{c}	2.18 ± 0.4^{d}	2.32 ± 0.4^{cd}	2.19 ± 0.3^{d}
8th „	**Coliform**	Mouldy	3.14 ± 0.5^{a}	2.90 ± 0.3^{b}	2.72 ± 0.8^{c}	2.65 ± 0.2^{cd}	2.52 ± 0.4^{d}
Day 0 - A	**Staph.**	4.55 ± 0.6^{a}	4.21 ± 0.4^{b}	4.20 ± 1.1^{b}	3.85 ± 0.2^{c}	3.80 ± 0.5^{c}	3.68 ± 0.4^{c}
Day 0 - B	**Staph.**	3.17 ± 0.3^{a}	0.64 ± 0.5^{b}	0.40 ± 0.3^{c}	0.40 ± 0.7^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
2nd wk	**Staph.**	5.06 ± 0.6^{a}	0.61 ± 0.3^{b}	0.57 ± 0.2^{b}	0.50 ± 0.3^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
4th „	**Staph.**	5.32 ± 1.2^{a}	1.20 ± 0.7^{b}	1.10 ± 0.4^{bc}	1.02 ± 0.8^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
6th „	**Staph.**	5.52 ± 0.4^{a}	1.70 ± 0.9^{b}	1.62 ± 0.7^{b}	1.33 ± 0.1^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
8th „	**Staph.**	Mouldy	2.50 ± 1.5^{a}	2.30 ± 0.1^{b}	1.82 ± 0.4^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
Day 0 - A	**Fungi**	4.52 ± 0.2^{a}	4.55 ± 0.3^{b}	4.56 ± 0.2^{b}	4.60 ± 0.3^{b}	4.62 ± 0.4^{b}	4.50 ± 0.5^{b}
Day 0 - B	**Fungi**	3.11 ± 0.4^{a}	1.80 ± 0.7^{b}	1.68 ± 0.4^{b}	1.24 ± 0.1^{c}	1.10 ± 0.6^{c}	0.67 ± 0.6^{d}
2nd wk	**Fungi**	5.28 ± 0.7^{a}	2.20 ± 0.6^{b}	2.17 ± 0.6^{b}	2.14 ± 0.3^{b}	1.71 ± 0.2^{c}	1.24 ± 0.1^{d}
4th „	**Fungi**	5.41 ± 1.1^{a}	2.82 ± 0.2^{b}	2.86 ± 0.8^{b}	2.71 ± 0.7^{b}	2.46 ± 0.8^{c}	1.60 ± 0.3^{d}
6th „	**Fungi**	5.70 ± 1.3^{a}	3.30 ± 0.4^{b}	3.24 ± 0.5^{b}	3.26 ± 0.4^{b}	2.98 ± 0.9^{c}	2.18 ± 0.1^{d}
8th „	**Fungi**	Mouldy	3.94 ± 0.3^{a}	3.85 ± 0.7^{a}	3.85 ± 0.8^{a}	3.67 ± 0.3^{b}	2.74 ± 0.4^{c}

Mean ± standard deviation of triplicate experiments and 2 replicates of each sample (6 readings of each Sample)
Using superscript $^{a, b, c, d, e, f}$., means in the same rows with different superscript are significantly different ($p < 0.05$).

KEY:
A = before smoking
B = after smoking

The isolation of *Staphylococcus* in smoked samples on day 0 may be attributed to post processing contamination. However, *Staphylococcus* was killed by the treatments 3-5% potassium sorbate and 4-5% citric acid. Fungi counts were also reduced in all the treatments and at the end of the 8-week storage time; however, the sample treated with 5% potassium sorbate showed 0 counts till the 4th and 6th weeks of storage. The control samples were high throughout the period of storage and the sample was even completely covered by mould at the end of the 8-week storage. This result were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the minimum fungal load during storage and presence of profuse mould growth after day 8 in the control.

It is of interest to observe that in spite of the slightly reduced moisture contents (from 2nd to 6th week) in almost all the samples microbial load still increases dramatically. This suggests that one single factor may not account for these microbial changes. Cross contamination, pH, purity of preservatives are among other factors that can influence microbial changes. The bacterial contamination of hot smoked fish just out of the smokehouse is usually below 10^3 per gram (Doe, 1998). The TVC of the most of the treated samples were all below $5x10^5$ CFU/g to the 6th week which is below m in a three-class attribute plan and signifies good quality. Low levels of coliform bacteria were detected and the pathogens *S. aureus* counts were below 10^3 in all the treated samples The control however, has TVC higher than $5x10^5$ CFU/g in the second week and higher than the recommended limit 7.0 log CFU/g (ICMSF, 1986) after the 4th week. In addition the coliform count already exceeded 10^3 even immediately after smoking. This finding is of concern as a result of the associated public health implications. For example, generally, hot smoked fish are consumed in the

tropics with little or no further processing, thus, they fall into the high-risk category of foods (ICMSF, 1986; FDA, 2001). Hence there is a need for the use of appropriate percentage of choice antimicrobial agent.

Table 2: Microbial Load of Catfish Treated With Potassium Sorbate Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
Day 0 - A	**TVC**	6.60 ± 0.4^{a}	5.48 ± 0.4^{b}	5.46 ± 0.3^{b}	5.42 ± 1.6^{b}	5.12 ± 0.4^{c}	5.07 ± 0.9^{c}
Day 0 - B	**TVC**	4.59 ± 1.2^{a}	3.61 ± 0.7^{b}	3.50 ± 0.8^{b}	3.47 ± 0.5^{b}	3.10 ± 0.3^{c}	2.04 ± 1.3^{d}
2nd wk	**TVC**	6.04 ± 0.3^{a}	4.14 ± 0.8^{b}	4.06 ± 0.1^{b}	3.98 ± 0.7^{b}	3.65 ± 0.5^{c}	2.72 ± 0.3^{d}
4th „	**TVC**	6.52 ± 0.8^{a}	5.00 ± 0.3^{b}	5.01 ± 0.4^{b}	4.84 ± 0.3^{c}	4.30 ± 0.2^{d}	3.43 ± 0.7^{e}
6th „	**TVC**	7.35 ± 0.2^{a}	5.71 ± 0.1^{b}	5.68 ± 0.2^{b}	5.50 ± 0.2^{c}	4.71 ± 0.8^{d}	3.90 ± 0.1^{e}
8th „	**TVC**	Mouldy	6.72 ± 0.2^{b}	6.64 ± 0.9^{b}	6.35 ± 0.3^{c}	6.21 ± 1.4^{c}	4.54 ± 0.4^{d}
Day 0 - A	**Coliform**	4.60 ± 0.9^{a}	3.95 ± 0.7^{b}	3.76 ± 0.1^{c}	3.74 ± 0.8^{cd}	3.61 ± 0.5^{cd}	3.58 ± 0.2^{d}
Day 0 - B	**Coliform**	3.54 ± 1.0^{a}	1.55 ± 0.5^{b}	1.40 ± 0.4^{bc}	1.32 ± 0.3^{c}	1.24 ± 0.4^{c}	0.93 ± 0.4^{d}
2nd wk	**Coliform**	4.10 ± 0.1^{a}	1.72 ± 0.3^{bc}	1.88 ± 0.6^{b}	1.61 ± 0.7^{c}	1.55 ± 0.7^{c}	1.10 ± 0.2^{d}
4th „	**Coliform**	4.43 ± 0.4^{a}	2.08 ± 0.2^{b}	2.00 ± 1.4^{b}	1.76 ± 0.3^{c}	1.62 ± 0.8^{c}	1.27 ± 0.3^{d}
6th „	**Coliform**	5.17 ± 1.0^{a}	2.50 ± 0.8^{b}	2.42 ± 0.5^{b}	2.23 ± 0.5^{c}	2.11 ± 0.1^{c}	1.92 ± 0.7^{d}
8th „	**Coliform**	Mouldy	2.81 ± 0.1^{b}	2.42 ± 0.2^{c}	2.54 ± 0.2^{c}	2.50 ± 0.3^{c}	2.20 ± 0.1^{d}
Day 0 - A	**Staph.**	4.55 ± 0.6^{a}	3.88 ± 0.1^{b}	3.74 ± 0.5^{bc}	3.71 ± 1.0^{c}	3.74 ± 0.2^{bc}	3.65 ± 0.5^{c}
Day 0 - B	**Staph.**	3.17 ± 0.3^{a}	0.40 ± 0.7^{b}	0.32 ± 0.7^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
2nd wk	**Staph.**	5.06 ± 0.6^{a}	0.60 ± 0.4^{b}	0.45 ± 0.3^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
4th „	**Staph.**	5.32 ± 1.2^{a}	1.0 ± 0.3^{b}	0.84 ± 0.1^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
6th „	**Staph.**	5.52 ± 0.4^{a}	1.60 ± 0.9^{b}	1.25 ± 0.4^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
8th „	**Staph.**	Mouldy	2.10 ± 0.2^{b}	1.80 ± 0.5^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
Day 0 - A	**Fungi**	4.52 ± 0.2^{a}	4.12 ± 0.7^{b}	4.02 ± 0.06^{b}	4.03 ± 0.7^{b}	3.71 ± 0.4^{c}	3.28 ± 0.3^{d}
Day 0 - B	**Fungi**	3.11 ± 0.4^{a}	1.21 ± 0.4^{b}	1.22 ± 0.5^{b}	1.05 ± 0.3^{c}	0.46 ± 0.6^{d}	0.0 ± 0.0^{e}
2nd wk	**Fungi**	5.28 ± 0.7^{a}	1.73 ± 0.2^{b}	1.84 ± 0.1^{b}	1.55 ± 0.1^{c}	0.54 ± 0.5^{d}	0.0 ± 0.0^{e}
4th „	**Fungi**	5.41 ± 1.1^{a}	2.59 ± 0.6^{b}	2.61 ± 0.6^{b}	1.92 ± 0.9^{c}	0.62 ± 0.4^{d}	0.0 ± 0.0^{e}
6th „	**Fungi**	5.70 ± 0.3^{a}	3.36 ± 0.9^{b}	3.25 ± 0.5^{bc}	2.14 ± 0.2^{c}	1.26 ± 0.2^{d}	0.22 ± 0.1^{e}
8th „	**Fungi**	Mouldy	3.78 ± 0.1^{b}	3.61 ± 0.02^{b}	2.57 ± 0.5^{c}	1.42 ± 0.8^{d}	0.36 ± 0.03^{e}

Mean ± standard deviation of triplicate experiments and 2 replicates of each sample (6 readings of each sample). Using superscript [a, b, c, d, e, f]., means in the same rows with different superscript are significantly different ($p < 0.05$).

KEY:

A = before smoking

B = after smoking

BACTERIAL ISOLATES

All treated smoked sample were negative for *E. coli and Streptococcus sp.* However, the control and the fresh fish treated samples showed the following bacteria flora *Bacillus coagulans, B. cereus, Klebsiella ozanae, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, and Streptococcus sp,* while the fungi isolated include *Penicillium verrucosum, Aspergillus niger, A. candidus, A. flavus and A. nidulan* while the smoked untreated sample (control) were dominated by the following organisms *B. coagulans,* (about 70% of the isolates) while the remaining being *S. aureus,* and *Streptococcus sp*. The treated sample showed the microbial load in the following pattern; 1% and 2% potassium sorbate of the fish samples contains the following spp *B. coagulans, S. aureus, K. ozanae, A. candidus and A. nidulan* while in 3% and 4% potassium sorbate treated samples have the following isolates *B. coagulans, K. ozanae and A. nidulan while* 5% treatment have only *B. coagulans.* While 1, 2 and 3% citric acid treated samples had *B. coagulans, K. ozanae, S. aureus, A. niger, A. nidulan, A. candidus, A. flavus, and Penicillium verrucosum. But* 4 and 5% citric acid contains the *B. coagulans, K. ozanae, A. niger, A. nidulan, and P. verrucosum.*

Proximate Analysis

The proximate analysis of the treated raw and Smoked catfish are presented in Figure 1 to 8, there were no significant ($p \leq 0.05$) differences in Protein (17.8 - 18.6%), Fat (3.9 – 4.30%), and Moisture contents (78.2 - 79.4%). The moisture content of fresh sample was 78.2%. In the treatments the moisture contents ranged from 78.2 - 79.4%. Moisture content of catfish decreased sharply after the smoking process and this decrease was due to loss of water during smoking (Asiedu *et al*., 1991). Also the study reveals that the average protein content increases after smoking, and increases till the 4th week and later decreases till the end of the 8th week of storage. There was an inverse relationship between the moisture and protein content in the smoked samples. The initial increase in protein content in smoked fish and till the 4th week may be due an increase in the dry matter content per unit of weight following sample dehydration during smoking and reduction in the moisture contents during the early part of the storage before autolysis becomes pronounced. These results shows that storage time causes a decrease in the protein content of smoked catfish which agreed with earlier work of Ufodike and Obureke (1989) where there was decrease in crude protein of preserved *Oreochromis niloticus.* These workers attributed the decrease to hydrolysis of protein during the process of autolysis in the fish muscle. However, the treated samples show some corresponding higher value of protein more than the control especially as the concentration of the preservatives increases from 1-5%. This increase may be due to the effects of the preservatives which slow down autolysis in the fish muscles and consequently slow down the protein break down.

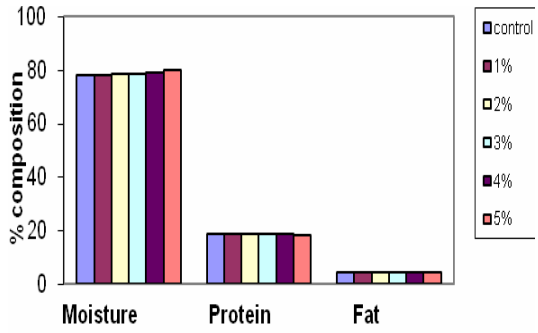

Figure 1. **Proximate** composition of Fresh Catfish Treated with Citric acid

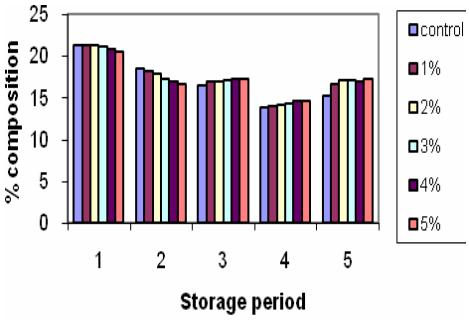

Figure 2. Moisture Contents of Smoked Catfish Preserved with Citric Acid

Note, in x-axis 1= Day 1, 2= 2n d Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

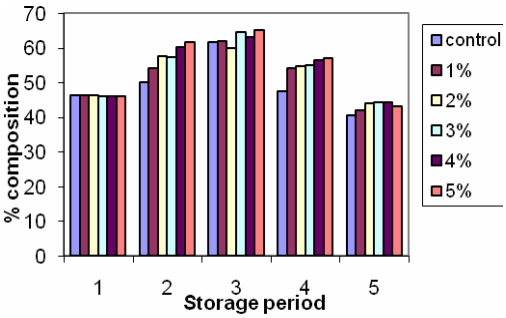

Figure 3. Protein composition of Smoked Catfish Preserved with Citric Acid

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

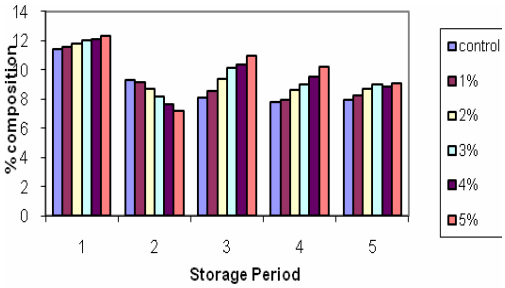

Figure 4. Fat composition of Smoked Catfish Preserved with Citric Acid

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

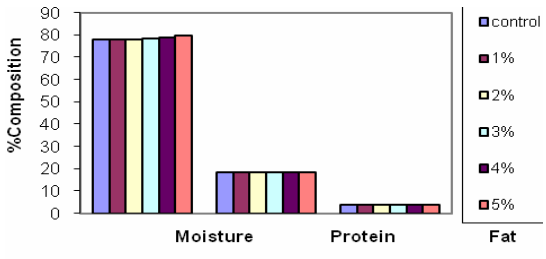

Figure 5. Proximate Analysis of Fresh Catfish Treated with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

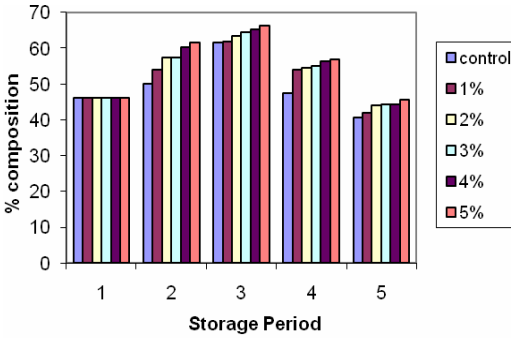

Figure 7. Protein Composition of Smoked Catfish Preserved with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

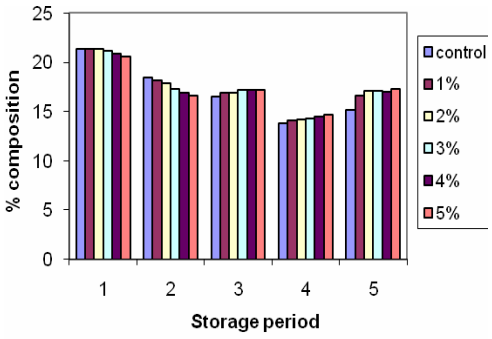

Figure 6. Moisture Contents of Smoked Catfish Preserved with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

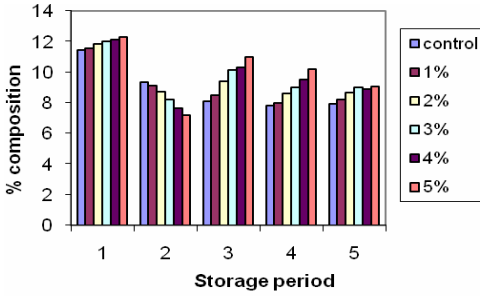

Figure 8. Fat composition of Smoked Catfish Preserved with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2$^{n\ d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

CONCLUSION AND RECOMMENDATION

This study has reveals that the samples treated with Potassium sorbate and Citric acid before smoking showed significant reduction and maintained a low level throughout the 8th weeks of storage. However, potassium sorbate proved to be better than citric acid in comparison. Potassium sorbate can be used as a first choice preservative in smoked catfish without adversely affecting quality in terms of lipid oxidation, color, microbial and nutritional quality and citric acid may be used in the absence of potassium sorbate. The use of 3% potassium sorbate as a choice antimicrobial agent is hereby recommended since it has been found to keep smoked fish in wholesome state for 8th week, reducing the TVC to 6.35 log CFU/g, the coliform to 2.64 log CFU/g, staphylococcus count to 0.0s and fungi to 2.57 log CFU/g at the end of 8th week storage. This will ensure prolonged shelf life and safe consumption of smoked fish of ICMSF standard of smoked fish quality.

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LiTiZn - Ferrite Radome for Satellite Communication

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Abstract: The magnetically switchable LiTiZn-Ferrite Radome's dispersion characteristics is presented for the satellite communication. A thin layer of LiTiZn-ferrite is used as superstrate or radome layer which control the radiation, reception, and scattering from a printed antenna or array by applying a dc magnetic bias field in the plane of the ferrite, orthogonal to the RF magnetic field. In this analysis absorbing and transmission power coefficient is calculated to obtain the power loss in radome layer and transmitted power through respectively. The absorbing power coefficient verifies the switching behavior of radome for certain range of applied external magnetic field (H_o) which depends on the resonance width parameter (ΔH) of ferrite material. By properly choosing the bias field, quasi TEM wave propagation in the ferrite layer can be made to be zero or negative over a certain frequency range, results a switching behavior in the ferrite layer. [Nature and Science 2009;7(11):9-14]. (ISSN: 1545-0740).

Key words: Substituted Li-ferrite superstrate layer, absorbing and transmission power coefficient, quasi-TEM and magnetostatic wave.

List of Symbols

f_r	=	resonant frequency
δ	=	thickness of radome layer
α	=	attenuation constant
β	=	phase constant
β_o	=	propagation constant in vacuum
ε_r	=	dielectric constant
μ_{eff}	=	effective permeability
μ, k	=	permeability tensor components of μ_{eff}
K_d	=	ordinary propagation constant
K_e	=	extraordinary propagation constant
T	=	relaxation time
H_o	=	applied bias field
ΔH	=	magnetic resonance width of ferrite
ω	=	angular frequency of incident e-m-waves
ω_o	=	external magnetic field angular frequency
ω_m	=	internal magnetic field angular frequency
μ'	=	real part of permeability
μ''	=	dissipative part of permeability
χ'	=	real part of susceptibility
χ''	=	dissipative part of susceptibility
$4\pi M_s$	=	saturation magnetization
Υ	=	gyromagnetic ratio (2.8 MHz / Oe.)

1. Introduction

Ferrite materials have a permeability tensor, whose elements can be controlled by the direction and strength of a dc magnetic bias field. A certain frequency range, results an evanescent wave behavior in the ferrite layer, and a large attenuation of the wave transmitted through the layer due to the generation of quasi-TEM modes and higher-order modes of the magnetostatic surface wave mode which propagates transversely to the quasi-TEM mode. This reciprocal behavior include the ability to tune the operating frequency of a microstrip antenna, the generation of circular polarization with a single feed point, the dynamic wide angle impedance matching of a phased array , and the reduction of microstrip antenna RCS using a normally biased ferrite substrate. In this work we describe the dispersion characteristics of radome layer by evaluating the absorbing and transmission power coefficients (Pozar et al., 1993, 1992, 1988; Fukusako et al., 1998).

With the help of proposed analysis we can also conclude, how a ferrite radome or superstrate layer can be used in conjunction with a printed antenna as a bulk effect "switch," whereby the antenna can be turned "on" or "off" by applying an appropriate magnetic bias field. This effect makes use of the negative

permeability state of an extraordinary quasi TEM plane wave, propagating in a ferrite region. Applications include radar cross section reduction, EMP protection, and possibly a switchable polarizer. The idea of using the negative permeability effect of a ferrite for switching radome is not a new one (Dixit et al., 2000; Batchelor et al., 1997; Ufimtsev et al., 2000; Horsfield et al., 2000); but here a different approach is applying with new ferrite material LiTiZn-ferrite which is synthesized by Solid State Reaction Technique at Solid State Physics Laboratory, Timarpur, Delhi.

2. Synthesis of Radome Layer

LiTiZn ferrite synthesized from the basic components of lithium ferrites In this work a typical composition of LiTiZn ferrite having room temperature magnetization ($4\pi Ms$) of 2200 gauss (± 5%) & Curie temperature (Tc) of 500 ^{0}C (± 5%) & synthesized using solid state reaction technique (SSRT). The ingredients required for the preparation of these ferrites were calculated on the basis of chemical formula. A small amount of Mn^{3+} ion was also incorporated in the basic composition in order to suppress the formation of Fe^{2+} ions in the ferrites and to influence megnetostriction being a John Teller ion (Uitert et al., 1956; Kishan et al, 1985). In order to avoid Lithia at high temperatures of sintering, Bi_2O_3 (0.25 wt %) was added as sintering aid (Randhawa et al, 2007). Analytical grade chemicals were used for the preparation of the material. The stoichiometric ratio of the chemicals was thoroughly mixed in a polypropylene jar containing the zirconium balls & distilled water was used as a mixing agent.

Table1. The electrical and magnetic properties of LiTiZn ferrite substrate

LiTiZn Ferrite Characteristics	Values
Magnetic Saturation ($4\pi M_s$)	2200 Gauss
Curie Temperature (T_c)	385 K
Density (ρ)	4.21 grams/cm^3
Remanence	0.90
Coercivity	1.50
Dielectric Constant (ε)	16
Resonance Line Width (ΔH)	370 Oersteds
Loss Tangent ($\tan\delta$)	< 0.0005

The presintering of the mixed powder has been carried out at ~ 750^{o}C in a box furnace and soaking time was kept 4 hours. The sieved material was pressed in disk (antenna substrate) and toroidal shapes with the help of suitable dies and using hydraulic pressing technique at pressure of 10 ton/cm^2. The substrates and toroidals were finally sintered at 1050^{o}C for four hours. The heating and cooling cycle of the samples was carried out in the air atmosphere of furnace. The sintered sample so obtained was subjected to cutting, grinding, polishing etc. in order to get specific size and shape. The important material properties such as magnetic and electrical properties were studied.

The single-phase spinel nature of the samples was confirmed by X-ray diffraction (XRD) patterns obtained by using Cu-K_α radiation. The microstructure studies of the sample were carried out by scanning electron microscopy (SEM). Vibrating Sample Magnetometer (VSM) was used to determine the magnetic properties of the samples. For dielectric measurements, rectangular pellets of size 15mm × 6mm × 3mm were used. The dielectric measurements were conducted from 1 to 20 MHz. by a HP 4192 A impedance analyzer. The value of the real part of dielectric constant (ε') of the ferrite samples was calculated using formula $\varepsilon' = Ct/(\varepsilon_o A)$, (where ε_0 is the permittivity of free space = 8.854 × 10^{-12} F/m, C is the capacitance of specimen, 't' is the thickness of specimen in square meter). The density measurement has been done by a small experiment based on Archimedes' principle. Remanence and Coercive Force measure by B-H loop setup applied to coiled toroid sample at 50 Hz.

The Curie temperature for the LiTiZn ferrite samples has been determined by using a simple experimental setup based on gravity effect in the laboratory. The ferrite specimen is made to attach itself to a bar magnet through a mild steel rod due to the magnetic attraction and combination is suspended inside the furnace. A chromel-alumel thermocouple is attached with the sample holder to read the temperature of the specimen. As the temperature of the system is increased, at a particular temperature the specimen losses it spontaneous magnetization and become paramagnetic. This temperature is known as Curie temperature. At this temperature specimen fall downward due to gravity. The electrical and magnetic

properties of LiTiZn ferrite substrate is experimentally calculated are presented in table 1.

3. Theory of Operation

Consider a plane wave propagating in the perpendicular direction of radome layer with a magnetic bias field applied longitudinally. On the basis of magnetic field directions following waves are generated in the radome layer.

3.1 Magnetostatic mode of wave

MSW are generated when external magnetic field applied to the perpendicular direction of the magnetic vector of EM waves. MSW are two types **(1)** Surface MSW **(2)** Volume MSW. MSW will propagate perpendicularly on both sides to the EM wave's propagation [Lax et at, 1962].

Vol. MSW:

$$\mu_o\gamma H \leq \omega \leq \mu_o\gamma\sqrt{H(H+M_o)} \qquad (1)$$

Sur. MSW:

$$\mu_o\gamma\sqrt{H(H+M_o)} \leq \omega \leq \mu_o\gamma H(H+\frac{M_o}{2}) \qquad (2)$$

The absorption and transmission coefficients due to the generation of MSW in the ferrite slab are:

$$P = \frac{2\beta_o\varepsilon_r(\alpha\sin 2\beta\delta + \beta\sinh(2\alpha\delta))}{\left[\begin{array}{c}\beta_o^2\varepsilon_r^2\left((\cos\beta\delta)^2+(\sinh(\alpha\delta))^2\right)\\+(\alpha^2+\beta^2)\left((\sin\beta\delta)^2+(\sinh(\alpha\delta))^2\right)\\+\beta_o\varepsilon_r(\alpha\sin 2\beta\delta+\beta\sinh(2\alpha\delta))\end{array}\right]} \qquad (3)$$

$$T = \frac{8(\alpha^2+\beta^2)\beta_o^2\varepsilon_r}{\left[\begin{array}{c}[4\beta^2\beta_o^2\varepsilon_r^2+(\alpha^2+\beta^2+\beta_o^2\varepsilon_r^2)^2]\cosh(2\alpha\delta)\\+4\beta\beta_o\varepsilon_r(\alpha^2+\beta^2+\beta_o^2\varepsilon_r^2)\sinh(2\alpha\delta)\\-[4\beta^2\beta_o^2\varepsilon_r^2+(\alpha^2+\beta^2-\beta_o^2\varepsilon_r^2)]\cos(2\beta\delta)\\+4\alpha\beta_o\varepsilon_r(\alpha^2+\beta^2-\beta_o^2\varepsilon_r^2)\sin(2\beta\delta)\end{array}\right]} \qquad (4)$$

where

$$\alpha = \beta_o\sqrt{\left(\frac{\varepsilon_r}{2}\right)}\sqrt{\left[\sqrt{(\mu'^2+\mu''^2)}-\mu'\right]}$$

$$\beta = \beta_o\sqrt{\left(\frac{\varepsilon_r}{2}\right)}\sqrt{\left[\sqrt{(\mu'^2+\mu''^2)}+\mu'\right]}$$

and

$$\mu' = 1+\chi'$$

$$\mu'' = \chi''$$

where

$$\chi' = \frac{\omega_m T(\omega_o+\omega)}{(\omega_o-\omega)^2T^2+1}$$

$$\chi'' = \frac{\omega_m T}{(\omega_o-\omega)^2T^2+1}$$

with

$$T = \frac{2}{\sqrt{\times}\Delta H} \quad and \quad \beta_o = \frac{\omega}{c}$$

3.2 Quasi TEM mode of wave

As discussed in (Lax et al, 1962; Kabos et al, 1994; Sodha et al, 1981), for a biased ferrite slab, a normal incident plane wave may excite two types of waves (ordinary and extraordinary wave). In the case of normal incident magnetic field biasing ordinary wave is same as the plane wave in the dielectric slab. On the other hand, the extraordinary wave is a TE mode polarized parallel to the biasing direction with its phase propagation constant K_e. In the case of extraordinary mode, the propagation constant dependence on the basic parameters is given as

$$\gamma_e = \alpha_e + j\beta_e = j\omega\sqrt{\mu_{eff}\varepsilon_r} \qquad (5)$$

where μ_{eff} is the effective permeability

$$\mu_{eff} = \frac{\mu^2-k^2}{\mu}$$

$$\mu = 1+\frac{\omega_o\omega_m}{\omega_o^2-\omega^2}$$

$$k = \frac{\omega\omega_m}{\omega_o^2-\omega^2}$$

where $\omega_o = \gamma H_o$, $\omega_m = \gamma 4\pi M_s$, H_o is the bias field, $4\pi M_s$ is the saturation magnetization, γ is the gyromagnetic ratio as $\gamma = 2.8$ MHz./Oe. In the case of extraordinary wave mode, the propagation constant dependence on the basic parameters is given as

$$\left(\frac{K_e}{K_d}\right)^2 = \frac{(\omega_o+\omega_m)^2-\omega^2}{\omega_o(\omega_o+\omega_m)-\omega^2} \qquad (6)$$

It is seen that, when μ_{eff} is negative, the wave is decaying even if the material is lossless. The frequency range of negative μ_{eff} is:

$$[\omega_o(\omega_o + \omega_m)]^{1/2} < \omega < (\omega_o + \omega_m) \qquad (7)$$

The frequency limits define the approximate range within and around which the ferrite exhibit interesting microwave characteristics.

4. Setup

Figure 1 shows the arrangement of an experimental setup for the validation of the switchable ferrite radome effect.

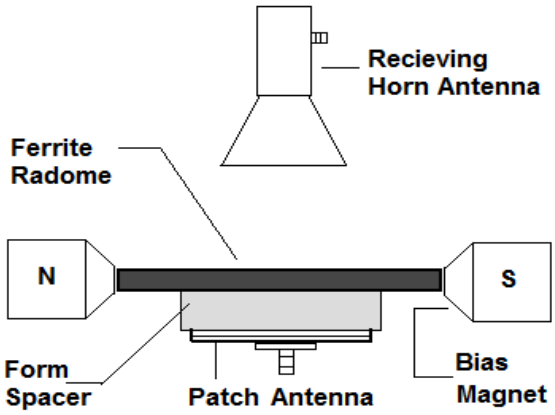

Figure 1. Setup for the measurement of radome power coefficients. Ferrite is 2 mm thick with 2200 Gauss saturation magnetization, '16' dielectric constant and 1800 Oe magnetic resonance width.

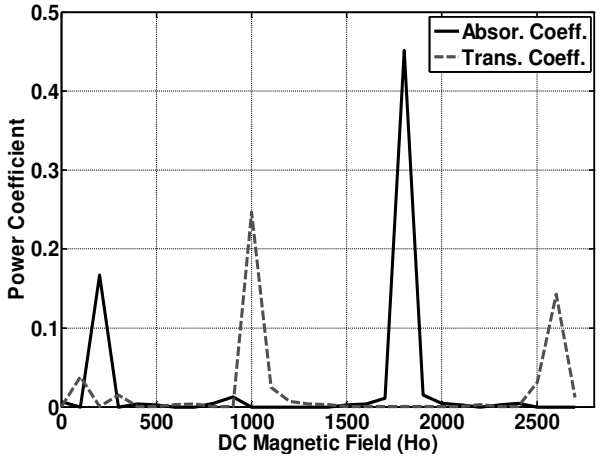

Figure 2: Comparison of transmission (T) and absorption (P) power coefficient with the varying DC magnetic field (H_o)

A 2 x 2 circular microstrip patch array was fabricated on a RT-duroid substrate, and operated at 10 GHz. A 1-cm foam spacer separated the array face from the ferrite radome. The ferrite layer was 44mm diameter, and was mounted between the poles of a laboratory electromagnet. An X-band waveguide-to-coax adapter was used as a receiving antenna, and was spaced about 5 cm above the ferrite layer.

As illustrated in Figure 1, a ferrite superstrate or radome layer can be placed above a microstrip antenna or array (Or any type of antenna, for that matter), and used as a switch. In practice such a ferrite layer could be spaced a small distance above the antenna, or placed directly over the antenna as a superstrate layer. Spacing the ferrite above the antenna may be preferable for ease of biasing, and also to minimize the direct interaction of the ferrite with the antenna elements (Bahl et al, 1980; Balanis et al, 1982).

5. Results

When the ferrite layer is unbiased, or biased to a state where $K_e > 0$, the antenna will transmit and receive as normal. When the ferrite is biased to the cutoff state where $K_e < 0$, however, an incident wave will be transformed to quasi-TEM and magnetostatic waves, which largely absorb and attenuate the incident RF waves.

From the graph figure 2 we can see, the absorbing power is max between 1700 Oe and 1850 Oe which is in good agreement of dispersion graph figure 3 plotted for LiTiZn-ferrite radome layer.

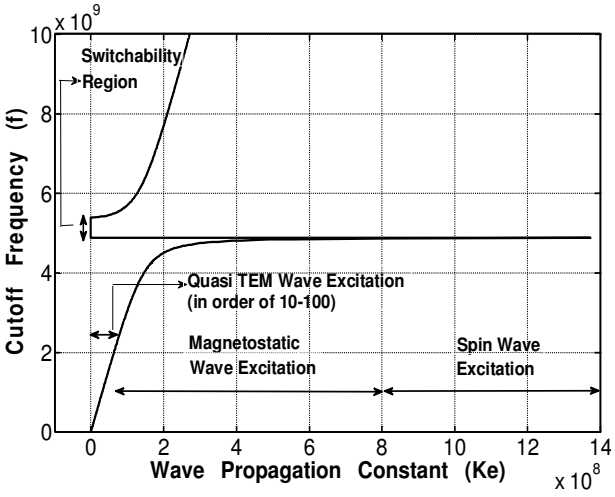

Figure 3: Dispersion curve (f vs. K_e) for incident plane wave perpendicular to biased radome layer

Dispersion graph depicts the switch off state of radome layer for cutoff frequency (f) around 5 to 5.5 GHz. From the figure 2, we can also observe the transmitted power coefficient variation with varying external DC magnetic field.

The amount of absorption and attenuation can be increased by operating the ferrite in a bias state to maximize power loss or by increasing the thickness of the ferrite layer (figure 4 and 5). If desired, dielectric matching layers could be placed on either side of the ferrite layer to reduce reflections. Magnetic and dielectric losses will have the effect of increasing the amount of attenuation; as compared to the lossless state (although at the point of maximum cutoff the attenuation may actually decrease slightly with the addition of magnetic losses).

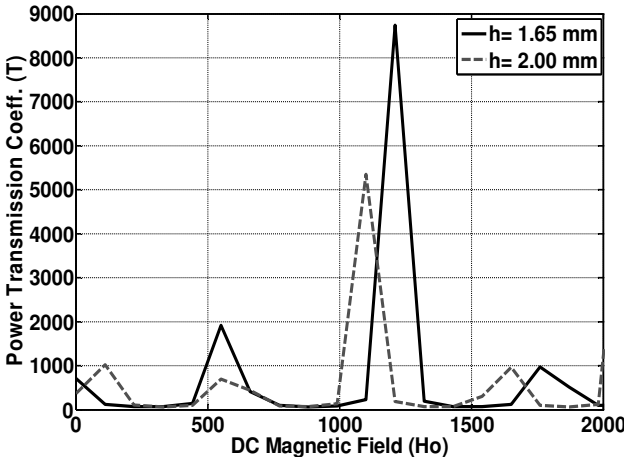

Figure 4: Transmission (T) power coefficient with the varying DC magnetic field (H_o)

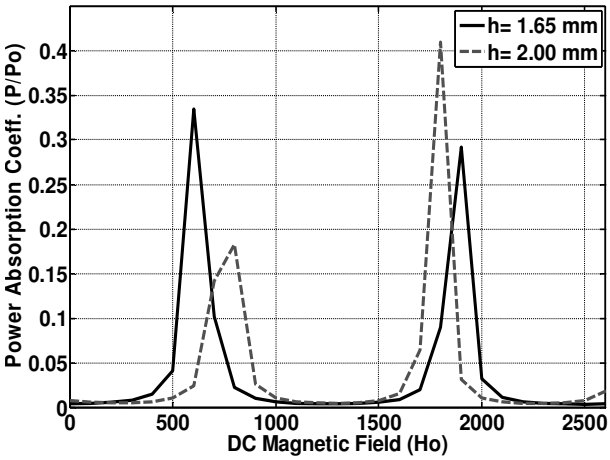

Figure 5: Absorption (P) power coefficient with the varying DC magnetic field (H_o)

Conclusion

The Dispersion Characteristics of thin layer radome of LiTiZn-ferrite under external DC-magnetic is presented. Resulted absorbing power coefficients graph, verifies the dispersion relation graph obtained by quasi-TEM cutoff frequency range. As discussed, this is a very simple approach which ignores reflections at the ferrite-air interfaces, as well as multiple reflections between the ferrite and antenna layers, but it is found to give a reasonable justification of the attenuation through the radome layer. More sophisticated (e.g., full-wave) analysis may be necessary if the ferrite layer is in direct contact with the antenna or array. It is seen that the frequency where maximum attenuation occurs can be tuned by adjusting the bias field. Also note that the attenuation is greater for higher frequencies, primarily because the ferrite layer looks electrically thicker.

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Establishment of the Unified Field Theory

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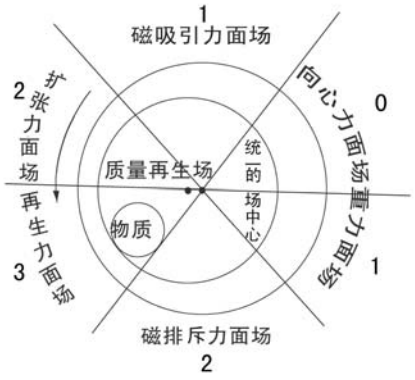
Abstract: There are many problems on natural science disturbing scientists. Einstein focused on finding fields of unified gravity, electromagnetic force, strong force and weak force, so he could not take the lead to create the Unified Field Theory to analyze which one is of bigger mass, and what had integrated all these forces to evolve new substances. As a result, the author tries to create the Unified Field Theory, its graphics and formulas in the purpose of correctly getting to know the causes in Nature and the ability of after-movement energy-change so as to benefit the mankind. [Nature and Science 2009;7(11):15-20]. (ISSN: 1545-0740).

Key words: Unified Field Theory; Oval charts and formulas; Application of the Unified Field Theory



The Unified Field Theory integrates the formation and state of movement and development of things into an oval chart, sets data according to each position shown in the oval model, and then demonstrates by theory, teaching you how propose, solve, and discuss different kinds of problems. First of all, it informs you of what a unified field is. For example, masses of forces surfaces of different fields marked in this oval model generate different energies on the same field. The one of bigger mass is the unified filed. Therefore, when proposing, solving and discussing things like galaxies, planets, atoms, plant seeds, animal cells, a country or a person, etc., we can deem the living-body structure as the unity of the unified field, and then discuss how a mass moves on this living-body field to generate ethers such as gravity, magnetic force, strong force, and weak force, etc.

What is the function of the unified field? We regard core objects that take up and consume much energy such as the pole of rotation of the earth, the core of atoms, the central government of a country or a living person as the unified fields. Then we can make accurate mathematical explanation to elements at each position on the oval model, e.g. the mass, speed, of the planets, and its distance from the core of the galaxy, etc.

This filed is formed by concentric movement, with its mass depending on the energy of the centripetal force on the force surfaces of the fields around. Its function is to locate objects around and unify them when the limit of their movements is reached, and provide or inherit wisdom and energy for the objects around.

Therefore, time and space begin at this time. Gravity, magnetism, electricity, light, etc. are formed one after another after self-autorotation and being pushed. They disappear also with the disappearance of self-autorotation, and time and space ends as well.

What is the Unified Field Theory? For example, when discussing problems like why the planet moves faster on one side of the galaxy core, but slower on the other side, the methods used in the process of demonstrating the unified field are called the Unified Field Theory, which is composed of the Unified Field Theory, an oval chart and the formulas of problem-proposing and problem-solving. With it, problems involved in the unified field can be discussed.

The large circle in the oval chart stands for the size of an object in the unified field.

The hole in the small circle of the oval chart is

called “Black Hole” at the core of the galaxy. It resembles the length of arms of a lever, or a woman’s womb, or copy tools of computers, weapons, etc., generating more energy after systematic accelerated movement. The mass of petrol is lost after being transformed, but the energy is conserved. As a result, the hole in the small circle and tools, etc. are called the mass-regeneration filed, which is used to generate more energy.

It has a uniform criterion of mass. The bigger the mass of the field, e.g. the fiercer the centrifugal force, or the larger the holes, r the heavier the tools and weapons people use, the more energy they generate.

On the contrary, every time when the mass of the field gets smaller, or when moving on the field of gravity or the field of centripetal force, the higher the object whirls, the slower it moves, and the less energy it generates.

The substances in the hole of the oval chart can also be regarded as the names of objects that generate energy. Every time it moves systematically, the bigger the mass, the more energy it generates; the smaller the mass, or the weaker the centrifugal force, the less energy it generates, or the less evolution of synthesis. Therefore, the movement is not only dependent on the surroundings, tools, and weapons, etc., but also restricted by its own mass. In addition, it cannot generate energy alone.

The six (relative) different fields of force surface in the oval chart represent the time of laws of motion of everything on the earth, including people, and they do ordered movement of a new round. The numbers stand for that during the process of development at each stage, the movement they do to generate energy is random. These six fields, just like the cores of the mass-regeneration field and the unified field, are divided into three kinds of masses--- big, medium, and small. The other chart is to illustrate the push and friction of centripetal and centrifugal forces in different spaces, i.e. the so-called *yin yao* and *yang yao* in the Eight Diagrams. When discussing about things, we have to be as accurate as what Zhouyi has predicted, combining all the data to work out the correct answer.

The top of the large circle in the oval chart stands for the starting point of the time of one object, or objects converged under this condition. It won’t change in energy. By a terminology in physics, we call it “the surface field of magnetic attraction”. We label the energy it generates from constant velocity motion as 1.

The protruding part of the top of the oval chart stands for the motion from the top to the bottom. We call it “the surface field of expansionary force”, and the energy it generates and converges is labeled as 2.

Under it, the acceleration inertia can generate more energy, e.g. to increase the ovality of the earth. We call it “the surface field of strong force”, or “the surface field of regenerative force”. The energy it generates is labeled as 3.

In short, movement in these two surface fields is in acclerrated motion because there is an inertia of push.

The very bottom of oval chart is the surface field of magnetic repulsive-force, standing for that the c entripetal force of a mass is stronger when it spins slowly and the centrifugal force is stronger when it spins quickly if a mass moves on this field and at this time. The energy generated has been decreased to 2. We call it “irregular motion”, which will result in fortuitous accidents.

The other side of the oval chart is a motion from the bottom to the top. The energy generated has decreased to 1. It does decelerated motion by inertia. We call it “the surface field of gravity”.

But on the top of such kind of field, since the objects have completely lost the ability to do work, and consumed energy by friction force and gravitation, so it does centripetal motion. Therefore, we call it “the surface field of centripetal force” or “the surface field of weak force”. The energy generated is 0.

In short, movement in these two surface fields is in deaccelerated motion because there is a centripetal force. And contraction as well as degeneration will happen.

Of course, the energy they have generated has done continuous accelerated centripetal movement, making the mass of the object in the center of the hole of the organizational structure of the joint force, generating more energy to do work after thrown by the centrifugal force after autorotation. If they move for a second time, the total mass of the system should be multiplied with the mass of the work done by throwing through acceleration inertia and the energy obtained from the distance away from the center, and then this new number should be multiplied with the energy of inertia one after another. After that, we can know energy evolved from different surface fields, giving rise to gradual acceleration of the speed of autorotation, such as expansion, photo-electro-magnetic or the element mass as shown in the positions on in different surface fields of the oval chart. The temperature changes periodically in energy, or does non-constant motion, etc

The object generating more energy doesn’t move in elliptic motion which consumes less energy but moves in circle which consumes more

energy. For example, quark cannot generate more energy, so it moves towards the ellipse. Under the influence of the centripetal force, its energy generates in an accelerated way. When it comes to the center, it makes gas or objects in the hole, or blue light with high temperature and larger mass than it, generate new groups or new elements. Or, when it reaches the central point of the hole, it will lose the energy to spin by acceleration of the system, thus integrating electromagnetic force, strong force, gravity, etc. generated from autorotation by the friction and gravity of bigger mass.

The oval chart above is used to unveil the fact that things in the microcosm do elliptic motion. It is the centripetal movement that has caused the generation of centripetal force which enables the things entered the center to be thrown away by the centrifugal force by the autorotation of the system. All things in the Nature use these two kinds of simple and different pushing forces and friction forces to make objects on any field of the unified field generate energy have autorotation energy to evolve and develop and anytime anywhere, gradually generating gravity, electromagnetic force, strong force, weak force, etc. In this way, making use of each of their descriptions, and combining the data mentioned above, it is possible and reasonable to propose, settle, and discuss different problems arising from the things on the earth.

Apart from using the oval chart to unveil mysteries of the past, present and future of all things on the earth which generate energy in motion, proposing and settling problems by the structure of a unified oval ball, the Unified Field Theory also sets up one formula:

$$Kg \neq m \cdot a2 \cdot t \begin{cases} \max \\ \\ \\ \min \end{cases}$$

This formula gives you an inspiration to propose and solve problems. This formula appears like a lever, enabling us to associate the mass of movement of all things which doesn't equal the energy they can generate.

"≠" tells the person who proposes and settles problems about the problem of limit of energy generated in motion in the system of the mass. With this limit, we can get to know the death of things and the speed of irregular periodical movement. Three things are proposed: ①"m"stands for the mass at the early stage of convergence, or the history of past. "t" stands for the energy generated after the convergence, or the present mass. "kg" stands for different occasions, different conditions, different results, or its future mass. ② Regardless of past, present or future, galaxies or atoms, animals or plants, macro or micro, "≠" means the contradiction between fast and slow. The speed contributes to the development of this contradiction, and time is the one to reconcile the contradiction.

Therefore, they are in compliance with the mass, acceleration, time, and the environment in one structure so as to propose and solve the problems. ③ "≠"also stands for the existence of the speed on the two sides of the autorotation pole, and the photon, electron radiated after the movement. So we should use different forms to discuss and different formulas to calculate. "t" (time) stands for the energy that can be generated after movement due to the existence of the regenerative field, and with what kind of mass to converge around the autorotation pole, in the regenerative field and other fields.

You can get to know the mass after their movement based on these conditions. The energy generated will be either the strongest or the weakest decided by the following three elements: first, the acceleration speed of autorotation; second, the speed in different situations on the two sides of the autorotation pole; third, the speed before or after the mass limit being unified. The relationship among them is unified randomly. If anyone of them converges or evolves and generates different amounts of energy, there will be numerous changes in the fields, just like new things keep on emerging in the Nature. Then discussions have to be held again one by one, or different formulas are used to calculate.

Therefore, this formula, like Chinese ideographic writing, contains both philosophy and inspiration for readers to associate, correctly calculating them and making out the causes of what does more work one by one. These objects, exceeding the bearing capacity of the mass of the system, result in "strong force" such as "electromagnetic force", nucleus (proton as well as neutron) and "weak force" when the atoms decay. Or, readers can propose a mathematical equation of this object to calculate physical quantities such as different forces, and intensity to solve the problems.

It is safe to say that with the Unified Field Theory and data from the oval chart showing you how to propose and solve problems, the researchers will get the insight to imitate out their own charts or fill the problems into the corresponding positions of the chart, then get to know what their nature is, or what kind of movement structure they belong to, e.g.

whether they do rectilinear motion, or do elliptic motion on a tilt. In this way, researchers will get to know their movement from a comprehensive, systematic, related, and developmental perspective so as to discuss random problems emerged such as how planets move in the galaxy as a unified field. Also, they can use simple methods to quickly solve the problem on fastness and slowness. At the same time, the view of transforming mass into energy and the quantum theory proposed by Newton and Einstein, as well as the superstring theory, etc. are summed up and arranged scientifically, making it clear that the one with a bigger mass or a proper method is the unified theory.

In the future, once the Unified Field Theory and the oval chart are accepted, a more magnificent scientific mansion of physics as strong as the framework of reinforced concrete will be built to propose and solve problems, leading people to put mass into a system to generate more regenerative energy, and to create a new moralized era featured by "great scientific development" so as to benefit the mankind.

Next, let's have a look at how the Unified Field Theory is used to propose and solve problems. I will just make a simple statement here, but you can infer the whole from a single instance. For more detailed information, please refer to passages of the series of the Unified Field Theory (45 all together), such as *The Great Parturition of Mother Universe*, and you can refer to the 162 problems solved by Wang Xiyu so as to further understand the scientific value of the Unified Field Theory.

Example 1: in the physical formula of the Unified Field Theory, we propose a limit of the energy that can be regenerated of the quality when in motion, and converge the biggest or smallest quality within the limit of "t" (time). The core of the unified field is like the central government, the quality-regenerative field is like the economic policy, and all the force fields are like different groups of people. When start moving at the same time, in what kind of state do they converge and move? Suppose each of them appears with the greatest quality in every field, and suppose the energy generated by the primitive quality is 1, that by the progressive quality is 2, by the advanced quality is 3, by the speculative quality is 2, by the backward quality is 1, and by the hard quality is 1, which constitute a system. When an advanced person who has operational management capability and enough money runs a factory or company, the business will develop very quickly at once, just like the objects that have accelerated and moved for a while in the quality-regenerative expansionary-force field and the regenerative field. If we use the figures, the energy they have generated is six (three times two) and nine (three times three), respectively. While those people who have no management capability or fund can only work for others. They are like the objects moving in the gravity field or the centripetal-force field, which get no help from any dark energy or dark matter. A year later, if we use the figures to express, apart from the daily-use articles, other profits they have gotten is three (three times one) and zero (three times zero).

From the Unified Field Theory, we can get to know that after years of energy accumulation, the quality of these people has evolved in this net-force structure by inertia, and the energy doesn't conserve. And they will invest their money in the things they are interested in. Suppose the primitive group does the business of land disposal and land leasing, the progressive group invest their money on real estate development, and the speculative group does any business that can get profit, such as buying or selling the real estate, and the backward and hard group accumulate money to purchase the living houses for future development.

Due to the development of the market and the different development of various groups of people, the backward and hard group who accumulate money to buy their houses cannot catch up with the increase of commodity prices, and then there will be situation of oversupply and dissatisfaction to the society. Therefore, the central government should firstly decide whether it is a planned-economy society or a market-economy society, whether their motion speed, structure, policy or strategy are injurious to the common benefit, and whether they have exceeded the greatest or the least limit, and then the government can flexibly use different methods, such as policies and laws to regulate and unify the unreasonable forces inside its movement structure. For example, we need to take comprehensive measure in the complex market economy, for its perniciousness is the same as the stocks. On the one hand, it damages the enterprise's profits; on the other hand, it does harm to the society. So the government should implement comprehensive treatment to those who illegally rent or sell land, who use the profit to run the real estate business, who irresponsibly apply loans from the bank, and also raise the salary of the low-income earners, provide subsidies for purchasing an apartment to the poor households, and take effective measures to the problems such as low-price public-land sales, and high-priced removal compensate, etc., so as to enable each component inside the matter to unify in the fierce movement or psychological world or legal system. It will be as regular as the six fields in the oval chart, being well-organized and harmonious. Things are run in this kind of condition, rather than the chaotic state of

no certain proportion, no certain conflict, and no certain difference.

If the central government can't formulate a new policy in time, or cannot unify by force, then its ruling ability will be zero, and the social stability will be disturbed.

Example: Why are there two different spiral arms of different speeds around the galaxy? We should solve this problem--- fast and slow, in the oval chart. Firstly, you should find out the causes of the problem to be solved from intuition and sudden enlightenment. The mass of all the planets have given rise to the difference of the degree of inclination of the galaxy, changing the normal amount of energy generated after movement of the planets around the core of the galaxy. For example, some planets of small mass, or planets of big mass that have moved from the surface field of expansionary force or the surface field of regenerative force field to the surface field of gravity or the surface field of centripetal force, all do upwardly elliptical movement on the slant of the galaxy. Since there is centripetal force in both the two surface fields, the energy of autorotation regenerated by the star core inside the planet will decrease. Consequently, the autorotation of the planets doing upward movement around the core of the galaxy is unified by the gravity and friction force, and each of them decelerates in inertia and gradually bends to the core of the galaxy and forms the density wave, making use of the repulsive force to pull and move forward. On the other side, the planets are doing downward movement on the galaxy slant, being pushed by the acceleration inertia, enabling the working star core to regenerate much more energy in the acceleration inertia through the cavity whenever it is pushed one step forward by the centrifugal force. It will apply more force to the internal rock of the regenerative-force field of the earth mantle, and consequently increases the energy of the movement of the plate on the sphere surface. Meanwhile, some of the small granular matters inside the earth's core will also be whirled and converged into numerous volcanic egg-shaped spheres of different sizes. Their masses of iron are constantly processed by the push and friction of centrifugal force and centripetal force, causing them to emit magnetism the same as the earth's core. After pushing the mantle wall, it is pushed by the centrifugal force continuously. The earth's core then keeps drifting and rising, no longer doing an elliptical movement consuming less energy as shown in the oval chart, while developing in to circular motion consuming more energy. Therefore, the intensity of autorotation is unified by the friction, gravitation and gravity with big mass in the core of the unified field, causing it to do upwardly accelerated movement of a shrinking ball, whirling from top down towards the south and north poles in the shape of the number "8" in cyclic motion, and converging with the plates in the mantle by the centripetal force from the earth's crust and the centrifugal force from the earth's core, and then, some of the mantle plates also emit magnetism. Since these movements are continuous, placing the top and bottom parts of the south and north poles spheres in a state of constant magnetism accumulation, so the magnetic fields of the South Pole and North Pole are formed. When it is during the day, there will be some space due to the conjunction of the mantle plates, but they cause many large holes in the magnetism layers (to know more, please refer to the internal structure of the perpetual motion machine). While the sun-facing plates around the earth's core do downward acceleration motion in the day, they do inward bending deceleration motion in the night. The small plates on the two sides of the sphere move regularly and orderly in the south-pole field and the north-pole field from left to right, interrupting the line of magnetic induction from the whirling motion of the earth's core to the elliptic motion of the mantel plates. Therefore, the mechanical energy changes into electric current, increasing the autorotation capability. Of course, the electrons inside the atom generate centripetal force, and the photons generate centrifugal force. When there is friction between two objects inside an atom, the one with weaker capability to bind the electron by the atom core will lose some electrons which will be transferred onto another object. Since it lacks electrons, it is positively charged. Conversely, the one which has gained extra electrons will be negatively charged. As a result, when the autorotation accelerates or regenerates different amounts of energy, there will be an uneven phenomenon that on one side of the galaxy, the objects move more quickly, and on the other side, the objects move more slowly.

In other words, spiral arms with different speeds on the two sides of the galaxy generate different amounts of energy because there is pulling force on the one side while pushing force on the other side. Besides, different masses of the planets on the two sides of the planet contribute to different amounts of energy. For example, on one side are the dark stars with a slower speed, which are at the early stage of evolvement, and on the other side are white dwarf stars with a quicker speed, which are at the late stage of evolvement. They converge, evolve, and generate; doing this again and again results in more energy generated, so the autorotation speed will certainly be quicker. In addition, different masses of the surface fields around the core of the galaxy have also exerted some impact, forcing the planets to move in their own surroundings and regenerate different amounts

of energy, thus resulting in different speeds of movement. Apart from these, the inclination degrees of the planets also decide the limit of the amount of energy regenerated by the planets. There will also be problems like uneven periodical movement on the two sides of the galaxy core, and difference of the oval motion orbit and inclination. To solve problems in this system, we cannot explain them clearly with current physical theories or rules.

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3. Ps. Books on Magic Skills written by Hong Kong people after the Spring Festival of 2007 has enlightened and encouraged me a lot

8/20/2009

Epigenetic (Protein) Pattern of Albino Rat *(Rattus norvegicus*) After Treatment with Homocysteine and Trimethylglycine

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Abstract: Hyperhomocysteineamia (hHcys) is thought to be a risk factor for cardiovascular disease. The epigenetic (protein) pattern due to the effect of hHcys in kidney and liver was profiled in the supplemented developing rats. A group treated with 0.6 mg / kg / b. w. Hcys for 8 weeks and trimethylglycine (Tri or Betaine) antioxidant treated group (0.6 g / kg / b. w.) were compared with four weeks age control group. The results indicated that, a great variety in protein pattern was obtained. Specific type of protein with M.W. of 214.29 K.d. was produced in the kidney samples treated with Trimethylglycine. Common types of protein with M.W. of 278.64, 157.07, 52.39, 39.31, 29.24, 24.49, 20.86, 20 and 18.87 K.d. were obtained in the same sample. Characteristic band with 59.58 K.d. was obtained in control the kidney after 8 weeks while three types of protein were obtained in the control liver with M.W. of 221.4, 28.25 and 18.07 K.d. The highest similarity index was between control and Trimethylglycine treated kidney group (0.76), but the lowest one was between the control and liver trimethylglycine treated group (0.38). [Nature and Science. 2009;7(11):21-27]. (ISSN: 1545-0740).

Keywords: Homocysteine, trimethyleglycine (betaine) and rats

Introduction

Hyperhomocysteineamia (hHcys) has been indicated as an independent risk factor causing a variety of pathological changes in different cells or tissues. Homocysteine **(**Hcys**)** is produced from S- adenosylhomocysteine **(**SAH**)** through the catalysis of **(**SAH**)** hydrolase, (Li *et al*., 2006). Hcys is derived from the essential amino acid methionine, during normal condition, excess Hcys is converted back to methionine or broken down be excreted (Stead *et al*., 2000). While the remethylation process of Hcys was 5-methyltetrahydrofolate and the activity of betaine homocystein S–methyltransferase (BHMT) as the methyl donor (Finkelstein *et al.*, 1971; Castro *et al*., 2002 and Finkelstein, 2007). In rat, the kidney is a major site for the removal of plasma Hcys House *et. al,* 1997 B & 1999. According to Stead *et al.,* 2000, the liver is a key organ of Hcys metabolism and potentially control the plasma Hcys level. Betaine was involved in Hcys metabolism as an alternative methyl donor, it is used in the treatment of homocystineuria in human as revealed by Schwab *et al*., 2002. Betaine is found naturally in most living organisms, it protects plants (Sakamoto and Murata, 2000), microbes (Rozwadowski *et al.,* 1991), marine and freshwater invertebrates (Konosu and Hayashi 1975) against osmotic stress and acts as an osmolyte in mammalian tissues as concluded by Garcia-Perez and Burg, 1991 and Burg, 1997. Betaine is formed in cells as an oxidation product of choline and can be obtained externally from food, (McCue and Hanson, 1992). Several nutrients are including folate, vitamin B-12 and vitamin B-6 influence the metabolism of Hcys, (Selhub and Miller, 1992; Cuskelly *et al.,* 2001 and Hu, 2002). The objective of this study is the revealence of the protein pattern after different treatments to asses the physiological state of rat as a response to this treatment. To achieve this purpose vertical slab electrophoresis process was performed and Polyacrylamide gel electrophoresis (PAGE) was used.

Material and Methods

The used albino rats *Rattus norvegicus* (4 weeks age and 50 -60 g in weight) were adapted to the laboratory conditions for 7 days before the study. Animals were divided into 4 groups, group I was control, group II was treated with Trimethylglycine (0.6 g / kg / b. w.) orally for 2 months (Wilcken *et al.,* 1985 and Schwab *et al.*, 2002) , group III received Homocysteine (Hcys) at the dose of (0.6 mg / kg / b. w.) orally for 2 months, (Masse *et al.,* 2003) and group IV received both Homocysteine and Trimethylglycine (Hycs + Tri) orally for 2 months. Animal's autopsy took place after 2 and 8 weeks. Kidney and liver organs were prepared to biochemical studies.

Proteins were separated through 8% Polyacrylamide gel electrophoresis (PAGE) according to method of (Davis, 1964). Electrode and gel buffer and polyacrylamide stocke were prepared according (Laemmli, 1970).

Staining solution (commsie brilliant blue) was used for 12-18 hr and destaining by destain solution. The gel were photographed, scanned and analyzed using Gel-Pro analyzer version 3.1

Calculations and data analysis

The similarity coefficient and genetic distance were calculated according to (Nei and Li,1979). As following:-

S.I = 2Nxy/ (Nx + Ny)

G. d = 1 – S. I

Where : S = similarity value, Nx and Ny are the number of bands in individuals x and y, N is the number of shared bands. The value produced by this index ranges from zero, representing no bands sharing, to (1), representing complete identity, while G.d is the genetic distance value.

Results

There is no different between protein of treated and control samples as a native technique.

Sodium deudosyle sulphate polyacrylamide gel electrophoresis (SDS PAGE) showed highly variation in kidney protein pattern. The tabulated data in table (1A) and fig. (1), revealed that , the total number in bands of control were 12 with Rf value ranged from 0.057 to 0.95 . While the total number of bands in the sacrificed animals after 2 weeks were 25, 22 and 22 for Tri, Hcys and Tri + Hcys, respectively. Data represented that, bands number 1, 4, 10, 11, 15, 16, 20, 22, 25, 26 and 27 were common bands with average M.W. 278.64, 157.07, 59.37, 52.39, 39.31, 35.13, 29.24, 24.49, 20.86, 20.04 and 18.87 K.d. for control respectively.

Table (1A): Molecular weight (M.W.), amount (%) and rate of flow (RF) for kidney of *Rattus norvegicus* fractionated protein for the treated and control groups after 2 weeks.

Samples	Control			Tri group			Hcys group			Tri+Hcys group			Average M.W.
Rows	M.W. ´	%	RF	M.W.	%	RF	M.W.	%	RF	M.W.	%	RF	
1*	278.57	1	0.057	278.71	0.4	0.057	278.71	0.9	0.057	278.57	1.8	0.057	278.64
2	221.43	1.8	0.088	-	-	-	221.43	5.3	0.088	221.43	4.3	0.088	221.43
3	-	-	-	214.29	4.3	0.091	-	-	-	-	-	-	214.29
4*	157.14	8.3	0.13	157.14	9.8	0.13	157	9.9	0.13	157	8.9	0.13	157.07
5	-	-	-	105	11.3	0.21	106.67	12.2	0.21	106.67	10.6	0.21	106.11
6	-	-	-	93.333	0.4	0.23	93.333	2.8	0.23	93.333	13.2	0.23	93.33
7	-	-	-	76.923	3.9	0.23	75.769	5.7	0.28	76.923	17.3	0.28	76.53
8	-	-	-	69.5	1.3	0.30	-	-	-	-	-	-	69.5
9	-	-	-	66.5	2.9	0.33	-	-	-	-	-	-	66.5
10*	59.583	9.3	0.38	59.167	0.7	0.38	59.583	0.8	0.38	59.167	1.3	0.38	59.37
11*	52.5	12.7	0.45	52.083	2.5	0.45	52.5	2.7	0.45	52.5	2.1	0.45	52.39
12	-	-	-	50	0.9	0.47	50	1.4	0.47	50.417	1.2	0.46	50.13
13	-	-	-	46.4	1.7	0.5	46.4	1.7	0.5	46.4	1.8	0.5	46.4
14	-	-	-	44.6	1.5	0.53	44.6	3.3	0.53	44.6	2.4	0.53	44.6
15*	39	12.8	0.57	39	5.2	0.57	39.75	3.2	0.57	39.5	0.3	0.57	39.31
16*	35.25	14.6	0.64	35.25	1	0.64	35	0.4	0.64	35.75	3.4	0.64	35.13
17	-	-	-	34	0.3	0.65	-	-	-	-	-	-	34
18	-	-	-	-	-	-	33.75	0.8	0.66	33.75	0.8	0.66	33.75
19	-	-	-	31.75	3.2	0.69	-	-	-	-	-	-	31.75
20*	29.038	17.2	0.73	29.038	1.4	0.73	29.038	4.2	0.73	29.846	3.2	0.73	29.24
21	-	-	-	27.885	0.6	0.76	27.885	1.2	0.76	27.692	1.2	0.76	27.82
22*	24	3.8	0.82	24.828	3.3	0.82	24.655	3.5	0.82	24.483	3.2	0.82	24.49
23	-	-	-	23.448	5.6	0.85	23.448	5.8	0.85	23.276	9.5	0.85	23.39
24	-	-	-	22.414	8.2	0.87	22.414	8.5	0.87	22.241	10.5	0.87	22.35
25*	20.862	4.5	0.91	20.862	13.2	0.91	20.862	9.1	0.91	20.862	0.9	0.91	20.86
26*	20	5.5	0.92	20	15.2	0.92	20.172	15.4	0.92	20	1.2	0.92	20.04
27*	18.966	8.5	0.95	18.793	1.2	0.95	18.793	1.2	0.95	18.966	0.9	0.95	18.87

* indicated for common bands % = Amount percent Rf = Rate of flow.

No characteristic bands for the control, while the bands no. 3, 8, 9, 17 and 19 with Rf value 0.091, 0.30, 0.33, 0.65 and 0.69 and M.W. 214.29, 69.5, 66.5, 34 and 31.75 K.d. were characteristic bands for the Tri treated animals. Band no. 18 appeared only in Hcys and Tri + Hcys treated animals with Rf value 0.66. Densitometric scanning of electrophoresis showed that, the 7th band in Tri + Hcys treated group had the highest density than the others. The quantitative mutation was recorded according to band % related to control samples. The highest quantitative mutation was recorded in band no. 15 in Tri + Hcys samples with M.W. 39.75 K.d. equal 42.6 times of control.

Table (1B): Molecular weight (M.W.), amount (%) and rate of flow (RF) of kidney fractionated protein for the treated and control groups of *Rattus norvegicus* after 8 weeks

Samples	Control			Tri group			Hcys group			Tri+Hcys group			Average
Rows	M.W.	%	RF	M.W..	%	RF	M.W.	%	RF	M.W.	%	RF	M.W
1*	278.57	o.9	0.057	278.57	3.9	0.057	278.71	0.8	0.57	278.57	1.3	0.057	278.60
2	-	-	-	-	-	-	-	-	-	242.86	6.3	0.076	242.86
3	221.43	1.8	0.081	221.57	7.5	0.081	221.71	5.5	0.08	-	-	-	221.57
4*	157.14	8.3	30.13	157	8.4	0.13	157	10.7	0.13	157.14	12.6	0.13	157.07
5	-	-	-	110	35.4	0.19	-	-	-	-	-	-	110
6	-	-	-	-	-	-	108.33	12.7	0.20	108.33	14.8	0.2	108.33
7	-	-	-	-	-	-	95	15.4	0.23	96.662	18.2	0.23	95.83
8	-	-	-	-	-	-	79.231	19.2	0.27	79.231	4.1	0.27	79.23
9	-	-	-	-	-	-	66	5.3	0.33	66.5	4.7	0.33	66.25
10	59.583	9.3	0.38	-	-	-	-	-	-	-	-	-	59.58
11*	52.5	12.7	0.44	52.917	2	0.44	52.917	1.1	0.44	52.333	3.8	0.44	52.66
12	-	-	-	-	-	-	50.417	1.2	0.46	50.417	0.6	0.46	50.41
13	-	-	-	-	-	-	48	1.2	0.49	47.6	2.9	0.49	47.8
14	-	-	-	-	-	-	44.6	4.3	0.51	44.6	3.7	0.51	44.6
15*	39	12.9	0.57	39.25	11.1	0.57	39.5	4.4	0.57	39.5	4.2	0.57	39.31
16	35.25	14.6	0.64	-	-	-	-	-	-	-	-	-	35.25
17	-	-	-	34.5	5.4	0.65	34.5	1	0.65	34.5	1.4	0.65	34.5
18	-	-	-	33.5	1.4	0.67	33.25	0.4	0.67	33.25	1.1	0.67	33.33
19*	29.038	17.2	0.73	29.231	4.3	0.73	29.231	4.2	0.73	29.423	3.8	0.73	29.23
20	-	-	-	-	-	-	28.077	0.5	0.75	28.269	4.1	0.75	28.17
21*	24	3.9	0.83	24.31	12.2	0.83	24.31	5.2	0.83	24.138	3.1	0.83	24.18
22	-	-	-	-	-	-	23.448	0.2	0.85	23.276	2.7	0.85	23.36
23	-	-	-	22.414	0.3	0.87	22.414	3.2	0.87	22.241	2.3	0.87	22.35
24*	20.862	4.6	0.91	20.69	4.3	0.91	20.862	0.8	0.91	20.69	1.3	0.91	20.77
25*	20	5.5	0.92	20	2.2	0.92	20.172	1.2	0.92	20	1.2	0.92	20.04
26*	19.966	8.3	0.95	19.966	1.6	0.95	19.31	1.5	0.95	19.138	1.8	0.95	19.59

* indicated for common bands % = Amount percent Rf = Rate of flow

In table (1B) and fig. (1), the total number of bands in treated animals after 8 weeks were 14, 22 and 22 for Tri, Hcys and Tri + Hcys respectively. Bands no. 1, 4, 11, 15, 19, 21, 24, 25 and 26 were common bands in all treated animal and control. Bands no. 10 and 16 with M.W. 59.583 and 35.25 K.d. specific to the control. Band no. 5 with M.W. 110 K.d. specific to Tri treated animals and recorded high denisty, while band no. 2 with M.W. 242.86 K.d specific to Tri + Hcys treated animals. There was a quantitative mutation recorded in band no. 11 in Hcys treated group with M.W. 52.66 K.d. reached to 11.5 times if compared to control.

The similarity index (S.I.) and genetic distance (G.D.) at the epigenetic level were recorded between control and other treated animals, table (1C). The highest value of 1 was found between Tri + Hcys and Hcys group, while the lowest value was 0.54 found between Tri and control group. The highest G.d. value of 0.46 was found between control and Tri group, while the lowest value of 0 was found between Hcys and Tri + Hcys group. From data in table (1D), the highest S.I. of 0.95 was found between Tri + Hcys and Hcys group, while the lowest value of 0.54 was found between Tri + Hcys and control group. The highest G.d. value of 0.46 was found between control and Tri + Hcys group, while the lowest value of 0.24 was found between control and Tri group Table (2A & 2B) and fig. (2), summarized the data collected from liver electrophoresis of all groups. In table (2A), six types of protein from each group under study were produced except for control, it was 11 bands. There were 4 common bands were no. 3, 8, 12 and 13 with average M.W. 138 , 44.22, 25 and 17.49 K.d for control group.

Table (2A): Molecular weight (M.W.), amount (%) and rate of flow (RF) of liver fractionated protein for the treated and control groups of *Rattus norvegicus* after 2 weeks

Samples	Control			Tri group			Hcys group			Tri+Hcys group			Average
Rows	M.W.	%	RF	M.W.	%	RF	M.W.	%	RF	M.W.	%	RF	M.W
1	-	-	-	-	-	-	228.57	11.4	0.049	228.57	9.3	0.049	228.57
2	221.43	7.7	0.053	221.43	9.5	0.053	-	-	-	-	-	-	221.43
3*	138	13.8	0.11	138	35.1	0.11	138	33.7	0.11	138	42.2	0.11	138
4	85	7.1	0.21	-	-	-	-	-	-	-	-	-	85
5	69.091	10.5	0.27	-	-	-	-	-	-	-	-	-	69.09
6	54.848	3.7	0.36	-	-	-	-	-	-	-	-	-	54.84
7	-	-	-	50	7.1	0.42	50	1.1	0.42	50	0.6	0.42	50
8*	44.105	9.4	0.48	44.263	30.2	0.48	44.263	38.7	0.48	44.263	29.6	0.48	44.22
9	35.833	17.8	0.61	-	-	-	-	-	-	-	-	-	35.83
10	33.056	9.7	0.64	-	-	-	-	-	-	-	-	-	33.05
11	28.25	0.7	0.71	-	-	-	-	-	-	-	-	-	28.25

12*	25.182	19.3	0.75	25	3.3	0.75	25	5.7	0.75	25	4.9	0.75	25.04
13*	17.077	0.3	0.88	17.885	14.8	0.88	17.692	9.4	0.88	17.308	13.4	0.88	17.49

* indicated to common bands % = Band percent Rf = Fractionated protein

In control group, there were 6 characteristic bands, were appeared their no. were 4, 5, 6, 9, 10 and 11, while band no 7 with M.W. 50 K.d was disappeared. Band no. 1 was a common band between Hcys and Tri + Hcys treated groups with Rf value 0.049 for both. Also band no. 2 was a common band between control group and Tri treated group with Rf value 0.053 for both. Densitometric scanning of electrophoresis showed that bands no. 3 and 8 in all three treared groups had higher density than others , table (2A). It is good to mention that, protein with M.W. 17.077 in control samples has a small band % (0.3) compared to Tri samples (14.8), i.e 49.3 times as compared with control samples.

In table (2B), the total number of protein types in control group bands, Tri treated group, Hcys treated group and Tri + Hcys treated group were 11, 14, 12 and 12 respectively. Bands no. 3, 5, 6, 7, 10, 11, 12 and 15 with average M.W. 140, 85.32, 75.02, 56.43, 44.06, 36.43, 32.52 and 23.29 K.d were common bands for all 4 groups.

Table (2B) : Molecular weight (M.W.), amount (%) and rate of flow (RF) of liver fractionated protein for the treated and control groups of *Rattus norvegicus* after 8 weeks.

Samples	Control			Tri group			Hcys group			Tri+Hcys group			Average
Rows	M.W.	%	RF	M.W.	%	RF	M.W.	%	RF	M.W.	%	RF	M.W
1	-	-	-	235.71	5.6	0.4	-	-	-	-	-	-	235.71
2	221.43	7.7	0.053	-	-	-	-	-	-	-	-	-	221.43
3*	140	13.8	0.11	140	1.1	0.11	140	6.1	0.11	140	17.3	0.11	140
4	-	-	-	126	7.3	0.18	126.29	7.1	0.18	126.29	2.5	0.18	126.19
5*	85	7.1	0.21	85.286	3.2	0.21	85.429	8.4	0.2	85.571	3.3	0.2	85.32
6*	75.091	10.5	0.25	75	8.3	0.25	75	12.7	0.25	75	13.6	0.25	75.02
7*	56.848	3.7	0.35	56.758	4.7	0.35	56.061	2.3	0.35	56.061	7.3	0.35	56.43
8	-	-	-	51.212	1.1	0.41	50.909	1.4	0.41	50.606	1.4	0.41	50.90
9	-	-	-	49.474	2.3	0.43	-	-	-	-	-	-	49.47
10*	44.105	9.4	0.47	44.053	4.6	0.47	44.053	1.4	0.47	44.053	7.9	0.47	44.06
11*	36.833	17.8	0.65	36.111	6.8	0.65	36.389	4.4	0.65	36.389	12.1	0.65	36.43
12*	32.056	9.7	0.66	32.5	3.4	0.66	32.778	24.1	0.66	32.778	9.2	0.66	32.52
13	-	-	-	29	22.7	0.69	29.25	9.2	0.69	29.25	2.4	0.69	29.16
14	28.25	0.7	0.71	-	-	-	-	-	-	-	-	-	28.25
15*	23.182	19.3	0.78	23.409	27.4	0.78	23.409	7.8	0.78	23.183	20.1	0.78	23.29
16	18.077	0.3	0.86	-	-	-	-	-	-	-	-	-	18.07
17	-	-	-	13.846	1.5	0.94	14.038	15.1	0.94	14.615	2.9	0.93	14.16

* indicated to common bands % = Band percent Rf = Fractionated protein.

Table (1C) : Similarity indices S. I. and genetic distance G. D. between treated groups and control kidney after 2 weeks

S. I. G. D.	Control	Tri group	Hcys group	Tri+Hcys group
Control	_	0.54	0.70	0.70
Tri group	0.46	_	0.78	0.85
Hcys group	0.30	0.22	_	1
Tri+Hcys group	0.30	0.15	0	_

Table (2C) : Similarity indices S. I. and genetic distance G. D Between treated groups and control of liver after 2 weeks

S. I. G. D.	Control	Tri group	Hcys group	Tri+Hcys group
Control	_	0.55	0.44	0.44
Tri group	0.45	_	0.83	0.83
Hcys group	0.56	0.17	_	1
Tri+Hcys group	0.56	0.17	0	_

There was one characteristic band for control group; it was no. 2 with M.W. 221.43 K.d. The rest treated groups agree with each other in all bands, except Tri treated group which contained 2 characteristic bands, their number were 1 and 9 with M.W 235.71 and 49.474 K.d respectively. Densitomeric scanning of electrophorogram showed that band no. 12 in Hcys treated group and band no. 15 in both Tri treated group and Tri + Hcys treated group had higher density than other bands. The highest quantitative mutation recorded in band no. 10 in Hcys samples, it equal 6.7 times if compared to control samples. From table (2C), the highest S. I. recorded between Tri + Hcys group and Hcys group , it reached one, while the smallest value of 0.44 was found between Hcys & Tri + Hcys and control group. Data from table (2D) revealed that, the smallest G.d. value of 0.05 was found between Hcys and Tri + Hcys group with M.W. 221.43, 28.25 and 18.07 K.d, (table 2B), were disappeared from all treated groups, this mean the inhibition of their genes to produce these types of protein.

Table (1D) : Similarity indices S. I. and genetic distance G. D between treated groups and control of kidney after 8 weeks.

S. I. G. D.	Control	Tri group	Hcys group	Tri+Hcys group
Control	_	0.76	0.60	0.54
Tri group	0.24	_	0.75	0.77
Hcys group	0.40	0.25	_	0.95
Tri+Hcys group	0.46	0.23	0.05	_

Discussion

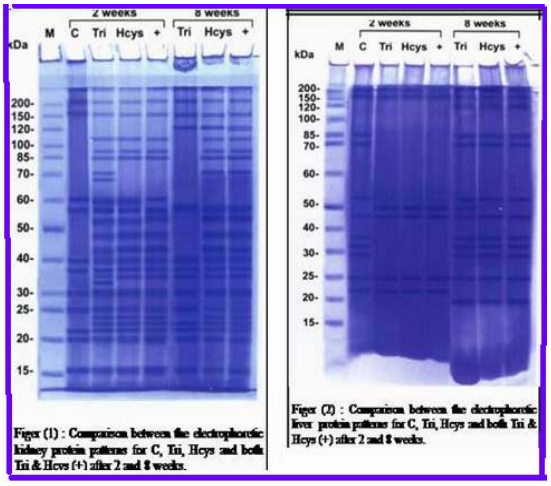
Nine new types of protein with average M.W. 106.11, 93.33, 76.43, 50.13, 46.4, 44.6, 27.82, 23.39 and 22.35 K.d were produced in the treated groups , as in table (1A) and three new types of protein with average M.W. 34.5, 33.33 and 22.35 K.d were produced in treated groups, as in table (1B), which mean the activation of some genes to produce these types of protein (Holdane, 1937). There were six types of protein with M. W. 85, 69.09, 54.848, 35.83, 33.05 and 28.25 K.d, as showed in table 2A, and three types of protein.

While there was one protein type with M.W. 50 K.d, (table 2A), and four types of protein with average M.W. 126.19, 50.90, 29.16 and 14.16 K.d were appeared in treated groups, which indicated that the activation of their genes to produce these types of protein. hHcys is very dangerous for the body and toxic for endothelium , it enhanced vascular smooth muscle cell proliferation, (Satta et al., 2006). In the present study the protein electrophoresis revealed differences between control and the treated groups and also between treated groups themselves. This is due to the production or the disappearance of different types of proteins.

Finkelstein et al., 1971; Breska and Garrow 1999; Castro et al., 2002 and Yap 2005, indicated that, the Betaine – homocysteine – S – methyltransferase 1 (BHMT 1) protein, with M.W. 44.9 K.d and the Betaine – homocysteine – S – methyltransferase 2 (BHMT 2) protein with M.W. 39.9 K.d were involved in the regulation of Hcys metabolism. Betaine and Hcys were converted to dimethylglycine and methionine respectively. This reaction is also required for the irreversible oxidation of choline. These two proteins were existed already in this study with M.W. 44.6 K.d in all treated groups, which represented to (BHMT1) protein. Also the common protein with M.W. 39.31 K.d, represented the BHMT2 protein, the two types of protein BHMT1 and BHMT2 were important in regulation of Hcys metabolism. The protein with M.W. 44.6 K.d which presented in Hcys group and Tri + Hcys group (band no 14) refered to the presence of BMHT1 protein and absent from Tri group indicates that, betaine supplementation decreased the plasma Hcys concentration as resulted by Brouwer *et al.*, 2000; Tangerman *et al.*, 2000 and Schwab *et al.*, 2002.

Table (2D): Similarity indices S. I. and genetic distance G. D. between treated groups and control of liver after 8 weeks.

S. I. G. D.	Control	Tri group	Hcys group	Tri+Hcys group
Control	_	0.38	0.66	0.69
Tri group	0.62	_	0.92	0.88
Hcys group	0.34	0.08	_	0.95
Tri+Hcys group	0.31	0.12	0.05	_



Figer (1) : Comparison between the electrophoretic kidney protein patterns for C, Tri, Hcys and both Tri & Hcys (+) after 2 and 8 weeks.

Figer (2) : Comparison between the electrophoretic liver protein patterns for C, Tri, Hcys and both Tri & Hcys (+) after 2 and 8 weeks.

From other obtained data, the protein of BHMT1 was represented in all treated groups with average M.W. 44.2 K.d and average M.W. 44.06 K.d in all treated groups. Finkelstein 2007, indicated that, all tissues posses the methionine cycle with methyltetrahydrofolate as the methyl donor, but only kidney, liver, pancrease, intestine and brain also contain the transsulfuration pathway. Also, Treberg and Driedzic (2007), suggested that, the kidney and liver appear to be the major sites of betaine synthesis.

Finally, it would be concluded that the protein pattern is changed in each of kidney and liver and quantitative mutation recorded in all tables leading to change in homocysteine pattern which causes different behavioral pattern in each of control and the treated groups.

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Effects of Salinity on Survival, Growth and Reproduction of the Water Flea, *Daphnia magna*

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ABSTRACT: Recent advancing industrialization and urbanization have increased salt concentrations in formerly-freshwater habitats. Freshwater animals are being affected, especially those like crustaceans that are unable to emigrate to escape the problem. *Daphnia magna* is mainly recognized as a freshwater cladoceran, but there are some strains that grow in brackish waters. There has been an increasing interest in using the freshwater crustacean *Daphnia magna* as a toxicological test species for water systems. The determined 48h-LC50s from studied salinities were 2.99, 3.92 and 4.82 ‰, for Sodium chloride (NaCl), synthetic sea water and filtered natural sea water respectively. The effect of salinity on reproduction, growth and survival rates of *D. magna* was studied under laboratory conditions. All individuals were fed with *Scenedesmus obliquus*. Salinity effects on daphnids was investigated at sublethal salinities LC10, LC15, LC20, LC25, LC30, LC35, and LC40 for the three tested saline waters. The number of progeny per female was the most at 0.44‰ (LC10) for synthetic sea water, at 1.67‰ (LC10) for NaCl, but for natural sea water at 2.48‰ (LC10) and 2.82‰ (LC15) were more or less like that of control. Time to the first brood was 9, 9 and 8 for the tested waters respectively indicating delay because of salinities effects when compared with those of control which was 7 days. Survival and growth rates of daphnids were decreased with increasing salinities of the three tested waters. Among three salinity series tested, daphnid survival rate was the highest in control; 97% followed by 80-90 % at LC10 after 21 days experimental period. The highest total reproduction 95±9 neonates per female (mean ±S.D.) were achieved at 0.44 ‰ (LC10) for synthetic sea water, over a period of 21 days. Data showed that, groups of *D. magna* reared in synthetic sea water 0.44‰ showed highest length and specific growth rate. Four essential amino acids were detected for *D. magna* reared in 0.44‰ synthetic sea water while for control group only two. Fatty acids profile of *D. magna* showed four groups of saturated fatty acids dominated by myristic acid (14:0). Moreover, unsaturated fatty acids (USFAs) represented by nine groups and the most abundant USFAs was linoleic acid (18:2n-6).From this study, it is concluded that the strain of *Daphnia magna* used in our study can not withstand high salinities; it may need a long period of time to accommodate to such high salinities to be maintained in aquacultures to be used as live food for higher crustaceans or various species of fish. Besides, low salinities at LC10 (0.44‰) for synthetic sea water, or below may enhance reproduction for this test organism. [Nature and Science. 2009;7(11):28-41]. (ISSN: 1545-0740).

Keywords: Salinity, Sodium Chloride, Synthetic and Natural Sea waters, *Daphnia magna*, Survival, Growth, Reproduction

INTRODUCTION

Since saline and freshwater environments require completely different adaptations if the animals inhabiting them are to retain suitable osmotic pressure and cell homeostasis, most aquatic organisms are unambiguously characteristic of either one habitat or the other (Young *et al.* 1989). Nevertheless, as salinity varies markedly in many habitats, such as estuaries or coastal lakes (Hall and Burns 2002, Schallenberg *et al.* 2003), local populations may be characterized by the presence of micro-evolutionary changes, the widening of tolerance ranges and greater phenotypic plasticity.

Freshwater invertebrates have been submitted over a long period of time to selection to cope successfully with the low osmotic pressure of their present habitat. Their adaptations to live in low salt concentrations and low osmotic pressure, however, are presently being subject to an unexpected test in waters enriched with salts due to ongoing industrialization and urbanization, e.g. with mine waters and storm drain runoff from streets treated with salt in winter. As the tolerance (or lack of tolerance) to high salinity in freshwater animals is poorly recognized, particularly in invertebrates which are not mobile enough to swim away from affected habitats.

Zooplankton production plays an important role in the functioning of aquatic ecosystems by making part of the production of phytoplankton available to higher trophic levels. Crustaceans, which often dominate the zooplankton are the major herbivores in many aquatic communities and are the main food for

bigger crustaceans, various species of fish and representative from many other taxa.

Cladocerans are very important components of zooplankton, usually restricted to freshwater environments (Arnér and Koivisto, 1993) with salinity values lower than $1gL^{-1}$ (Hart *et al*., 1991) or conductivity values less than 500 mS cm^{-1} (Hebert *et al.*, 2002). The genus *Daphnia* is freshwater in its origin and distribution (Peters, 1987; Teschner, 1995); for North America, there are 34 species in freshwater environments and only one for saline lakes; *Daphnia salina* (Hebert *et al*., 2002). *Daphnia* are hypertonic to the medium and the fluxes of water and solutes with the surrounding water could be considerable, but they have reduced their osmotic loads through the impermeability of their bodies and the low internal concentration of solutes, being sodium pumping from the epithelial cytoplasm to the hemolymph the major mechanism for osmoregulation in freshwater cladocerans (Peters, 1987). According to Arnér and Koivisto (1993), although it is possible to find *D. magna* in rock pools with salinity values up to 12.5% in the Baltic Sea, they experimentally determined that the best development was achieved at 4%. Schuytema *et al.* (1997) also concluded that the best growth of *D. magna* occurs at salinity values lower than 4‰. The freshwater Cladocera that successfully colonize brackish environments are smaller in size and have a reduced reproduction (Arnér and Koivisto, 1993). Cowgill and Milazzo (1990, 1991) demonstrated that reproduction, population growth rate, and survival in *D. magna* decreased as NaCl concentration increased in the range of 0.08–6000 mgL^{-1}.

Materials

Experimental animals and food:

A freshwater *D. magna* strain that has been successfully grown in our laboratory of Hydrobiology in National Research Center for more than 19 years in synthetic freshwater media (Fayed and Ghazy, 2000) was used as the test organism for this study.

Gravid females were transferred at regular intervals to 1-L glass beakers, in which the culture medium; synthetic freshwater medium (pH; 7.9, total hardness; 90 mg/L as Ca CO_3, alkalinity; 34 mg/L as Ca CO_3, conductivity; 260µmhos) was renewed 3 times a week and were checked daily for the release of neonates to be used in starting experiments. In these beakers, the animals were fed 3 times a week with $14X10^7$ cells/ml of the green micro alga *Scenedesmus obliquus*, it was previously determined that this cell concentrations is an optimal food dosage for this strain (Ghazy,1997). The algal culture was renewed once a week to maintain the algae solution

There is relatively little information available on the responses and adaptations of freshwater organisms penetrating into brackish water. Among cladocerans, a great majority are exclusively freshwater animal, although a few genera such as *Podon, Evodne, Bosmina* and *Penilia* , have recently colonized brackish and marine environments and some species like *Moina hutchinsoni* and *Daphniopsis pusilla* live in saline lakes (Potts and Durning, 1980). In previous studies *Daphnia magna* has been found in springs of high salinities up to 62‰ (Ghazy, 2003).

Martínez-Jerónimo and Martínez-Jerónimo (2007) also stated that *Daphnia magna* is mainly recognized as a freshwater cladoceran, but there are some strains that grow in brackish waters. Some species have been observed in salinities up to 4 ppt, and salinities of 1.5 to 3ppt are common in pond cultures in the orient.

Even though salinity is one of the niche dimensions that affect the distribution of *D. magna*, there are only a few experimental studies on the salinity tolerance of *D. magna* (Lagerspetz, 1955; Cowgill and Milazzo 1990, 1991).

The aim of this study was to investigate the effect of salinity of each of sodium chloride as a model compound, synthetic sea water and natural sea water, on survival, growth and reproduction of *Daphnia magna* and to establish the maximum salinity level in which *Daphnia* can survive and reproduce to be used as a natural food in aquaculture where *Daphnia magna* is of high nutritive value for aquatic animals.

MATERIALS AND METHODS

in good condition. The algae and the daphnids were kept at a temperature 22± 2°C with a light period of 16 L: 8 D both during culturing and experimental periods.

Facilities and protocols:

The experiments were carried out in 250-ml glass beakers contained 100 ml synthetic freshwater media for control and inoculated with 10 neonates < 24 h.

The effect of salinity treatments were conducted using three test waters; sodium chloride (NaCl) solution, Synthetic sea water (Instant Ocean® Salt, Aquarium Systems,France) and natural filtered sea water. Every treatment ran in parallel with control in three replicates, each replicate contained 10 neonates in 100 ml test water in 250 ml glass beakers. The natural or artificial test water was diluted with the synthetic freshwater media to the respective test salinity. Test media were prepared by diluting saline water with synthetic freshwater media until the

required salinities were recorded with a salinity-conductivity-temperature Meter (YSI Model 33).

Temperature in a 70x60x30 cm aquarium in which test beakers were conducted, was maintained constant by automatic heater (thermostat), Model "hydor",Italy. A mercury thermometer was used to measure temperature in test containers to be at 22±2° C. Natural day length during the experiment period was 16L: 8D. Synthetic freshwater media was used as dilution water and for control.

Methods

Acute tests:

Acute toxicity testing were in triplicates where groups of 10 < 24 h-old daphnids are placed in 250-ml beakers, each containing 100 ml medium and subjected to test conditions for 48 h. Tests were run without food addition. The number of live organisms after the elapse of 48h is recorded. Control test is run in parallel. Salinities series 0, 2, 3, 4, and 5‰ (parts per thousand, ppt) for NaCl solution, 2, 4, 6, 8, 10, 20 ‰ for synthetic sea water and 3.5, 4, 4,2, 5.8, 6.1 ‰ for filtered natural sea water, were studied.

Chronic tests:

Ten neonates (<24h-old, standard length of 1.60 to 2.00 mm) were placed in each 250 ml - glass beakers containing 100 ml of synthetic freshwater for control or saline water for each treatment which were renewed with addition of fresh food three times a week .These experiments lasted for 21 days. Tests were run with food addition three times a week during changing test water.

Salinities effects on daphnids was investigated at series: 1.67, 1.86, 2.04, 2.20, 2.35, 2.51, 2.66 ‰ for NaCl, for synthetic sea water 0.44, 0.67, 0.93, 1.24, 1.60, 2.03, 2.54 ‰, and for natural filtered sea water at 2.48, 2.82, 3.12, 3.40, 3.68, 3.95, 4.42 ‰ which were corresponding to sub-lethal salinities LC10, LC15, LC20, LC25, LC30, LC35, LC40 for each series, determined from acute tests.

For chronic tests, three times a week, *Daphnia* were removed from their container and placed immediately into a new prepared synthetic freshwater media, as control and different salinity-adjusted treatments containing algal food, Scenedesmus *obliquus* at $14x10^7$ coenobia/ ml.

Survival, growth and reproduction rates of daphnids were recorded three times a week. The survival rate was calculated by dividing the numbers counted every time by the number of neonates at the beginning of the experiment.

Growth was determined from the body lengths which were measured under the microscope with an ocular micrometer (160 X magnification) from base of caudal spine to the anterior edge of the head.

Growth is described as the increase in body length over time. Growth in crustaceans is a discontinuous process, i.e. the succession of molts (= exuvia, ecdyses) is separated by intermoult periods. Each time an individual moults , the old integument is shed and a rapid, extensive growth occurs during the short period before the standard length at subsequent moults was tested in function of salinity using a repeated measures analysis of variance (ANOVA).

The age at release of first brood was noted. After every reproduction the offspring were counted and taken away until end of experiment to calculate the number of progeny per *D. magna* female.

Biochemical analysis:

At the end of experiment the biochemical composition (proximate analysis), amino and fatty acids of *D. magna* were determined for the best concentration of synthetic sea water LC10 (0.44‰) and control (0‰).

Proximate analysis: Protein content of daphnids was determined according to Daughaday *et al*. (1952). Lipid content was determined according to the method of Knight *et al*. (1972). Ash and moisture were analyzed according to the method of AOAC (1999).

Analysis of amino acids: The sample was ground and filtered. The residue was washed with a few ml of 75% ethanol and the volume was made up to 100 ml. Several amino acids were examined using a HPLC system (HP1050) with a UV detector at 254 nm. The separation was accomplished with an APS, NH2, (5 µm. 4 ×250 mm) column. The mobile phase consists of 32% (methanol/water), 60/40 with 0.3 ml acetic acid. The flow rate was 0.9 ml/min. The temperature of column was 45°C, while the injection volume was one µl according to the method of (Christian, 1990).

Analysis of Fatty acids: Lipids were extracted from daphnids using the procedure of Folch *et al*. (1957) by homogenizing them in a mechanical blender with a mixture of chloroform and methanol (2:1 v/v). To prevent oxidation, crystals of hydroquinone were added to all samples. The chloroform extract was evaporated at 55ºC under vacuum and the residue weighed.

Following the extraction of lipids from daphnids, methyl esters of fatty acids were prepared for subsequent use in gas-liquid chromatography. Lipid extracts were converted to their methyl esters according to Hartman and Lago (1973).

Analysis of methyl esters were performed on a

CG-17 Gas Chromatography (CG Instrumentos, Sao Paulo, Brazil), equipped with a flame ionization detector.

A stainless steel column, 2m x 5mm, packed with chromosorb W coated with 18% (by wt) of diethylene glycol succinate (DEGS) was used. The operating conditions were as follows: column temperature, 195ºC; sample vaporizer temperature, 225ºC; detector temperature, 245ºC. The carrier gas used was nitrogen, at a flow rate of 40 ml/min. Injected sample size were in the range 2.0-3.0 ml. Fatty acids were identified by comparison with the retention time of standards and by equivalent chain length (Ackman, 1969).

Statistical Analyses:

Probit Analysis was used to calculate the 48h-LC50s for acute tests and 21day - LC50s for chronic tests on *Daphnia magna*, from studied salinities as described by Finney's method (1977). The terminology recommended by Sprague (1969), lethal concentration (LC) was used for survival and, as given here, represents an interpolation from three or more partial-effect concentrations.

Data were analyzed by ANOVA using the SAS ANOVA procedure (SAS, 1988). Fisher′s least significant difference test was used to compare treatment means.

RESULTS AND DISCUSSION

Median lethal concentrations:

The probit estimated 48h-LC50 for NaCl was 2.99 ‰, the tested concentrations ranged from 0 to 6 ‰, for Synthetic sea water and natural sea water 48h-LC50s were 3.92 and 4.82 ‰, and their tested concentrations ranged from 0 to 22‰ and 0 to 6.2, respectively (Table 1) and at the highest salinity, in all the tests, all neonates died during 48 hours. Mortality rates are also elevated where the salt concentration is high, though susceptibility to salt differs both between species and between clones of the same species (Grzesiuk& Mikulski, 2006). The probit estimated 21d-LC50s for the three tested waters were 2.54, 2.27 and 3.48 ‰ respectively (Table 1).

Table (1): Comparison between effects of salinities of each of sodium chloride (NaCl), synthetic sea water and natural filtered sea water in acute (48h) and chronic (21 days) tests

Toxicity (LC)	Salinity (‰)					
	NaCl		Synthetic Sea Water		Natural Sea Water	
	48h-acute test	21day-chronic test	48h-acute test	21day-chronic test	48h-acute test	21day-chronic test
LC10	1.66	1.70	0.44	0.43	2.48	2.14
LC15	1.86	1.83	0.67	0.58	2.82	2.34
LC20	2.04	1.95	0.93	0.76	3.12	2.52
LC25	2.20	2.05	1.24	0.94	3.40	2.69
LC30	2.35	2.15	1.60	1.15	3.68	2.85
LC35	2.51	2.25	2.03	1.37	3.95	3.00
LC40	2.66	2.34	2.54	1.63	4.23	3.16
LC45	2.82	2.44	3.16	1.93	4.52	3.31
LC50	2.99	2.54	3.92	2.27	4.82	3.48

The effect of salinities on the survival, growth and reproduction rate of *D. magna*:

Effect of NaCl salinity:

Table (2) and Fig. (1) shows the effect of different sub-lethal concentrations of NaCl on survival rate of *D. magna* at the end of experimental, generally it was found that the survival rate decreased with increasing the cultured period and also with increasing the concentrations from 0‰ (control) to 2.66 ‰ (LC40). Groups of *D. magna* cultured in 0‰ showed the highest significant ($P < 0.01$) survival rate represented by 97% after 21 days. While the lowest significant survival rate ($P < 0.0001$) for those cultured in the highest concentration, 2.66‰ represented by 43% after 21 days. There were no significant differences between control and LC10 groups. Strong negative correlations was observed from the first 2 days of the experiment $r = -0.69$ and increased till the end of experiment to reach $r = -0.99$ at $P<0.005$.

Table (2): Effect of salinity as sodium chloride, NaCl on % survival, growth and reproduction rate of *Daphnia magna* cultured in static renewal system for 21 days.

NaCl addition to exposure medium (‰)	% survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day (mean±SD)	Mean length of adult females at 21st day in mm (mean±SD)	Average weight of adult females at 21st day (mg)	
					Wet weight	Dry weight
Control	97[a]	7	(40±24)[a]	(3.74±0.06)[a]	8.7	0.322
1.67LC10	90[ab]	9	(54±9)[b]	(3.73±0.08)[a]	8.3	0.375
1.86LC15	83[bc]	9	(49±6)[a]	(3.70±0.00)[a]	7.6	0.369
2.04LC20	77[c]	9	(49±7)[a]	(3.61±0.07)[b]	7.5	0.325
2.20LC25	70[c]	9	(48±8)[a]	(3.59±0.29)[b]	7.5	0.288
2.35LC30	60[d]	9	(45±9)[a]	(3.55±0.20)[b]	7.2	0.272
2.51LC35	50[ef]	9	(33±7)[c]	(3.42±0.13)[c]	6.4	0.230
2.66LC40	43[f]	9	(32±3)[c]	(3.38±0.07)[c]	6.2	0.220

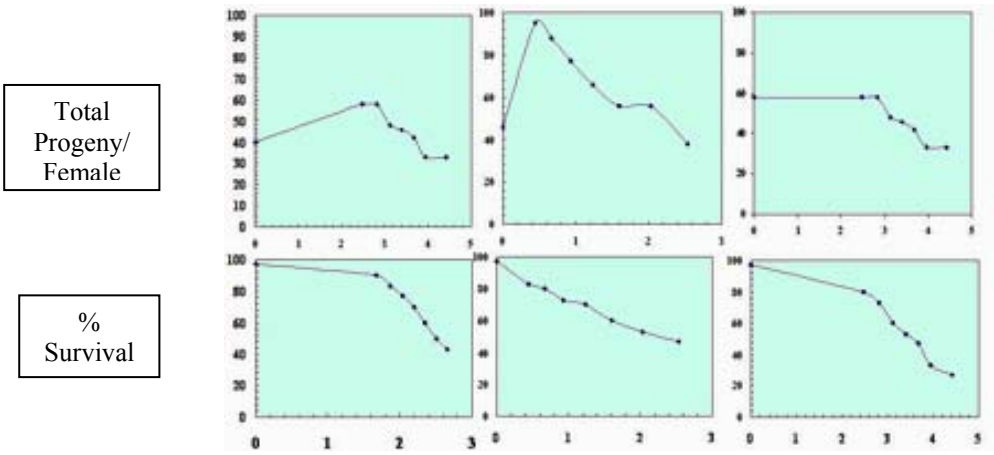
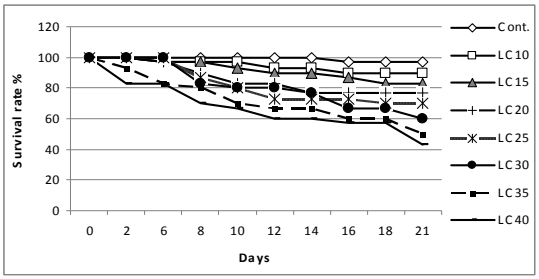
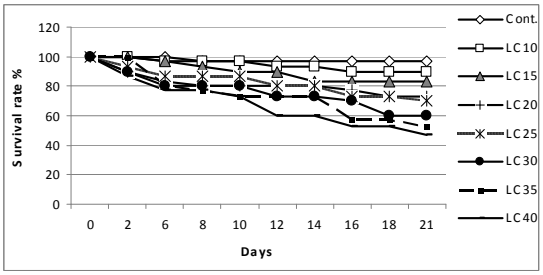


Fig. (1) Survival and Reproduction of *Daphnia magna* grown at different salinities at the 21st day

Table (2) and Figure (3) illustrate the effect of different concentrations of NaCl on growth rate of *D. magna* at the end of experiment. Generally it was found that the length of *D. magna* increased with increasing period of culture for all concentrations. At the end of experiment, control group (0‰ salinity) showed the highest significant (P<0.001) lengths which represented by 3.74mm. While the lowest significant (P<0.001) lengths were observed for group cultured at the highest NaCl concentrations (2.66 ‰) corresponding to LC 40, which represented by 3.38 mm after 21 days. There were no significant differences (P<0.001) between control group and `groups cultured in concentrations 1.67, 1.86 and 2.04‰ corresponding to LC10, LC15, LC20, respectively.



Synthetic Sea Water



Nature Sea Water

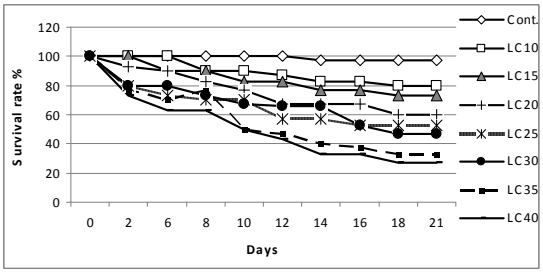


Figure (2): Effect of different concentrations of NaCl, synthetic sea water, and nature sea water on survival rate (mean±SE) of *D. magna* during the experimental periods (21 days).

Salinity affected reproduction of females during the experiments the number of progeny per female at 21 day for NaCl salinity (Table 2 and Figure 1) was highest (54 neonates/female) at 1.67‰ corresponding to LC10, but not differ significantly (P>0.01) than those reared on 1.86‰, 2.04‰, 2.20‰ and 2.35‰. The lowest number of progeny (32 neonates/female) was observed for those reared at the highest concentration (2.66‰) corresponding to LC40. So it was noticed that the number of progeny per female decreased as salinity increased. Arnér and Koivisto (1993) demonstrated that the freshwater Cladocera that successfully colonize brackish environments are smaller in size and have a reduced reproduction. Cowgill and Milazzo (1990, 1991) demonstrated that reproduction, population growth rate, and survival in *D. magna* decreased as NaCl concentration increased in the range of 0.08–6000 mgL^{-1}.

Effect of synthetic sea water salinity:

Effect of different salinity treatments of synthetic sea water on survival rate of *D. magna* during the experimental period is in Fig. (2). In general the survival rate decreased with increasing the culture periods and increasing the concentrations of salinity, the highest survival rate (97%) noticed for control groups, followed by those cultured in LC10. Also, survival decreased gradually as the concentration of salinity increased until the highest concentration (2.54‰) corresponding to LC40, where the survival rate was 47%. From the first two days data showed negative correlation r = -0.57 and increased as the experiment progress r = -0.93 at P<0.005 (Table 3 and Fig. 2).

From Figure (3), it is clear that the length of *D. magna* increased with increasing culture period for all concentrations. It was found that the highest length; 3.88 mm was observed for group reared in 0.44‰ (LC10) which differed significantly (p<0.001) than those reared in the other concentrations. While the lowest length was found for those reared at the highest concentration; 2.54‰ corresponding to LC40 represented by 3.28 mm.

Mean total progeny per female, over the experiment period ranged from 38 to 95 neonates/female .The maximum significant count (P<0.01) was at LC10, and represented by 95 neonates/female, which differ than the other concentrations even the control group. The age at first reproduction was 9 days for all treatments but was 7 days for control (Table 3 & Fig. 1).

Table (3): Effect of synthetic sea water salinity on % survival, growth and reproduction rate of *Daphnia magna* cultured in static- renewal system for 21 days.

Synthetic sea water addition to exposure medium (‰)	% survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day mean±SD)	Mean length of adult females at 21st day in mm (mean±SD)	Average weight of adult females at 21st day (mg)	
					Wet weight	Dry weight

Control	97[a]	7	(46±7)[a]	(3.35±0.27)[a]	7.8	0.288
0.44LC10	83[bc]	9	(95±9)[b]	(3.88±0.13)[b]	7.9	0.292
0.67LC15	80[c]	9	(88±7)[c]	(3.74±0.13)[c]	7.8	0.288
0.93LC20	73[c]	9	(77±7)[d]	(3.42±0.21)[a]	7.4	0.273
1.24LC25	70[c]	9	(66±8)[e]	(3.40±0.3)[a]	7.0	0.265
1.60LC30	60[d]	9	(56±9)[f]	(3.40±0.10)[a]	6.9	0.259
2.03LC35	53[de]	9	(56±7)[f]	(3.35±0.13)[a]	6.3	0.255
2.54LC40	47[e]	9	(38±6)[g]	(3.28±0.2)[a]	5.5	0.225

Such delays in the onset of reproduction have been observed for salt-stressed horseshoe crabs *Limulus polyphemus* (L.) (Ehlinger and Tankersley 2004), copepods of the species *Gladioferens imparipes* Thompson (Payne and Rippingale 2001) and cladocerans *Daphnia carinata* King (Hall and Burns 2002) and *D. magna* (Arner and Koivisto 1993). Salinity can cause both delayed maturity and a smaller size at first reproduction, as Teschner (1995) showed for *Daphnia magna*.

Effect of natural sea water salinity on *D. magna*:

For natural sea water treatments, the salinity could affect the survival rate of *D. magna* (Fig. 1). At the end of the experiment, the highest significant survival rate (P<0.0001) for control group (0‰), followed by those cultured in the lowest salinity, 2.48‰ (LC10) were represented by 97 and 80%, respectively. While the lowest significant survival rate (27‰) at (P<0.0001) was observed for those cultured in the highest salinity of 4.42‰ corresponding to LC40 (Table 4).

Figure (3) indicate the effect of different salinities of natural sea water on the growth rate of *D. magna* during the experimental period. It was found that the length of *D. magna* increased for all different concentrations with increasing culture period.

For control group (0‰ salinity) the daphnids lengths was 3.76 mm at the end of experiment which differed significantly (P<0.01) than the groups cultured in different concentrations except for group cultured in concentration 2.48 ‰ corresponding to LC10, it was found that length of *D. magna* not significantly differed (P>0.01) between control group and those cultured in 2.48‰ (LC10). While the lowest significant length (p<0.01) was observed for those cultured in the highest concentration represented by 3.23 mm (Table 4). The highest production of offspring per female at the end of experiment (58 neonates/female) were observed at 0, 2.48 and 2.82‰, which did not differ significantly (P>0.01). However, the production declined gradually with increasing salinity till reach the lowest significant value (P<0.01); 33 neonates/female at 4.42‰ (Table 4 and Fig. 1).

Thus the growth, survival and reproduction rate of the adult daphnids decreased in a high salinity environment. It is known that *D. magna* is a freshwater crustacean organism and it is adapted to low salinity condition, but on the other hand, it was found in a previous study (Ghazy, 2003) that a strain of *D. magna* could survive in salinities up to 62‰ in El-Imam El-Shaffie spring.

Table (4): Effect of natural sea water salinity on % survival, growth and reproduction rate of *Daphnia magna* cultured in static renewal system for 21 days.

Natural sea water addition to exposure medium (‰)	% survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day (mean±SD)	Mean length of adult females at 21st day in mm (mean±SD)	Average weight of adult females at 21st day (mg)	
					Wet weight	Dry weight
Control	97[a]	7	(58±2.8)[a]	(3.76±0.18)[a]	8.7	0.32
2.48LC10	80[b]	8	(58±3.0)[a]	(3.70±0.23)[ab]	8.1	0.30
2.82LC15	73[b]	8	(58±1.00)[a]	(3.69±0.25)[b]	7.5	0.28
3.12LC20	60[c]	8	(48±2.0)[b]	(3.52±0.22)[c]	7.2	0.28
3.40LC25	53[c]	8	(46±1.8)[b]	(3.48±0.25)[c]	7.0	0.26
3.68LC30	47[e]	8	(42±2.7)[b]	(3.36±0.05)[d]	6.9	0.25
3.95LC35	33[f]	8	(33±2.1)[c]	(3.35±0.11)[d]	6.6	0.23
4.42LC40	27[f]	8	(33±2.0)[c]	(3.23±0.07)[e]	6.1	0.23

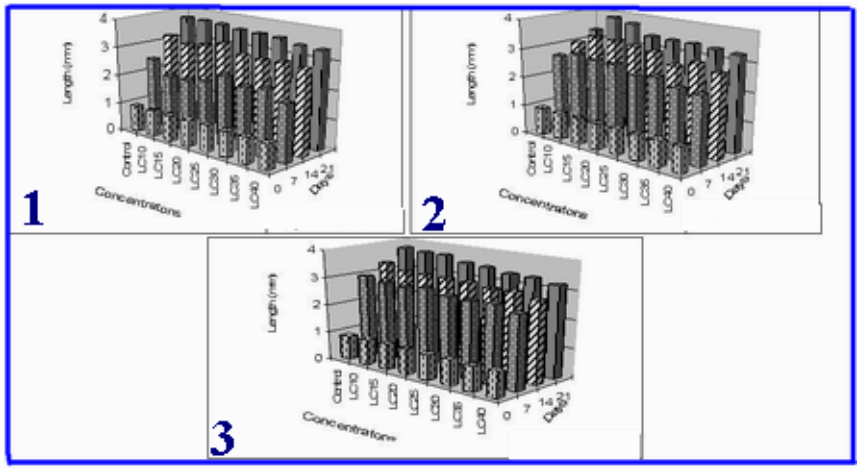


Figure (3): Effect of different concentrations of NaCl, synthetic sea water, and nature sea water on survival rate of *D.magna* during the experimental period (21 days), 1= Sodium Chloride, 2= Synthetic Sea Water, 3= Nature Sea Water

Comparing results in the three concentration ranges tested, we can conclude that the impairment effects on reproduction and survival under the current treatments were provoked by salinity stress. It has been demonstrated that various freshwater invertebrate species (including cladocerans) are more sensitive to NaCl salinity than to the effect produced by the array of chemical compounds present in sea salt (Kefford *et al*., 2004). In this study, *D. magna* is more sensitive to NaCl salinities than to that of synthetic- and natural sea waters. The present results might be useful for a better understanding of how salinity affected the survival, growth and reproductive responses of a freshwater *D. magna* strain. These data provide support for the use of this strain, based on this capability to endure brackish waters, as test organism in toxicity assays performed in slightly saline conditions (up to 2.99, 3.92 or 4.82‰ for the three salinities studied).

Casey *et al.* (2000) stated that the LC50 lethal salt concentration for *Daphnia magna* varies from 5491 to 5736 mg NaCl L-1).

Also, for *D. magna*, Schuytema *et al*. (1997) determined a median lethal concentration (LC50) at salinity concentration of 6.6 gL_1 (measured conductivity, 10.0mScm_1). Cowgill and Milazzo (1990) estimated an LC50 for NaCl at 6.034 gL_1, and pointed out that the ‘‘non observable effects level’’ (NOEL) for NaCl was approximately 1.2 gL_1. In the present study, 48h-LC50 was determined to occur at the NaCl concentration of 2.99‰, and was lower than previously reported values. This situation could be related to a greater sensitivity to salinity by the experimental strain we used, a plausible situation since this strain has been grown with outstanding reproductive results in freshwater conditions for more than 15 years. On the other hand, Arnér and Koivisto (1993) reported that *D. magna* grew in salinities of 4‰ and 8‰.

Kikuchi (1983) stated that the gills and digestive tracts of crustaceans are their basic osmoregulatory organs, with changes in salinity capable of modifying gill morphology in *Daphnia*, for example. These changes affect the so-called dark cells in particular, these being rich in mitochondria and possessed of an elaborate tubular system and modified cell membrane. It is probable that they play an important role in osmoregulation.

Aladin (1991) described round nuchal (neck) organs in *Daphnia magna* embryos, whose cytoplasm is seen to be very condensed and capable of intensive cellular absorption of salt on account of high permeability to ions. Adult *Daphnia* do not rely on such neck organs, relying instead on the absorption of salt with food.

Williams (1998) remarked that *D. magna* in slightly brackish waters has a narrow range of salt tolerance, whereas varieties found in highly saline lakes display a wide tolerance to this factor, as observed in *Moina hutchinsoni*, a cladoceran that flourishes in saline lakes in North America at up to 40 psu, but *M. hutchinsoni* can be grown under laboratory conditions at salinity values as low as 4 psu with similar results to those recorded at a higher salinity (Martínez-Jerónimo *et al*., 2004; Martínez- Jerónimo and Espinosa-Chávez, 2005).

Considering its tolerance to salinity, Green *et al*. (2005) established that *D. magna* is a euryhaline species that is particularly tolerant to salinity conditions in brackish lakes; nevertheless, they concluded that the reproductive and/or survival rates of cladocerans are reduced at higher water conductivities. In our study, we demonstrated that the freshwater strain we used has in fact a relatively small range of tolerance to studied salinities; nevertheless there was a strain of *D. magna* has been acclimated to thrive at the upper salinity values (Ghazy,2003).

It was found that two published examples of behavioral responses to salinity in freshwater crustaceans were studied. First, Baillieut and Blust (1999) found that high salinity level reduced swimming speed in *Daphnia magna*. Harder (1968) observed aggregation behavior in zooplankton responding to increased salinity but did not suggest any advantages in terms of fitness.

Grzesiuk and Mikulski (2006) stated that the effect of salinity can be modified by other abiotic factors, albeit with the pattern of these modifications varying. A strong interaction between effects of temperature and salinity on survival of *Daphnia magna* has been demonstrated, a high temperature compounding the harmful effect of the salinity (Casey *et al.* 2000). Even where it does not reduce lifespan, salinity may limit individuals' growth rates, with freshwater animals transferred to a brackish environment found to grow more slowly: as with *Daphnia carinata* (Hall and Burns 2002) and *D. magna* (Teschner 1995, Arner and Koivisto 1993).

Biochemical composition of *Daphnia magna*

The protein, lipid, ash and Moisture content of *D. magna* reared in 0‰ (control group) and 0.44‰ corresponding to LC10 synthetic sea water are given in Table (5).

Table (5): Biochemical composition of *Daphnia magna* reared in 0‰ (control) and 0.44‰ (LC10) synthetic sea water (g/100g wet weight)

Treatment	Control (0‰)	Synthetic sea water LC10(0.44‰)
Total protein	4.18±0.63	5.2±0.85
Total lipid	1.09±0.135	1.15±0.02
Moisture%	81±2.5	79±3.1
Ash%	8.7±1.22	8.8±1.5

Values are given as means ± SE for triplicate determination

Both groups of *D. magna* had high moisture (81% and 79%, respectively). Protein, lipid and Ash content slightly increased for *D. magna* reared in 0.44‰ than those reared in control group and are represented by 5.2(g/100g wet weight), 1.15(g/100g wet weight) and 8.8%, respectively. A similar results was obtained by Habashy (1998) suggesting that the composition of the same species (*D. magna*) was 6.9 (g/100g wet weight) for protein content, 0.91 (g/100g wet weight) for the total lipid, 8.9% Ash and 84.6% for moisture. In this respect, other studies recorded the protein content of *Daphnia carinata* and *Moina australiensis* is 54.34% and 64.80%, respectively (Kibria *et al*., 1999) and in *Daphnia* sp. it is reported to be 49.70% (Yurkowski and Tabachek, 1979; Watanabe *et al*., 1983), whereas for *Moina* it varies between 59% and 77.85% (Tay *et al*., 1991). The present investigation showed that protein content of both groups of *D. magna* were 4.18 and 5.2 g/100g wet weight which is lower than reported earlier (Tay *et al*., 1991) which may be due to analytical methods used.

On the other hand, these results differ from those of Das *et al*. (2007) who observed some variation in the moisture, total protein and a considerable variation in the lipid level between un- enriched and enriched *Moina*. The highest lipid content of 20.03% was observed for those enriched with cod liver oil emulsion and the difference between this study and our investigation may be due to the difference in the type of foods and enriched process (Watanabe *et al*., 1982; Leger *et al*., 1987). In this respect, Macedo and Pinto-Coelho (2001) observed that the lipid level of *Moina* varied from 11.4% to 19.9% and this due to feeding effect of different algal diets.

In addition, Mitra *et al*. (2007) recorded lipid content in mixed zooplankton from different ponds varied from 10.79 to 14.55% DM (dry matter) and were inversely related to water temperature. Watanabe *et al*. (1983) analysed various zooplankton; *Daphnia* containing 13% and *Moina* 12-27% lipids whereas in *D. carinata* and *Moina australiensis* it ranged from 7.29-7.73% (Kibria *et al*., 1999).

The amino acid profile of *Daphnia magna* reared in 0‰ (control group) and 0.44‰ synthetic sea water which corresponding to LC10, are shown in Table (6).

It was found that four essential amino acids were detected for *D. magna* reared in 0.44‰ synthetic sea water, while for control group only two essential amino acids (lysine and phenylalanine) were detected. Aspartic acid showed the highest value for both groups and represented by 32.06% for *D. magna* reared in 0.44‰ and 4.43% for control group. On the other hand, aspartic acid, tyrosin and cysteine constituted more than 50% of the total amino acids for group reared in 0.44‰ synthetic sea water. While phenylalanine represented the lowest value (0.974%) for control group and glutamic (0.591%) for those reared in 0.44‰ synthetic sea water. Tryptophan showed approximately similar values for both groups of *D. magna*. Other previous studies revealed that both *D. carinata* and *Moina australiensis* contained appreciable levels of both essential and non-essential amino acids for fish (Kibria *et al*., 1999). However, Yurkowski and Tabachek (1979) and Hepher (1988) reported lower values of some essential

amino acids for *Daphnia* and *Moina* sp.

Table (6): Amino acids composition of *Daphnia magna* (% relative concentration

	Amino acids	Control (0‰)	Synthetic sea water (0.44‰)
EAA**	Lysine acid	1.25±0.01	1.84±0.08
	Phenylalanin acid	0.974±0.003	1.785±0.05
	Leucine acid	ND*	0.6850±0.01
	Isoleucine acid	ND*	0.695±0.11
	Tryptophan acid	1.119±0.01	1.241±0.01
Non-EAA***	Tyrosin acid	ND*	6.985±0.52
	Aspartic acid	4.43±0.02	32.06±0.58
	Alanine acid	1.47±0.04	ND*
	Glutamic acid	ND*	0.591±0.06
	Glycine acid	ND*	1.31±0.18
	Cysteine acid	ND*	3.79±0.65
EAA, Non-EAA	Total AA	9.243	50.982
	∑ EAA	2.224	5.005
	∑ Non-EAA	7.019	45.977
	EAA/Non-EAA	0.32	0.11

* Not detected; **Essential amino acids; ***Non-essential amino acids;
Values are given as means ± SE for triplicate determinations

Table (7): Fatty acids composition of *Daphnia magna* (mg/100 gm)

	Fatty acids		Control		Synthetic sea water	
			mg/100 g	%	mg/100 g	%
SFA*	Lauric acid	12:0	1.203±0.12	0.89	1.265±0.03	0.91
	Myristic acid	14:0	2.122±0.01	1.58	2.231±0.02	1.6
	Palmitic acid	16:0	0.465±0.06	0.35	0.489±0.06	0.35
	Stearic acid	18:0	1.225±0.07	0.91	1.288±0.11	0.92
USFA**	Myristolic acid	14:1n-6	1.221±0.13	0.91	1.284±0.06	0.92
	Palmitoleic acid	16:1n-7	8.176±0.58	6.1	8.597±0.19	6.16
	Oleic acid	18:1n-9	4.857±0.47	3.63	5.107±0.58	3.66
	Linoleic acid	18:2n-6	64.457±0.59	48.12	67.778±0.58	48.57
	Linolenic acid	18:**3**n-**3**	0.488±0.01	0.36	0.514±0.06	0.37
	Arachidonic acid	20:4n-6	47.46±0.58	35.43	48.577±0.09	34.81
	Eicosapentaenoic acid (EPA)	20:5n-3	0.733±0.01	0.55	0.77±0.06	0.55
	Docosahexaeaenoic acid (DHA)	22:6n-3	1.293±0.06	0.97	1.36±0.06	0.97
	Erucic acid	22:1n-9	0.262±0.06	0.2	0.276±0.04	0.2
SFA, USFA	Total FA		133.962		139.536	
	∑ SFA		5.015		5.273	
	∑ UFA		128.947		134.263	
	∑ n-6		113.138	100	117.639	100
	∑ n-3		2.514		2.644	
	∑ PUFA		115.652		120.283	
	n-6/n-3		45.003		44.49	
	EPA/DHA		0.57		0.57	

* Saturated fatty acids; ** Unsaturated fatty acidsValues are given as means ± SE for triplicate determinations

On the other hand, Watanabe *et al*. (1983) reported that *Artemia* nauplii of different origin had different amino acids profiles. Mitra *et al*., (2007) detected all the ten essential amino acids with low level of methionine in mixed zooplankton samples collected from fertilized earthen ponds.

Moreover, fatty acids play a major role as an energy source, affect cellular membrane structure and function, are important for cell growth differentiation and metabolism, improve resistance to stress (starvation and osmotic shock) and regulate gene expression (Kamler *et al*., 2008)

The fatty acids composition of *Daphnia magna* cultured in 0‰ salinity and concentration 0.44‰ of synthetic sea water were shown in Table (7). noticed that myristic acid was the dominant saturated fatty acids followed by stearic acid and lauric acid for control group (0‰S) and those cultured in concentration 0.44‰S, whereas, palmitic acid was found in trace amount. It was found that *D. magna* in both control and those reared in 0.44‰ contain high level of unsaturated fatty acids linoleic acid; omega-6 (48.12% of total fatty acids for control group and 48.57% for those reared in 0.44‰S) and arachidonic acid (20:4n-6) 35.43% for control group and 34.81% for concentration 0.44‰S, this finding in agreement with that obtained by Aman and Altaff (2004) who stated that *Mesocyclop aspericomis* contain high level of these unsaturated fatty acids. Also, the present study is in agreement with Das *et al*. 2007 who reported that the linoleic acid in sunflower oil-enriched *Moina* is much higher than un-enriched *Moina*. It indicates the role of linoleic acid on the growth enhancement of *M. rosenbergii* (Guary *et al*., 1976; kanazawa *et al*., 1977b, 1979c and Read, 1981). Zooplankton contain high levels of arachidonic acid which help in the growth and survival of larvae of turbot fish as documented by Bell *et al*., (1995), Sargent *et al*.(1995) and Tidwell *et al*., (1997). Also in the present study, it was found that *Daphnia* in control and those reared in 0.44‰, not have adequate amount of eicosapentaenoic acid (EPA) (0.55‰ of total FAs) or docosahexaeaenoic acid (DHA) (0.97‰ of total FAs) were found in small amounts. This result was in agreement with that obtained by Lim *et al*. (2000) who found very small amounts of EPA (2.3 mg/g^{-1} dry weight) and DAA (0.2mg/g^{-1} dry weight) in *Artemia* and Das *et al* (2007) with *Moina*. From data presented total fatty acids were similar or slightly different for *D. magna* reared in concentration 0.44‰S (139.536mg/100gm) than those cultured in control (133.962mg/100gm); this similarity may be due to the similar food conditions as reported by Bengtson *et al*. (1991), who stated that the fatty acid composition of *Artemia* nauplii is considered to be more environmentally than genetically determined and reflect the fatty acid profile of the diet direct received by the original adult population (Lavens *et al*., 1989).

On the other hand Das *et al*. (2007) reported that the enrichment of *Moina mircrura* with different oils improve the eicosapentaenoic acid (EPA) and DHA which affect positively on growth and survival of *M. rosenbergii* larvae fed on it. *Daphnia* like *Moina* may be a beneficial alternative to *Artemia* especially in developing countries where imported *Artemia* are costly and sometimes scarce. *Daphnia* spp. is known to be suitable live food source for raising fish and prawn larvae (Masters, 1975; Rasawo and Radull, 1986; Habashy, 1998). However, Abdel Rahman (1996) proved that enrichment of *Artemia* with HPUFA greatly increase growth and survival of fish and shrimp larvae. Fujita *et al*. (1980) clarified that the high mortality observed in red sea bream culture is due to the lack of HUFA in *Artemia* nauplii given as a single food.

Like *Artemia*, *Moina* and *Daphnia* does not meet the requirement of the predator crustaceans with respect to EPA and DHA though it contains 60-70% protein (dry wet). This nutritional quality can be enhanced with HUFA (Das *et al*., 2007).

CONCLUSION

In conclusion, the salinity significantly influenced the growth, survival and reproduction rates of *D. magna* and 0.44‰ of synthetic sea water was the optimal salinity.

Reproduction and survival rates of *D. magna* which lived at the same salinity during study differ with different salinities. The influences of salinity on cumulative progeny per female differed. All rates were greater at LC10 than those at higher salinities. However, daphnids survival rate was best at 0‰ followed by LC10 for the three saline waters studied and decreased gradually with increasing salinities. This study indicated that reproduction of daphnids was favored at the low salinity and survival was favored in control.

The salinity could influence to some extent the time of first reproduction but increased the number of progeny per female at low salinities.

It can be concluded that the best reproduction of *D. magna* occurs at salinity values lower than 3‰.

D. magna like *Moina* can be used as a substitution of *Artemia* in aquaculture. This is because it contains suitable levels of protein, amino acids and unsaturated fatty acids (USFA), and we recommend that its nutritive value can be enhanced by enrichment processes. USFA have been found to be critical for maintaining high growth, survival and reproduction rates and high food conversion

efficiencies for a wide variety of marine and freshwater organisms.

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3/9/2009

Influence of Drying Methods, Extraction Time, and Organ Type on Essential Oil Content of Rosemary (*Rosmarinus officinalis* L.)

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Abstract: Rosemary (*Rosmarinus officinalis* L.) is belonging to *Lamiaceae* family which grows wild in most Mediterranean countries. In order to investigate the influence of different methods of drying, extraction time, and type of organ on the essential oil percentage of this plant an experiment was carried out in college of agriculture of Karaj during the year 2009. Three drying methods investigated were oven drying (45ºC), shade drying and sun drying. Four extraction times were: 1, 2, 3, 4 hours and three organ type were leaf, stem, mixed leaf and stem. Essential oil was obtained by water distillation method. Results showed that effect of drying methods, extraction time, and organ type on the essential oil percentage were significant. The maximum essential oil percentage (1.8%) was obtained to leaf sample, 3h of extraction, and shade drying. While the minimum essential oil percentage (0.12%) was obtained to stem sample, 1h of extraction, and oven drying. [Nature and Science. 2009;7(11):42-44]. (ISSN: 1545-0740).

Key words: *Rosmarinus officinalis* L.; Drying Methods; Extraction; Essential oil

1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity (Wang et al, 2008., Peng et al, 2005., Oluwatuyi et al, 2004). Rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent. Major constituents described for the oil are α-pinene, 1, 8-cineole and camphor. The post-harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold. Drying has been one of the most important processes in pre-processing of agricultural products. Aromatic plants are often dried before extraction to reduce moisture content. The aim of drying is to reduce the moisture content of the product from actively growing in the field to a level that prevents deterioration of the product and allows storage in a stable condition. Proper drying of medicinal plants is fundamental to the achievement of a high quality product. A literature search was undertaken on effects of different methods of drying on essential oil content and chemical composition of the essential oil plants. The results showed that drying method had a significant effect on oil content and composition of aromatic plants (Okoh et al, 2008., Asekun et al, 2006., Ahmadi et al, 2008., Ronicely et al, 2008., Diana et al, 2008). Also duration of essential oil extraction affected on the quantity and quality of essential oil. Jamshidi et al. (2004) reported that essential oil percentage and essential oil component of fennel were affected by duration of essential oil extraction. The objective of this study was to evaluate the influence of drying methods, extraction time, and type of organ on essential oil content of *Rosmarinus officinalis* L.

2. Materials and Methods

Rosmarinus officinalis L. were collected in college of agriculture of Karaj in during the year 2009 (longitude: 50° 59´E, Latitude: 35° 47´N, height of sea level (m): 1312/5, average annual rainfall (mm): 230, and mean annual temperature: 14.3 °C). The fresh plant materials were carefully separated into leaves, stems, and mixed of stems and leaves. Three drying methods investigated were oven drying, shade drying and sun drying. One of samples (100gr) was dried at room temperature and shade for 5 days. One of samples (100gr) was dried at sun for 3 days and other sample (100gr) was dried at oven (45ºC) for 24 hours. This work performed for each sample with three replications. In order to extraction of essential oil, 100gr from each sample powdered and then essential oil isolated by water distillation for 1, 2, 3, 4 hour and to three replications. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and calculated average of essential oil yield for three replications.

3. Results and Discussion

Results showed that drying methods, extraction time, and type of organ had a significant effect on the essential oil content of Rosemary. The maximum essential oil percentage (1/8%) was obtained in leaf,

shade drying, and 3h. The minimum essential oil percentage (0.12%) was obtained in stem, owen drying, and 1h. The samples that dried in shade had the higher essential oil as compared to samples that dried in sun and owen. Also leaf samples as compared to stem and mixed leaf and stem had the higher essential oil (Table 1). The oil content of shade-dried *Roman chamomil* flowers was found to be larger (1.9% w/w) than there of sun-dried (0.4%) and oven-dried at 40 ºC (0.9%) (Omidbaigi et al, 2004). The drying method also had a significant effect on the proportion of the various components. It was also reported that the chemical composition, physical properties and antioxidant activities of yam flours were affected by drying methods to different extents. Yuan Zhang and Zhezhi Wang (2007) reported that in *Glechoma longituba* different drying methods caused some variation of the relative proportions of the components and the higher amount of germacrene D (19.0%) was obtained by shade-drying. Only sun drying brought about significant losses of the major compounds (a-cadinol, germacrene B, germacrene D-4-ol, and a-caryophyllene) in the essential oil when compared to the fresh plant material. Yuan Zhang and Zhezhi Wang showed that it could be concluded that drying of leaves of *G. longituba* under normal air and at room temperature conditions is most suitable for a high-percentage of sesquiterpene, especially for germacrene D, but the oven-drying and silica gel-drying method are recommended for fast drying and similar components compared to the fresh plant material. Fatemeh Sefidkon et al. (2006) reported that the highest essential oil content of *Satureja hortensis* (1.06%) was obtained to owen- dried sample and the lowest essential oil content (0.87%) was obtained to sun- dried sample. In the *Mentha longifolia* L. only oven drying brought about significant losses of the major compounds (menthone, pulegone and 1,8-cineole) in the essential oil when compared to the fresh plant material (Asekun et al, 2007). The maximum essential oil percentage was obtained at 3h duration of extraction and with increasing duration of extraction not observed increasing in essential oil percentage. Barazandeh, M.M. (2005) reported that duration of essential oil extraction had significant effect on essential oil content in *Eucalyptus globules* and essential oil percentage was increased with the increasing of duration of extraction. Jamshidi et al. (2004) suggested that in *Foeniculum vulgare* Mill the highest essential oil percentage was obtained in 2.5hour.

Table 1. Effect of drying methods, extraction time, and type of organ on the essential oil content of *Rosmarinus officinalis* L.

Time (hour)	Shade drying			Sun drying			Owen drying		
	Leaf	Stem	Mixed leaf and stem	Leaf	Stem	Mixed leaf and stem	Leaf	Stem	Mixed leaf and stem
1	0.9	0.21	0.7	0.8	0.19	0.5	0.7	0.12	0.4
2	1.3	0.3	0.9	1.2	0.26	0.7	1.1	0.22	0.7
3	1.8	0.45	1.3	1.62	0.35	1.1	1.5	0.3	0.9
4	1.8	0.46	1.3	1.63	0.36	1.1	1.5	0.3	0.9

4. Conclusions

Rosmarinus officinalis L. is one of the most interesting research plants because it is between medicinal and aromatic plant. The post harvesting process (drying methods and extraction time) have a great importance in the production of essential oil and influenced on the quantity and quality of essential oil. Generally results showed that by shade drying, leaf samples and 3 hour a highest of essential oil percentage was obtained.

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8/10/2009

Crop Protection Problems in Production of Maize and Guinea Corn in Northern Guinea Savanna of Nigeria and Control Measures

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Abstract: The cultivation of Maize and Guinea corn in the northern Guinea Savanna of Nigeria is faced with lots of Crop protection problems which hinder full scale production of these crops in that ecological zone. The problems range from biotic factors like vertebrate and invertebrate pests, disease pathogens, nematode and weeds, to abiotic factors such as nutrient deficiencies, environmental conditions (climatic, edaphic), and agronomic, logistic or social problems. Addressing the various problems militating against the production of maize and Guinea corn in this zone will further help strengthen the national food reserve base and alleviate the devastating effects of the global food crisis particularly in Nigeria. [Nature and Science. 2009;7(11):45-51]. (ISSN: 1545-0740).

Key words: Maize, Guinea-corn, biotic, abiotic, problems, control.

Introduction

Maize (*Zea mays* L.) and Guinea corn (*Sorghum bicolor* (L) Moench) are important food crops in Nigeria, widely grown in the savanna regions of the country. These crops form the staple foods for most of the population especially in areas adaptable for their production. Green maize (fresh grains) is eaten roasted or boiled on the cob. The ripe grains (of maize or sorghum) are cooked in combination with pulses or milled and boiled as porridge (Yoruba = Eko, Hausa = Kamu, Ibo = Akamu). Sorghum (Guinea Corn) uses vary from drinks to 'tuwo'. The stems are used for fuel and building of fences and local huts. Maize and guinea corn are used as basal ingredients of livestock feeds. They are rich in Carbohydrates. In spite of the importance of these cereals as sources of food for human consumption, their production is concentrated in the hands of peasant farmers whose average hectarage is very small, approximately 0.5 – 1.0 hectare per farmer. The technologies are basically traditional farming methods and systems.

An estimated one million hectares of land was planted to maize in the country in 1989/1990 and over 40% of this was cultivated in the northern states (NAERLS, 1982). This figure has been increasing steadily ever since, with the help of irrigation especially in the drier parts of the north (Sahel and Sudan). Average yield per hectare in the northern savannas on peasant farms is about 0.6 metric tonnes, while commercial farms average is about 2.0 metric tonnes/ha. Guinea corn, on the other hand, is grown in an estimated 300,000 hectares of land north of the Niger and Benue rivers, especially in areas generally too dry for consistent and reliable maize production. Average yield in both peasant farms and commercial setup is 0.40 metric tonnes/ha and 1.0 metric tonnes/ha respectively. Varieties of these cereals (maize and sorghum) planted in these areas are both local, improved local and hybrids. Plant breeders in I.A.R. (Institute for Agricultural Research, Ahmadu Bello University, Zaria) have produced suitable varieties adapted to different ecological zones of the savanna where the crops are grown. Suitable yields of the improved crops have also been packaged. However, a number of constraints (crop protection problems) militate against the production of those crops. These are discussed below and solutions proffered on identified problems.

Crop Protection Problems

Crop protection problems refer to all the biotic and abiotic factors which impede our quest to achieve self sufficiency in food production. These problems are common to both maize and sorghum in the savanna areas even though their importance may vary greatly. Some problems are confined to a single zone, others are generalized.

1. Biotic Factors

About 6 percent of the total food currently produced in this country is lost to pests and diseases. Maize and Sorghum are susceptible to various pests (vertebrate and invertebrate) and diseases (bacterial, fungal, viral, nematode infections) in different ecological zones of the northern savanna.

Pests

Vertebrate Pests

Samaru **(**Lat. $11^0\,11^{11}$N, Long. $07^0 38^{11}$E) is in the Savanna region which consists of derived Savanna (referred to as northern and southern Guinea zones),

Sudan and Sahel ecological zones. These zones are hosts to various species of monkeys, birds, rodents and other wild animals which cause extensive damage to maize and sorghum fields.

Monkeys moving in groups can cause up to 70% loss in peasant's maize fields and about 30% loss in commercial farms if their activities are not checked (Amadi, 1988 personal communication). Damage is done early in the morning between 6.00 am and 9.00 am; and between 4.30 pm and 6.30 pm. Succulent maize cobs are removed from the stalks and eaten up while excess harvest are littered on the fields and along bush paths. Monkeys menace on the farms can be checked by trapping and employment of hunters to track down the animals. Widening of farm paths to allow for regular traffic and placement of scare-crows in strategic areas in the farm may also be helpful.

Birds especially *Quelea quelea* and Doves cause great damage to Sorghum heads. Adult Quelea birds may not be as destructive as the young ones newly weaned. The adults feed on wild seeds of grasses until exhausted before raiding cereal fields. Damage done by the newly independent young Quelea (from 3 weeks of age) arises from extreme hunger, since their parents no longer feed them. The young Queleas are extremely persistent in their attack and may continue feeding even when the Sorghum stem they are on is shaken by hand. They may also pay a deaf ear to shouts or sounds produced by farmers to scare them away. Quelea birds can be controlled by using flame-throwers, explosives or aerial spraying of organo-phosphorus pesticides. The use of resistant varieties of Sorghum (those with hard kernels and more tannin content e.g. red types) may prove helpful in some localities.

Rodents particularly rats and grass cutters cause extensive damage to both maize and sorghum in the northern and southern Guinea Savannas. Rats and bush fowls attack newly sown seeds and young germinating seedlings causing wide gaps in crop rows. These gaps when extensive cause severe yield reduction and necessitate supplying to fill the gaps at extra costs. Seed dressing chemicals such as Apron Star, Apron plus etc, should be used to treat seeds before sowing to control these pests. Rats and grass cutters may cause damage on maize grains on the field. Rats climb up the stalk, reaching the cobs and feed on the grains while grass cutters cut the stem a few centimeters from the ground, subjecting the stalk to lodging. They later feed on the falling immature cobs. Rats also feed on stored grains of maize and sorghum thereby reducing its quality and quantity. Control of rats is by using bait poisons (both in the field and store) and by fumigation with phostoxin tablets during storage.

Invertebrate Pests

These include all arthropod insects, molluscs, etc. which attack maize and sorghum plants inflicting heavy losses to the farmers. Different stages of the plant growth (e.g. seedling, vegetative, flowering and heading) are susceptible and various parts (e.g. roots, stems, leaves, flowers and grains) are attacked resulting in colossal losses. Termites and mole crickets destroy seeds in the soil causing wide gaps within the crop rows and poor crop establishment. The roots of seedlings and mature plants may be attacked by termites resulting in extensive damage to the cereals. Control is by seed dressing chemicals and use of Dieldren sprayers on the habitat of termites.

The major insect pest problems on cereals in the field are the stem borers, (*Busseola fusca* and *Sesemia calamistis*); shoot flies (*Atherigona* spp); grasshoppers (*Zonocerus variegatus*) and army worms (*Spodoptera exempta* and *Helicoverpa armigera*). The stem borer attack is usually more serious in late maize than the early ones. These borers feed inside the plant stems and are well protected from both their natural parasites and insecticides. They cause two types of damage to the plants. First, is mechanical damage due to consistent feeding in the stem, weakening it, and thus rendering the stems susceptible to lodging (stem breaking or falling down) and withering (dead heart). Secondly, stem borers may cause characteristic perforations or windows on leaves called 'fenestrations' seen when the sheath opens exposing the perforations (NAERLS, 1982). This type of damage reduces the photosynthetic area of the leaves resulting in poor cereal yield, especially during high infestation. Stem borers can be controlled economically by cultural methods. This involves removal and destruction of infested plants and plant residues. Pesticides with contact and systemic action are very effective at the initial stage of infestation to get rid of the larvae before burrowing into the stems.

Similarly, *Sessemia calamistis* a polyphagous insect most associated with young seedlings can cause extensive tunneling of adult plants stems resulting in 'dead' heart and chaffy heads in sorghum. Control is similar to *B. fusca. Zonocerus variegatus* when occurring gregariously causes extensive defoliation of cereals. Spraying with Fernithrothion 50 EC, Endosulfan (granules) or Trichlorphon (granules) can effectively check its menace on the field. Other grasshoppers attacking maize and sorghum albeit sporadically include *Locusta migratoria* L., *Schistocerca gregaria* L. (desert locust) and *Oedaleus* spp. All these are gregarious pests which can stripe the plants of their vegetation leaving the stalk bare. Control is similar to that of *Zonocerus* spp.

Army worms, *Spodoptera* spp and *Helicoverpa armigera* occur sporadically but may destroy the crops completely. The larvae are gregarious during outbreak and they feed for about three weeks. Outbreak is associated with alternating wet and dry spells (Misari, 1993 personal communication). These worms cause severe yield reduction on cereals by feeding on developing grains cutting them into smaller bits. Deep ploughing immediately after the season's harvest exposes the pupae to direct sun-rays resulting in desiccation of the pupae. Chemical control using Uppercott ® (Cypermethrin + Dimethoate) gives a good control.

Sorghum shoot fly, *Atherigona socata* Rondani attacks young seedlings as soon as the plants emerge from the soil and can last for about six weeks. The larvae feed on the central bud of young shoot, causing the death of the growing points ('dead heart' effect). Fenithrothion 50 EC at the rate of two litres per hectare can be applied for control. Sorghum midge, *Contarina sorghicola* lays its eggs on flowering heads and on hatching, the larvae feed on developing ovaries. Control can be achieved by prompt spraying of the sorghum heads as soon as the pests are detected with a good insecticide.

Beside field pests, maize and sorghum are seriously attacked by storage pests. The most important storage pests include grain weevils (*Sitophilus zeamays*) and *Rhizopertha dominica* for maize crops; *Tribolium casteanum* or *T. confucium*, *Trogoderma* spp, *Sitotroga cerealella* and *Sitophilus* spp for sorghum. In some cases, infestation takes place on the field and continues in the store. Some others are confined to the store while infestation may be by insects already present where the cereal grains had previously been stored or by crops infestation between granaries during storage.

For control of storage pests, strict adherence to hygiene in the store as well as provision of air-tight cover is essential. Mixing or storing old grains with new ones during storage should be discouraged. Cereals stored for seed or consumption beyond one month should be fumigated with phostoxin or treated with Actellic e.c.

Diseases

Diseases play an important role in the reduction of the potential yield of cereal crops. Agents causing diseases include bacteria, fungi, viruses, nematodes, weeds and nutrient deficiencies. The geographical distribution of cereal diseases in the savanna ecological zones is influenced by temperatures (high/low), moisture (humidity), cultural practices and the type and diversity of germplasm used.

i) Pathogen Problems

In a survey for incidence and severity of diseases in both the northern and southern guinea savanna of Nigeria, Adeoti (1992) reported the occurrence of the common foliar diseases such as the rust, *Turcicum* blight, *Curvularia* leaf spot and *Maydis* blight induced by *Puccinia* spp; *Helminthosporium turcicum*; *Curvularia* spp and *H. maydis* in the order of severity. The 'Pokkha boeng' disease induced by *Fusarium moniliforme* was also found to be severe in many areas where it occurs (Adeoti, 1992) and the percentage yield loss ranges between 5 and 30%. Other important maize diseases occurring in the savanna ecological zones include smut (*Ustilago maydis*), Downy mildews, Maize leaf fleck and Maize streak. Similarly, some rusts, smuts and blight diseases have been recorded on Sorghum plants. These include common rust (*Puccinia graminis*) f.sp. *Sorghii*, loose smut (*Sphacelotheca cruenta*), Cover smut (*Sphacelotheca sorghii)* and Head smut (*Sporisorium reiliana*) (Adeoti, personal communication).

Control of most fungal, viral and bacterial diseases of maize and sorghum can be by the use of resistant varieties, seed dressing with Furadan or Apron plus; elimination of alternate host (for rusts); crop rotation, removal and burning of infected plants and spraying with systemic fungicides such as a mixture of Benomyl and Dithane M45, Delsene, Rovrus (for 'Pokkha Boeng' disease) and so on.

ii) Nematode Problems

Several species of nematode have been reportedly associated with both soil and root of sorghum and maize in the savanna ecological zones. These species include *Pratylenchus* spp, *Aphelenchoides* spp, *Tylenchus* spp, *Helicotylenchus* spp, *Ditylenchus* spp and *Scutellonema* spp (Chindo, 1991 personal communication). Infected plants fall down from the root level and on examination, the plant roots are shortened, tiller profusely with round stubs at the tips. Control of nematodes is achieved by the use of Furadan 3G and other fumigant nematicides e.g. Ethylene Di-bromide (EDB), Dichloropropenes (Telone) and Dichromochloropropane (Nemagon). Manufacturer's recommendations should be adhered to for effectiveness.

iii) Weeds

Weeds constitute a special class of pests which seriously limit the production of the major crops on any scale. They compete with the crops for nutrients, air, light and moisture. The most noxious of these weeds are the parasitic ones particularly striga spp (known as witch weed and "wuta wuta" or "kuduji"

in Hausa). There is still no "universally applicable" and most effective control for striga despite several years of research in Nigeria. However, some inexpensive control measures including crop rotation, the use of tolerant varieties, generous fertilizer application and hand pulling before flowering can be applied to ensure satisfactory crop yield.

Other 'stubborn' weeds which also reduce cereals yield in the savanna include *Rottboellia* spp, *Pennisetum purpureum*, *Cyperus* spp, *Dactylon* spp and some broad leaved plants (compositae). Stomp, Round up, Fusilade and 2, 4-D respectively offer good control of these weeds. Hand weeding is effective but must be timely and repeated thrice before the crops mature to ensure economic yield. Since most cereals are shallow-rooted, it is essential to ensure that no mechanical damage is done to the crops roots during hand weeding. In erosion prone sites, earthing up or remolding of ridges may be required to prevent excessive exposure of the roots to the sun.

iv) Nutrient Deficiencies

Maize and sorghum are high nutrients demanding crops than other cereals (rice, millet and wheat). These crops require both the major nutrients (N, P and K) and the secondary nutrients (S, Mg, Ca, B, Fe, Cl, Cu etc.) in adequate amount to ensure good root establishment, vigorous and healthy growth and increased yields. Healthy seedlings and plants are less susceptible to pests and disease attack. Deficiencies of vital nutrients cause yield reductions through poor plant development and growth, thereby, predisposing the plants to pests and disease attack.

Plant nutrients are supplied as fertilizer formulations. The demand for fertilizers in Nigeria has increased in recent years forcing the Federal Government to remove fertilizer subsidies to the Nigerian farmers. The result is escalating prices of the product making it difficult for peasant farmers to purchase enough for their crop needs. Consequently, most field crops especially maize and sorghum planted all over the savanna ecological zones exhibit symptoms of nutrient deficiencies such as chlorosis, stunted growth, poor root development, early leaf fall, delayed flower opening, hasty maturity, improper setting of grains, poor resistance to disease agents and low yield. Since the prices of different brands are prohibitive, the Federal government may reconsider its stand on fertilizer subsidy in order to encourage farmers to produce more food crops. Availability of these fertilizers at the right time is also essential.

2. Abiotic Factors

Crop protection problems in the savannas can be precipitated by various abiotic factors including climatic, edaphic, agronomic, logistic and social contributors.

Climatic Problems

The areas north of Niger and Benue rivers can be classified as mainly savannas. The savanna consists of the southern and northern Guinea zones, Sudan and Sahel zones. The main characteristics of these ecological zones include poor rainfall (distribution and quantity), high temperatures, humidity, drought, high wind velocity and harmattan, etc. In the last ten years, the onset, distribution and even total amount of rainfall in the savanna zones have been erratic, resulting in crops failure (NAERLS, 1982). Maize crop is more water demanding than sorghum and the uncertainty in rainfall pattern and distribution affected the crop severely. If drought occurs at the time of silking, the result is poor pollination and serious loss of grain, even when the plants look well grown and healthy.

The Sahel zone (Katsina, Sokoto, Maiduguri, Kano, Potiskum, Nguru etc.) are particularly vulnerable to this problem where average maize yield on rainfed crop is below 400kg/ha as compared to national average ranging between 1000kg/ha and 2500kg/ha. Similarly, sorghum crop though tolerant to drought may be susceptible to drought during the reproductive growth stage. Late season drought causes sorghum midge and head bug outbreak.

Rainfall shortages and drought can be solved by constructing more dams for irrigation. Breeding of drought tolerant /resistant varieties of maize and sorghum as well as closer rows may reduce soil moisture loss at the end of the season. High humidity, day length and high wind velocity affect maize and sorghum yields. High humidity encourages pests and diseases attack; short day-length affects the photoperiod requirement of maize (usually about 12 hours) for high yield; while heavy wind causes lodging especially on tall local varieties as well as facilitating pests and diseases movement.

These problems can be ameliorated by planting resistant varieties and adopting relevant agronomic practices. Wind breaks/shelter belts may be established in strategic locations to check wind movement.

Edaphic Factors

Soils in the savanna parts of the country consist of sandy loam, clayey-loam and loess (wind deposited sand). Organic matter contents are generally low (< 0.5%) and plant nutrients are critically low. In some places, soil water availability is very critical and in some others water logging is

common-place (e.g. Fadama). Soils of the savanna are generally alkaline in nature but in some cases, soils with low pH values have been reported (UAC Agro, 1989 unpublished). Erosion due to wind and running water also create problems in some localities. These edaphic factors constitute an impediment to crop production in the savannas and can be remedied by various soil amelioration processes. These include application of cow dung, poultry droppings, farm yard manure, and leaf dropping of shelter trees (to improve the organic matter content and improve the physical properties of soil). Wind erosion can be checked by establishment of shelter belts in wind prone areas (Kano, Sokoto, Daura, etc.). Erosion due to running water (flood) can be checked through construction of water channels (gutters); embankments and levees; encouraging vegetation cover in susceptible areas. Soils with low pH can be reclaimed through liming to improve its nutrient availability to the crops.

Agronomic Factors

Various agronomic or cultural practices may predispose crops to attack by pests and diseases, in the following ways:

(1) Sowing dates influence grain yield through number, head weight and length of total growth cycle. Ogunlela (1985) reported a marked reduction in grain yield when sowing of photosensitive variety of sorghum (L.187) was delayed beyond June at Samaru than at Mokwa. A major cause for sorghum failure under delayed sowing was shoot fly (*A. soccata*) attack. Adapted sorghum varieties should not be sown later than late June in the northern Guinea savanna to ensure good yield; and a little later in the southern Guinea savanna (Ogunlela, 1985). However, early sowing for early maturing grain varieties causes crop to mature during the rains leading to the problem of grain mould. Similarly, late sowing for late maturing varieties runs the risk of drought or early cessation of rains (Ogunlela, 1985).

(2) Planting depth also affects incidence of pests and diseases. Deep planting causes the seed to rot while shallow planting subjects the seed to predation by birds, rodents, termites; and may weaken the roots of seedlings. Solution to this problem is to plant at the recommended depth, usually between 2.5cm and 4.5cm on ridges or flat.

(3) Crops grown by hand labour and using wider row spacing encourage pests and diseases attack. High nitrogen predisposes crop to disease and lodging. Continuous cropping (monoculture) of these cereals throughout the year permits the maintenance of a high inoculum potential. Similarly, the practice of leaving maize in the field long after maturity tends to increase losses from ear rots, stalk rots and even pilfering.

(4) Seed Bed Preparation - Poorly prepared seed bed encourages shallow rooting, poor seed establishment, lodging and wilt due to soil water unavailability to plants. Good seed bed preparation is therefore essential to ensure good crop establishment and high yield. Deep plowing, harrowing and ridging facilitate water penetration, exposes eggs and diapausing pupae of pests to desiccation by the sun and ensures weed and erosion control in the field.

(5) Removal of crop residue - Maize and sorghum stalks left over on the farm after harvest is a source of pest and disease attack next planting season. Their removal and burning will ensure protection of crops from this source of infestation. Guinea corn stalk used for fencing or building should be properly dried in the sun before use.

(6) Crop density and close spacing-This may be used to reduce pest infestation on the field by denying insect pests the opportunity to make soil contact during their life cycles due to extensive canopy cover.

(7) Farming systems - In some areas, farmers plant sorghum, groundnut and okra; maize, cowpea and pepper; or maize-soyabean in the same parcel of land in a single growing season. Each crop combination requires different agronomic practices, nutrients, sowing and harvesting periods. For a successful handling of a combination of crops, understanding of the farming systems is very essential. Invariably, this becomes a source of worry to the farmer due to poor planning and execution of work plans for each crop in the combination to ensure good yield. Crops with similar pests and diseases (e.g. sorghum and maize) should not be planted in a crop combination in order to avoid the perpetuation of their common pests.

(8) Timeliness is very important in crop protection programmes; a little delay can jeopardize efforts and render completely unprofitable all that have been incorporated into the farming enterprise. For instance, stem borer is an insect pest of economic importance on cereal crops. Larvae of these insects are found in the whorls feeding on the young unexpanded leaves and later bore into the stem. Control programme for these insect pests should be directed at the larvae while feeding in the whorls. If spraying is delayed and the larvae have bored into the stem, the use of contact insecticides to control the insects at this stage is no longer feasible (Amatobi *et al*., 1988).

(9) Similarly, some insect pests that attack produce in storage usually commence infestation while still in the field. These fields to storage pests (e.g. *Sitophilus* spp on maize and sorghum) may

cause extensive damage to stored produce if harvesting is delayed on the field. Timely harvesting of these crops is recommended to avoid further yield losses during storage.

(10) Pesticides are chemical formulations for the prevention and control of crop pests and diseases. Pesticides include herbicides, insecticides, fungicides, bactericides, nematicides, various protectants and growth regulators. A clear understanding of the mode of action is essential to ensure effective use. However, most Nigerian farmers seldom understand what pesticides to apply or look for to solve a specific field problem. Invariably, these farmers fall easy prey to Agro-chemical hawkers who are more interested in making money than solving the farmers' problems. Even when the correct pesticide is purchased by the farmer, the pesticide may not be effective due to staleness, late application after significant damage has been done to the crop; poor follow up of the recommended application schedule, incorrect method of application, sole reliance on pesticides in situations where other methods are more effective and fear of toxic effects on crops and man (Srivastava, 1974). Another constraint to pesticide use in the northern savanna is the lack of large amount of water required for conventional application. Even when water is available, cost of labour in carrying and applying large quantities of water can be quite high. The above problems can be solved by training and posting more extension workers to the villages to assist farmers solve their crop protection problems. Water shortages can be checked by digging wells or sinking bore-holes in the farms and by formulation of more ultra low volume (ULV) pesticides with hand sprayers.

(11) Use of Resistant varieties - Farmers in the northern savanna seldom plant improved resistant varieties of maize and sorghum due to its high demand for fertilizers and good management practices before high yield is guaranteed. Again, local preferences (for example use of tall sorghum variety stems for fencing/building; or preference for red coloured to white sorghum) may restrict the adoption of resistant varieties in spite of its attendant benefits. Solution to this problem lies in effective extension services and mass literacy campaign to change farmer's orientation. Cost of purchase of resistant crop varieties must be farmer pocket- friendly

Logistic Problems in Crop Protection

Farmers in northern Nigeria seldom plan for their crop protection needs. Their pre-occupation is to clear and till the land, plant the seeds, apply fertilizers, remove unwanted vegetation and harvest the crop. This sequence has exposed a major problem inherent in our farm management system, at least, at the peasant farmer's level. It is only when crop protection problems surface (albeit when much harm have been done to the crop) that a 'fire brigade approach' to control pest is initiated. Crop protection problems such as pests, diseases and early cessation of rains should be anticipated and planned for, to ensure success.

Logistic problems have been recognized as a major hindrance to a virile and effective crop protection programme. For instance, farmers may recognize a problem situation in the field but may not understand its causes. This calls for training of more extension workers and sending them to the rural suburbs to assist farmers. Difficult field problems should be communicated to the research scientists for solution. Unfortunately, the number of trained extension workers is inadequate and ill-equipped. Most extension workers prefer living in the urban areas than staying in the rural areas where pipe-borne water and electricity are lacking. This situation can be reversed by training two extension workers from each village in northern Nigeria who will live among the rural people to render extension services to them.

Another logistic problem in crop protection is lack of monitoring group and ineffectiveness of plant quarantine and sanitation programmes in the country. To this end, it is suggested that the state governments in the northern savanna should as a matter of necessity establish pests and disease monitoring groups as well as effective plant quarantine laboratory in each state capital. At the national level, a system of quick response (within 48 hours) to a reported case of outbreak of pests and diseases should be adopted to salvage crops and prevent total failure and great loss to farmers.

Social Problem

Plant protection problems have its social aspect. For instance, an individual farmer cannot take effective measures against pests which ravage over a large territory; in which case a joint action with his neighbours is necessary. An example is illustrated with grass hopper (*Z. variegatus*) infestation. Preventive measures should be taken in the locations where the eggs are laid, such as burning old tree stumps, heaps of uprooted weeds and avoiding damp places in general. Eggs are most effectively destroyed by raking them out so that they dry in the sun. Nest sites are comparatively rare, usually one or two per hectare (Amatobi, 1984). For effective control, the destruction of all nest sites over a large area by all farmers is necessary. One farmer acting alone has little effect, whereas joint action can reduce the succeeding population of grasshoppers by 70 – 80% (Amatobi, 1984).

Another social problem in crop protection is inadequate price incentives to farmers particularly when inclement weather strikes. Price incentive is necessary to guarantee farmers good reward for their efforts. Also, excess produce should be purchased by the state governments and stored as strategic grain reserves. All these would encourage farmers to produce more to feed the teeming population in the country.

CONCLUSION

Plant protection is currently considered as being synonymous with the use of pesticides whose utilization is the only barometer for ascertaining achievement in this respect. Other control methods which are relatively easy to adopt should be explored and exploited. A number of pests can effectively be checked by manipulation of cultural practices, for example, depth of planting, soil and water management, soil amendments (for nematodes), and dry season deep plowing for killing insects and pathogens in the soil. Growing maize and sorghum in areas where the environment is unsuitable for pests and diseases attack and where the crops have relative advantage for high yield potential is essential for good economic returns from the farm.

Besides, since the savanna ecological zones have diverse weather conditions and varied farming systems, pest and disease problems, the severity of their occurrence and control strategies differ according to conditions. By studying the occurrence and spread of pests and diseases in these agro-ecological zones for more than 3 years, a fairly good idea of the pest and disease situation can be worked out for that particular area.

Establishment of plant clinics at district or local government level, staffed with competent pathologists and an entomologists will ensure sound surveillance service with the aim of supplying technical assistance to the farmers and village extension workers in terms of diagnosis, control, correct use of pesticides and use of disease free planting materials.

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Combined effect of soil solarization and neem amendment on survival of *Macrophomina phaseolina* sclerotia and growth of soybean

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Abstract: Combined effect of soil solarization and neem products (leaf, bark and oil cake powders and neem oil) amendments on the survival of *Macrophomina phaseolina* sclerotia in soil was studied. Propagules of *M. phaseolina* treated with different neem products gradually decreased with increase in duration of soil solarization as compared to control. Minimum number of sclerotia/g dry soil was recorded in 1% neem oil and 10% cake powder amended soil. Maximum viable sclerotia were detected in unsolarized control soil followed by bark powder amended soil. Soil solarization effectively caused a decline in propagules of *M. phaseolina* by 20% in comparison to unsolarized control soil after 30 days. However, the effectiveness of solarization got potentiated upon addition of different neem products. Neem cake powder posed the most toxic effect in decreasing the survivability of test pathogen sclerotia. The bacterial population was higher in solarized soil as compared to unsolarized control soil. Moreover, the bacterial counts increased after addition of neem cake powder in solarized soil. The fungal population was found to be almost equal in leaf and oil cake powders amended soils. Seedling emergence (%) of soybean in solarized and nonsolarized soils was similar but the total number of infected plants in solarized soil was lesser than unsolarized control. Combined effect of solarization and cake powder amendment minimized the number of infected plants by 60% and increased the seedling growth and biomass as compared with control.
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Key words: Soil solarization, Neem products, Sclerotia survival, *Macrophomina phaseolina*, Soybean growth

1. Introduction

Soil solarization is a non-chemical method for controlling soil borne diseases and pests other than the use of plant products as pesticide (Katan, 1981). Soil borne plant pathogens of some vegetable and fruit crops have been controlled partially by pesticides. However, the use of soil fumigants is often undesirable due to unfavorable effect on animals or human beings, resulting in toxicity on plants and soil, complexity in treatment and expensive high cost. Soil solarization captures the radiant energy of sun, thereby causing physical, chemical and biological changes in the soil. Transparent polythene sheet placed on moist soil during the hot summer months increases soil temperature to a level lethal to many soil-borne plant pathogens (Dwivedi and Dubey, 1987).

Soybean (*Glycine max* L. Merr.) is one of the major oil yielding plant found not only in India but throughout the world due to the presence of high calorie protein and edible oil. *Macrophomina phaseolina* Tassi (Goid.) is one of the pathogens that cause charcoal rot of soybean resulting in great economic loss. However, neem (*Azadirachta indica* A. Juss.) has a great potential for controlling various phytopathogenic fungi due to presence of a variety of bioactive compounds (Tewari, 1992). Effectiveness of neem extracts and oil as a fungicide has been reported by several workers (Dwivedi and Dubey, 1986; Sharma and Basandrai, 1997; Lokhande et al., 1998; Dubey et al., 2009).

M. phaseolina was reported to cause significant inhibition in germination of soybean in tarai region of Uttarakhand along with some other phytopathogenic fungi (Uma et al., 1999). Integration of soil solarization with neem product to minimize the charcoal rot disease of soybean caused by *M. phaseolina* aroused the interest to find out an ecofriendly, non-chemical technique. In the present study an attempt has been made to evaluate the combined effect of soil solarization and amendment of different neem products on the survival of *M. phaseolina* sclerotia and growth of soybean seedlings.

2. Material and Methods

2.1. Preparation of neem powders

Fresh and healthy leaves were collected from mature neem trees, washed with distilled water, dried in shade and powdered by using a mixer/grinder (Model Maharaj, Whiteline). Bark was gently removed

from the same tree, dried in shade and powdered as above. Seed cake was collected from a local Expeller mill and powdered. Neem oil procured from the Expeller mill was used as such.

2.2. Harvesting of *M. phaseolina* sclerotia *in vitro*

The sclerotia of *M. phaseolina* were harvested by cellophane disc method (Ayanru and Green, 1974). The cellophane paper discs were cut according to the size of Petri dishes and boiled for 30 min to remove plasticizers. The cellophane discs were gently spread onto the surface of Petri dishes containing solidified Potato Dextrose Agar (PDA) medium, inoculated with an agar block (5 mm diam.) of *M. phaseolina* and incubated at 30±1 ^{o}C for 5 days. On the sixth day the cellophane paper was gently removed from the surface of medium and sclerotia were harvested and placed onto sterile filter paper to dry. Dried sclerotia were mashed with mortar and pestle and filtered by a sieve of pour size 150 µm.

2.3. Survival of *M. phaseolina* in soil amended with neem products

Soil (5.5 kg) was taken from crop field and water was added to make up 50% of moisture holding capacity. Soil was autoclaved and mixed properly with 500 mg sclerotia produced as above. 250 g of soil was kept in 13 polythene bags (300 g capacity). One bag was kept as such for control. The others were amended separately with 1%, 5% and 10% of different neem products i.e. leaf, bark and cake powders. However, the neem oil concentrations applied in the present study were ca. 0.1%, 0.5% and 1.0%. All the bags were tied with a thread, punctured for gaseous exchange and incubated for 7 days at room temperature. Thereafter, 50 mg of soil (containing fungal inocula) taken out from each bag was sprinkled over solidified water agar (2%) + rose Bengal (50 mg/l) + Streptomycin (30 mg/l) medium in sterile Petri dishes in triplicates. Plates were incubated at 30±1 °C in dark for 5 days. After incubation colony forming units (CFUs) of *M. phaseolina* appeared in each plate were counted.

2.4. Effect of solarization on survival of *M. phaseolina* sclerotia in soil treated with neem products

Solar heating of soil mixed with each neem product was carried out for 30 days following the methods described by Dwivedi and Dubey (1987) and Dubey (1992). Five experimental plots (1×1 m) were selected in an agricultural area in Haldwani (Nainital district). The plots were well ploughed, evenly leveled and made free from plant debris. Three small pits (6 cm depth) for each treatment were dug in each plot. Bark powder, leaf powder and oil cake powder (@. 200 g/ha) were properly mixed with soil separately in three plots. Sclerotia (500 mg) were transferred to 15 polythene bags (300 g capacity). The bags were tied with thread and punctured with needle to facilitate moisture, heat and gaseous exchange. One bag was put in each pit and covered with soil. Two plots served as control out of which one control plot and three treatment plots were covered with 45 µm thick transparent polythene sheets. The plots were well irrigated and surface of polythene sheet was gently pressed so that it may get attached with soil surface. The edges of sheets were buried in soil to avoid the loss of moisture, heat and volatile emanating from soil. Soil moisture was regularly maintained to about 90% of its moisture holding capacity by supplying water at 3 days intervals.

After solar treatment for 10, 20 and 30 days polythene sheets were removed and bags from a pit of each plot (control unsolarized, control solarized and three neem products-treated plots) were taken out and after sampling the pits were covered as mentioned above. All samples were powdered separately with a pestle and mortar under aseptic conditions and 50 mg of sclerotia were sprinkled over the surface of sterilized and cooled 2% water agar medium and rose Bengal and Streptomycin containing in Petri dishes. Plates were incubated at 30±1 °C for a week. On the 7th day colonies of *M. phaseolina* growing on the surface of agar plates were counted and population of fungal propagules was determined on colony count basis.

2.5. Effect of neem products on microbial community of soil

Soil samples (100 g) were separately mixed with 5 g of neem products i.e. leaf, bark, cake powders and the oil (0.5 ml). The soil samples were kept in sterile polythene bags, tied with thread, punctured with needle and incubated for 7 days at room temperature. Microbial community of soil was estimated by dilution plate method. Soil fungi were isolated from 10^{-3} dilution and incubated at 25±1 ^{o}C for about a week, whereas bacteria were isolated from 10^{-5} dilution and incubated for 4 days at 30±1 °C. The colony forming units (CFUs) of fungi and bacteria g^{-1} dry soil were calculated as follows: CFUs = (Average number of colonies appeared in culture plate × 100) × Dilution factor/Weight of the oven dry soil.

2.6. Effect of soil solarization and neem products amendments on growth of soybean seedlings

After completion of solarization process soybean seeds (variety PK 416) were sown in all the five plots (control unsolarized, control solarized, leaf powder, bark powder and cake powder amended plots). After

10 days of sowing the numbers of seeds germinated were counted and seedling emergence (%) was observed using the following formula: Seedling emergence (%) = (total number of seeds germinated)/ total number of seeds sown × 100

After 30 days of sowing the soybean seedlings were removed carefully from each plot and the roots were washed gently in running tap water to remove the adhered soil particles. Healthy and diseased seedlings were categorized into two categories (infected and uninfected) on the basis of visual observation using 10 x ocular lens. Root and shoot length of each seedling and the root/shoot biomass were measured on oven dry basis at 85 °C for 24 h.

3. Results

3.1. Survival of *M. phaseolina* sclerotia in neem products amended soil

Minimum number of viable sclerotia g^{-1} dry soil was detected in 1% neem oil and 10% neem cake powder amended soil (Table 1). Maximum viable sclerotia (560 sclerotia g^{-1} dry soil) were detected in control soil, followed by 1% bark powder-amended soil (500 sclerotia g^{-1} dry soil). Neem oil was most effective in controlling the sclerotial viability, while the bark powder was the least effective.

3.2. Effect of solarization on sclerotial survival of *M. phaseolina* in neem products amended soils

Initial population of *M. phaseolina* was 440 sclerotia g^{-1} dry soil, which gradually decreased with increase in duration of solarization. Increase in temperature drastically caused a decline in population of sclerotia from 300 (unsolarized control) to 240 (solarized control) (Table 1). Further decline in number of sclerotia was potentiated after neem products amendment in soil. Only 60 sclerotia g^{-1} dry soil was survived in cake-amended soil, whereas control soil revealed 300 sclerotia g^{-1} dry soil. The cake powder was found to be the most effective in minimizing the sclerotia from 240 (control) to 60 (cake powder-amended soil) followed by leaf powder-amended soil (80 sclerotia g^{-1} soil) after the completion of solarization period (Table 1).

Table 1: Viable number of *M. phaseolina* sclerotia in solarized and unsolarized soils amended with different neem products.

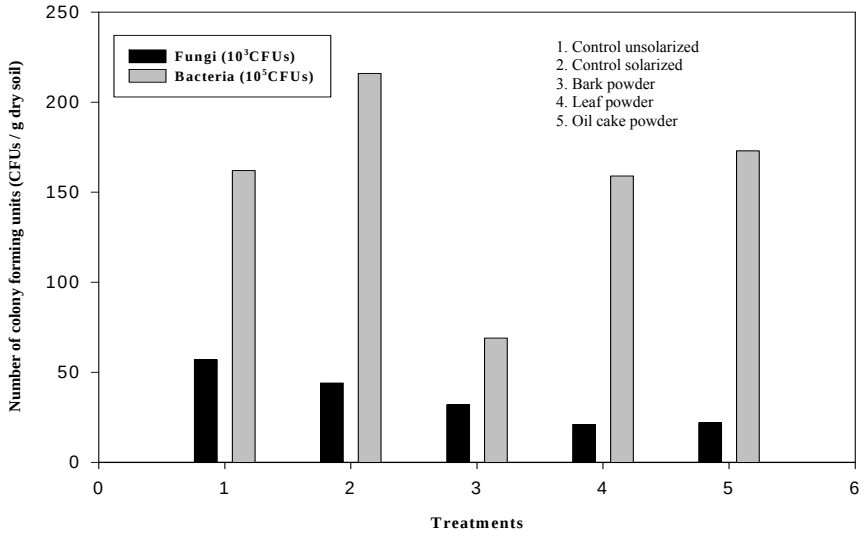
Treatment with neem products	No. of viable sclerotia g^{-1} dry soil[!]						
	Survival in lab conditions				Solarization of field		
	Concentration of neem products (%)				Duration of solarization (days)[+]		
	No.	1%	5%	10%	10	20	30
Control unsolarized	560	-	-	-	380	360	300
Control solarized	-	-	-	-	340	300	240
Bark powder	-	500	400	420	340	280	260
Leaf powder	-	320	240	160	240	140	80
Oil cake powder	-	400	340	140	200	160	60
Oil*	-	300	260	140	-	-	-

*****Concentration of oil was 0.1%, 0.5% and 1.0% instead of 1%, 5% and 10 %.
[!] Initial population of *M. phaseolina* was 440 g^{-1} dry soil ±1.
[+] Temperature recording on sampling dates:
10 days – air, 30 °C; unsolarized soil, 33 °C; solarized 39 °C
20 days – air, 29 °C; unsolarized soil, 30 °C; solarized 37 °C
30 days – air, 30 °C; unsolarized soil, 35 °C; solarized 41 °C

3.3. Effect of solarization on fungal and bacterial populations in neem products amended soils

Changes in bacterial and fungal population were measured after 30 days of solarization which revealed that the addition of neem products increased the bacterial population in solarized soils. The most abundant CFUs (173×10^{-5} g^{-1} dry soil) of bacteria were found in cake powder amended solarized soil (Fig 1). The maximum number of fungi was observed in unsolarized control (57×10^{-3} CFUs g^{-1} dry soil) followed by solarized control (44×10^{-3} CFUs g^{-1} dry soil) and bark powder amended soil (32×10^{-3} CFUs g^{-1} dry soil). The number of fungi in leaf and oil cake amended soil was almost equal.

Figure 1: Effect of different neem products on population of soil fungi and bacteria after soil solarization.



3.4. Effect of solarization and neem products amendments on growth of soybean seedlings and disease incidence

Though the emergence of seeding was equal in unsolarized and solarized control, yet disease incidence was higher in unsolarized control (85%) followed by solarized control (70%). The highest number of seed germination was observed in cake powder amended soil followed by leaf powder amended ones (Table 2). Addition of bark powder resulted in poor seedling emergence (52%) and higher disease incidence (61%) as compared to leaf and cake amendments whereas, 70-85% disease incidences were recorded in control solarized and unsolarized plots. Most of the seedlings were either disease-free or showed lesser disease incidence (1-30%). Cake powder was found to be the most effective where only 34% seedlings were infected as compared to leaf powder amended soil (43%) (Table 2). Soybean seedlings showed better growth in solarized than unsolarized soils. The maximum plant biomass yield was recorded in oil cake powder-amended soil followed by leaf powder amended ones (Table 2).

Table 2: Effect of soil solarization and neem amendments on emergence and growth of soybean seedlings.

Treatment	Growth of soybean seedlings				
	Seedling emergence (%)	Plants infected (%)	Root length (cm)	Shoot length (cm)	Dry weight (Plant biomass) (g)
Control soil					
Unsolarized	72	85	10.7	19.2	0.72
Solarized	72	70	11.0	21.3	0.92
Solarized amended soil					
Bark powder	52	61	13.0	23.4	0.95
Leaf powder	64	43	20.2	27.3	1.13
Oil cake powder	68	34	26.8	38.0	1.27

Values are the mean of 15 plants; Number of soybean seeds sown in 1×1 m^2 plots was 25.

4. Discussion

During the course of present study the field trial received huge rain fall i.e. 5 times between 2-10 days, 4 times between 10-20 days and ones between 20-30 days of solarization. The maximum temperature of mulched soil was recorded to be 41°C at 6 cm depth. Dubey (1992) has earlier reported the temperature range of 50-55.5 ^{o}C upto 6 cm depth in solarized plots amended with fungicides. Interestingly the effectiveness of solarization got potentiated upon addition of different neem products like powders of leaves, bark and seed cake. *M. phaseolina* is a high temperature loving fungus. Mihail and Alcorn (1982) have reported the thermal death range of the test fungus between 50-55 °C. However, it can tolerate temperature up to 50-52 °C for about 120 h (Paharia and Sahai, 1970). Beyond 50 °C the fungal sclerotia become inactivated within 24 h, but they are not killed (Bega and Smith, 1962). So it is possible for *M. phaseolina* to tolerate this temperature range even after a slight inactivation of some of sclerotial cells by thermal killing, because all the cells of a sclerotium function as independent unit (Wyllie and Brown, 1970). Besides thermal killing of soil-borne pathogens, plastic mulching operates its function by restoring the soil volatiles and increasing the population of antagonistic microorganisms after the completion of solarization.

Our findings revealed that the total numbers of bacterial CFUs were increased in solarized control soil, whereas the fungal counts were decreased as compared to unsolarized control. The reason for higher bacterial population in solarized soil than the unsolarized ones may be explained as perpetuation and proliferation of thermotolerant bacteria surviving after solar heating of soils. Neem products consist of toxic chemical constituents such as nimolicinol, azadirachtin, azadirachtol, nimlinone, nimbocinol, nimocin etc. with varying amounts in different plant parts (Tewari, 1992). The neem products are also subjected to microbial decomposition after their amendments in the soil. The combined effect of decomposition of neem products for 30 days in soil along with solarization process might have caused the difference in soil microbial population. Moreover, soil amendments with farmyard manure and nitrogen have been reported to increase the effectiveness of solarization by reducing the population of *M. phaseolina* (Lodha, 1995). Gaur et al. (1997) also reported that the population of *Pythium ultimum* causing damping off of linseed was decreased to 10.64 fold log cfu/g soil (90.6 %) after 30 days of soil solarization as compared to 1.16 fold reduction (14 %) in nonsolarized plots.

Though survival of *M. phaseolina* sclerotia declined to a greater extent after 30 days of solarization in the present study, a considerable number of propagules were detected in soil. It is well known that magnitude of temperature, light intensity, soil texture, soil moisture and the soil depth also play important role on the survival of microorganisms (Katan, 1981). It may be attributed that soil texture and loss of volatile fungistatic substances after lifting the polyethylene sheet during the time of sampling and watering may be the factors involved in helping sclerotial viability. The results of present study are in conformity with those of Dwivedi and Dubey (1987), Lodha and Solanki (1992), Dubey (1992) and Lodha (1995).

Increased plant biomass due to amendment of neem products may be attributed to the inhibitory volatiles emanating from decomposing powders of neem plant parts in solarized soil. These gases would have allowed changes in microbial population with increase in thermotolerant antagonistic microbes, which in turn would have caused inhibition in disease incidence caused by *M. phaseolina* and enhanced plant growth. In the present study, reduction in population of *M. phaseolina* might be the result of the triple actions of neem amendments, antagonistic activity of thermophillic microorganisms and the thermal inactivation of *M. phaseolina* sclerotia. Reduction in coconut disease caused by *Theiloviopsis paradoxa* and *Gonoderma lucidum* and the improvement in yield with application of 5 kg neem cake/palm have also been reported (Rao et al., 1992; Bhaskaran, 1993).

In conclusion our finding suggests that integrated effect of soil solarization and amendments of neem products might have played combined role in reducing the survival of *M. phaseolina* sclerotia and also the disease incidence of charcoal rot in soybean seedlings. Such disease control measure can also be efficiently used in the regions where field soil solarization is not feasible for longer duration.

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Study on accountable factors for physiological and biochemical variations in normal and variant *Cinnamomum tamala* (Nees and Eberm) seedlings

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Abstract: The morphological variant seedlings premeditated were discrete from the normal plant seedlings in many characteristic features. Leaf pigments of both the plants did not differ much in leaf pigments (Chl a, Chl b and total chlorophyll content) wherein slight increase in carotenoide content was recorded in normal plant seedlings than the variant. Carbohydrates (soluble sugars and starch), soluble proteins, total free amino acid content was recorded maximum in variant seedlings compared to normal plant seedlings. Electrophoretic profile of polypeptides and isoenzymes executed greater band intensity of variant seedlings in comparison to normal plant. Chl a fluorescence measurements displayed slender increase in ABS/RC and DI/RC in the membrane model of the variant plant and in the leaf model, increase in ABS/CS_O, TR_O/CS_O, ET_O/CS_O, DI_O/CS_O and inactive reaction centers over normal was displayed by the variant plants. [Nature and Science 2009;7(11):58-64]. (ISSN: 1545-0740).

Keywords: Morphology, Pigmentation, SDS - PAGE, Isoenzymes, Fluorescence, Heat dissipation index.

Abbreviations: N (normal), V (variant). ABS/RC, DI/RC, ABS/CS_O, TR_O/CS_O, ET_O/CS_O and DI_O/CS_O

Introduction:

Survival of a species depends on its adaptability which in turn depends on a proper balance between flexible changes in the plants subjected to varying environmental stresses (Gairola, 1990). The adaptability in plants is measured in terms of the morphological, physiological and biochemical manifestations in a particular environment, these are closely interrelated (Bradshaw, 1965).

An individual genotype assumes particular characteristics in a given environment. However, in a diverse environment it may remain the same or may change. The stability, which is genetically determined, can vary from one genotype to another. It has been shown that the stability levels are specific for individual characteristics within a single genotype and are not common for all characters of a single genotype (Williams, 1960).

Plants being stationary can not escape through environmental stresses and thus are capable of structural and functional modifications. These modifications in a plant under a particular environment are under biochemical regulations which are ultimately controlled by the enzymes (Gairola, 1990). The environmental modifications of growth or phenotype are known to play an important role in the adaptation of plants to changing environment (Onipchenko, 2004).

Cinnamomum tamala (Nees and Eberm) commonly known as Tejpat or Indian cassia/Indian lignea (Lauraceae), 25 ft. height, 4.5 ft. girth, evergreen tree, distributed in Eastern Asia, Indo - Malayan and the Pacific Islands (Brandis, 1998) and is a moderate sized evergreen tree distributed in tropical and sub - tropical Himalaya between 3000 - 8000 ft amsl. Leaves contain essential oil (Eugenol and Isoeugenol) and bark contains 70 - 80 % cinnamic aldehyde (Anonymous, 1950). Ayurveda describes the use of leaves of Tejpatra in the treatment of ailments such as anorexia, bladder disorders, dryness of mouth, coryza, diarrhea, nausea and spermatohea (Kapoor, 2000). It is commonly used in food industry because if its special aroma (Chang and Cheng, 2002). The main constituents of cinnamon tamala are alpha pinene, camphene, myrecen, limonene, eugenol, p - cymene, methyl - eugenol, eugenol acetate and methyl ether of eugenol (Smith *et al*., 2002; Saino *et al*., 2003).

In the present work, an attempt has been made to compare normal *Cinnamomum tamala* seedlings with its morphological variant seedlings in terms of some physiological and biochemical parameters (Plate 1). The aspects studied included leaf pigments, carbohydrates, soluble proteins, total free amino acids, electrophoretic profiles of polypeptides, isoenzymes and chlorophyll a fluorescence measurements. The in attendance communication aims the liable factors responsible for variation in normal and variant *cinnamon* seedlings.

Methodology

Sample collection:

Two year old normal *Cinnamomum tamala* seedlings and its morphological variant seedlings growing in glass

house conditions at High Altitude Plant Physiology Research Centre (HAPPRC) were used for the present study.

Plate 1. Normal and variant seedlings of *Cinnamomum tamala*

Morphological attributes:

Leaf length, leaf width, petiole length, right and left inter vein distance were recorded manually by using measuring scale, leaf thickness and petiole thickness were measured with the help of digital vernier caliper and leaf area was recorded by using digital leaf area meter.

Biochemical attributes:

Leaf pigments (chlorophyll and carotenoides) were estimated as per Holm, 1954. Soluble sugars were estimated as per Mc Cready *et al.* (1950). Total free amino acids were quantified by following the method of Moore and Stein (1954). Soluble protein estimation was carried out as per Bradford (1976). SDS - PAGE was worked out as per the method of Laemmeli (1970). Isoenzymes viz., peroxidase was separated on 7.5 % polyacrylamide gel as described by Davis (1964) and detected by Wetter (1982). Esterase was separated on 10 % Polyacrylamide gel as described by Bhadula and Sawhney (1987).

Physiological attributes:

Chlorophyll a fluorescence induction in both normal as well as variant seedlings was measured using a plant efficiency analyzer (PEA, Hansatech Ltd., U.K).

Results and Discussion

1. **Variations in morphological attributes in normal and variant seedlings**:

All the morphological attributes were recorded maximum for the normal plant seedlings compared to the variant seedlings (Table 1). The respective pattern could be ascribed to the quality and quantity of light intensity as has been earlier reported by Toole *et al*. (1956), Lokhart (1961), Goodchild et al. (1972) and Pandey and Sinha (1977). Soil physico-chemical environment is another determining factor as reported by many workers viz., Hillel (1972), Lal and Greenland (1979), Lal (1979a & b), Larson et al. (1989), Ghildiyal and Gupta (1991), Ouwerkerk (1991), Six et al. (2000), Saggar et al. (2001), Turrion et al. (2001), Jobbagy et al. (2001), Tessier et al. (2003), Ehrenfeld (2003), Westman et al. (2003), Haubensak and Parker (2004), Guo et al. (2004), Nziguheba et al. (2005).

Table 1. Variations in morphological attributes in normal and variant seedlings

Morphological attributes	Normal	Variant
Leaf area (cm²)	525.19±99.17	177.09±81.39
Leaf length (cm)	16.20±2.23	10.10±2.80
Leaf thickness (mm)	0.40±0.02	0.30±0.06
Petiole length (cm)	1.00±0.19	0.92±0.08
Petiole thickness (mm)	1.95±0.15	1.27±0.42
Leaf width (cm)	3.93±0.34	0.23±0.60
Right inter vein distance (cm)	1.25±0.11	0.03±0.16
Left inter vein distance (cm)	1.23±0.16	0.65±0.18

2. **Variations in biochemical attributes in normal and variant seedlings**

2.1. **Leaf pigments**:

The leaves of normal and variant plant seedlings did not differ much in Chl a, Chl b, total Chl and carotenoide content. The contents of Chl a, Chl b and total Chl were 0.4, 0.1 and 0.5 (mg/g fr. wt.) in normal leaves wherein variant contents were 0.4, 0.07 and 0.5, respectively. The normal and variant leaves did not differ much in their carotenoide contents which were recorded 0.33 and 0.30 (Figure1), respectively. Differences in the pigments between the two types were recorded. Such differences in the leaf pigmentation from one stage to another are known in the literature. A rapid increase in chlorophyll and carotenoide content during ontogeny of a leaf is a characteristic in an insertion gradient (Sestak, 1985). Ontogenetic changes in the contents of carotenoide have not been studied as often as those of chlorophylls (Sestak, 1978). Range of chlorophyll variation among the species is partly environmentally determined (Hornvedt, 1983).

2.2. **Carbohydrate content**:

Figure 2 executes that soluble sugar content was found maximum in variant plant seedlings (9.53 mg/g) in comparison to normal plant (7.8 mg/g). Morphological variability and biochemical differences in sugar level are important parameters which reflect variation due to environmental conditions rather than genetic variations. Like soluble sugars, starch content was found maximum in variant (32.03 mg/g) in comparison to normal plant seedlings (6.96 mg/g). Total free amino acids: The total free amino acid content was found maximum in variant leaves (3.63 mg/g) wherein 1.21 mg/g in normal seedling leaves (Figure 3). Soluble proteins: The soluble protein content in normal leaves were recorded 83.74 mg/g wherein variant leaves had 78.31 mg/g protein content (Figure 4). Miflin and Shewry

(1981) reported that protein act as the reserve on nitrogen and sulphur during germination and the amount of protein content is of adaptive significance. Thus high protein content in the leaves of variant indicates the adaptive significance to sustain harsh climatic conditions.

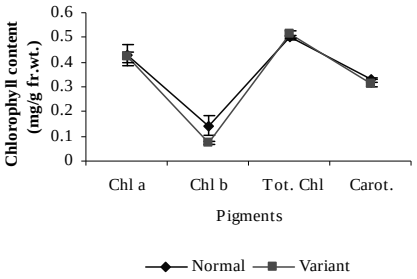

Figure 1. Variations in leaf pigments in normal and variant seedlings

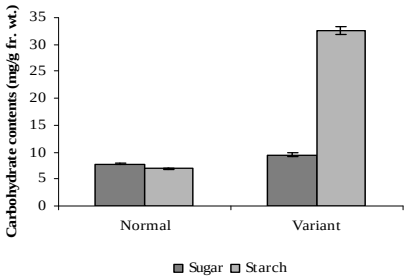

Figure 2. Variations in carbohydrate content in normal and variant seedlings

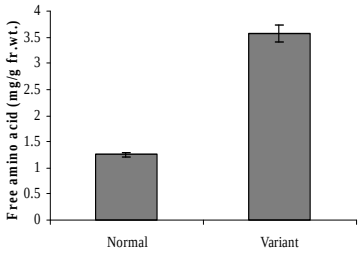

Figure 3. Variations in total free amino acid content in normal and variant seedlings

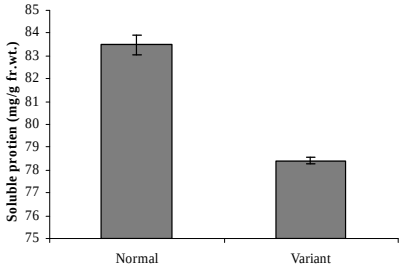

Figure 4. Variations in soluble protein content in normal and variant seedlings

2.3. **Polypeptide profile**:

In normal plant seedlings, appearance of polypeptide patterns was light in comparison to the variant seedlings. Normal as well as the variant had similar number of polypeptide bands i.e., five. Out of these bands the upper one is of high molecular weight and rests of the lower bands were of low molecular weight. The intensity of the first band in case of the variant was high in comparison to the normal plant (Plate 2). It is a well established fact that adaptation to any new environment can cause changes in the molecular configuration and in the activity of different enzymes (Straub, 1964). Considerable variations of polypeptide variation were also found in *Polygonum* species when acclimatized at three different altitudes (Prakash, 1999).

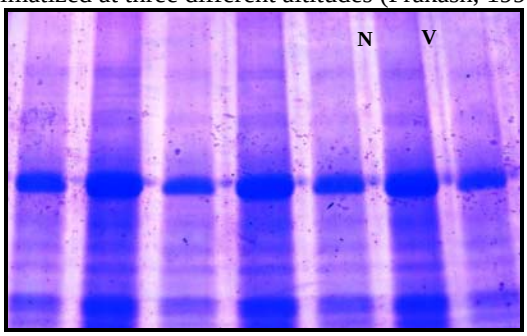

Plate 2. Polypeptide variation in normal and morphological variant seedling of *C. tamala*

2.4. **Isoenzymes**

2.4. a. **Peroxidase**:

Number of bands appeared in normal and variant was four, out of which three were of high molecular weight and the rest one is of low molecular weight (Plate 3). The placement of band appearance was similar in both the cases. However, the intensity of the third band from the migration point was high in case of the variant in comparison to the normal plant. Peroxidase is generally composed of a number of isoenzymes and is capable of catalyzing several types of metabolic activities and also involved in synthesis of ethylene. An increase in peroxidase activity probably represents an induced protective reaction delaying senescence (Birecka *et al*., 1977).

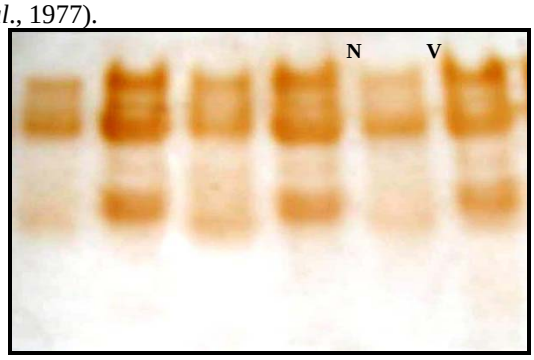

Plate 3. Peroxidase variation in normal and morphological variant seedlings of *C. tamala*

2.4. b. **Esterase**:

Number of bands appeared in normal as well as variant was three. The upper two were of high molecular weight and the lower one is of low molecular weight. The intensity of the first two bands in variant was high wherein low in the normal plant (Plate 4). Several isoenzymes including esterase have also been used in the analysis of genetic diversity of endangered species (Bousquenet *et al*., 1986; Godt and Hamrik, 1995). Isoenzymes variation has been used frequently to characterize germplasm collections (Brown, 1978; Goodman and Stuber, 1983; Souza and Sorrells, 1989).

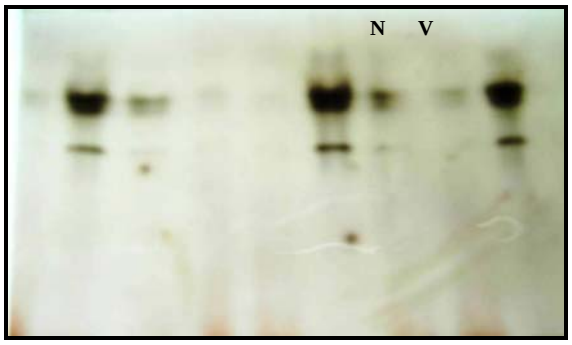

Plate 4. Esterase variation in normal and morphological variant seedlings of *C. tamala*

3. **Variations in physiological attributes in normal and variant seedlings**:

Fluorescence measurements:

Minor variations between variant and normal seedlings were observed for different fluorescence characters. However, the main difference was observed for performance index which was more for variant seedlings in comparison to the normal seedlings. Pipeline models obtained for chlorophyll a transient of normal and variant seedlings of *Cinnamomum tamala* revealed normal variations between both the types. In the membrane model, a little bit increase in ABS/RC and DI/RC was observed in variant seedlings compared to normal seedlings. In leaf model, also variant plant recorded a little increase in ABS/CS_O, TR_O/CS_O, ET_O/CS_O, DI_O/CS_O and inactive reaction centers over normal plants (Plate 5). Chl fluorescence has been proven to be very useful, non - invasive tool for the study of the photosynthetic apparatus and more specifically the behavior of photosystem - II (Papageorgiou, 1975; Krause and Weis, 1991; Govindjee, 1995; Joshi and Mohanty, 1995; Schreiber *et al*., 1995; Lazar, 1999; Strasser *et al*., 1999, 2000).

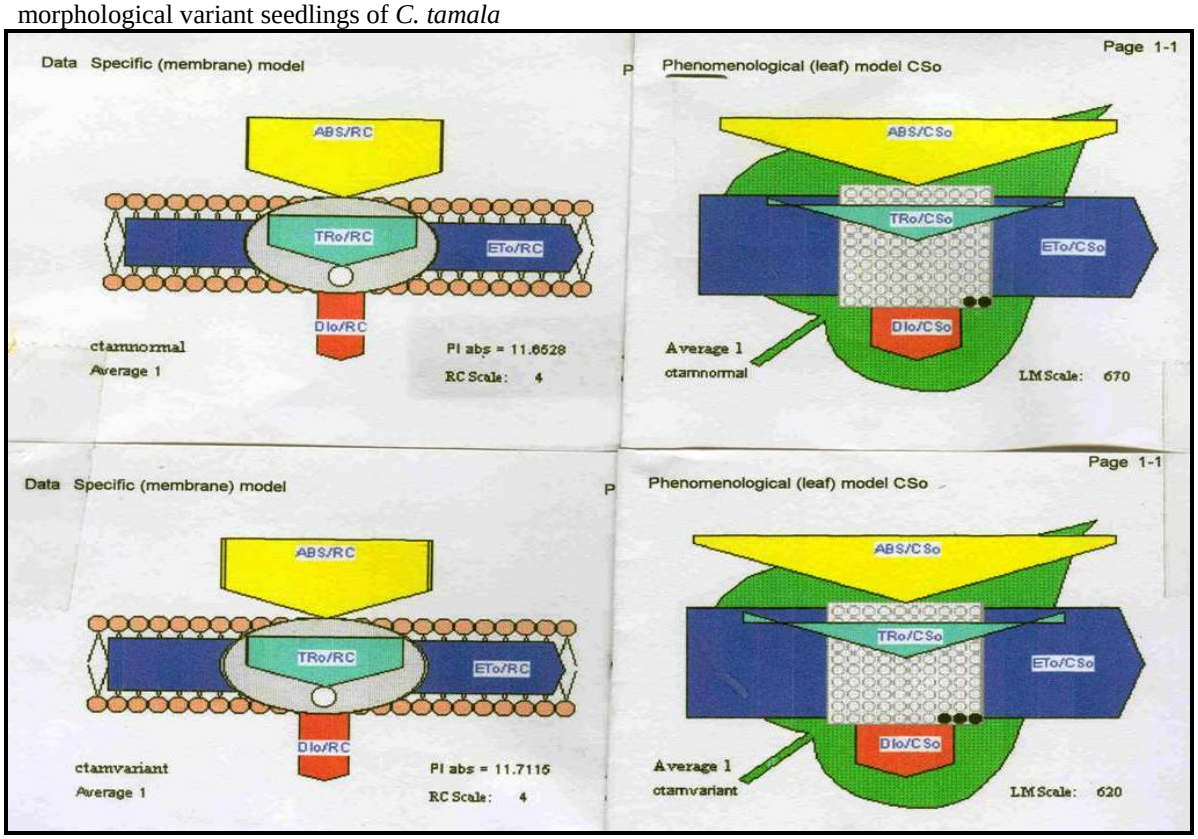

Plate 5. Pipeline models for specific fluxes (membrane model) and phenomenological fluxes (leaf model) for fluorescence characters of normal and morphological variant seedlings of *C. tamala.*

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A Preliminary Study on Genetic Variability in Hypoglycaemic Response to *Vernonia amygdalina* in Rats

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Abstract: The possibility that genetic factors play a role in sensitivity to the hypoglycaemic effect of *Vernonia amygdalina* (bitter leaf) was investigated through selective inbreeding in albino rats. Aqueous extract from the plant was administered orally using oro-gastric intubation and fasting plasma glucose concentration was monitored in the rats for 180 minutes. Percent Change of Glycaemia (PCG) was used as an index to measure the degree of glycaemia reduction following *V. amygdalina* administration. The initial PCG of the original unselected stock of animals (U) when treated with *V. amygdalina* was -4.5%, but after two generations of selective inbreeding (brother-sister mating) for sensitivity to the hypoglycaemic effect of the plant, the magnitude of PCG (-49.1%) was greater ($P<0.05$) in the sensitive F_2 offspring (i.e. S_2). Animals that were similarly inbred for resistance (R_2) had a PCG of -9.3% at F_2. a value which was significantly lower in magnitude than the PCG of S_2 animals ($P<0.01$). It was therefore suggested that since it was possible to develop two strains of rats that differ markedly in their sensitivity to the hypoglycaemic effect of *V. amygdalina* through selective inbreeding, the plasma glucose reducing action of *V. amygdalina* is probably mediated, at least partly, through genetic factors. The implication of the results of this study for the treatment of diabetes in conventional and herbal medicine is discussed.
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Key words: *Vernonia amygdalina*,; hypoglycaemic; variation; selective inbreeding

1. Introduction

Variation in sensitivity to drugs had long been recognized as one of the several genetically controlled polymorphisms seen in human populations (Vesell, 1979). Human variations in response to drugs such as primaquine sensitivity, isoniazid inactivation, and response to hydrogen peroxide are well known because they have been studied in considerable detail in view of their relevance to clinical medicine and public health (Evans et al, 1960; Marks and Blanks, 1965; Odeigah and Okunowo, 1989). As opposed to the case with most conventional drugs that are usually recommended in clinical practice, variations in sensitivity to plant medicinal products, especially those that are commonly used in traditional medicine have received very little or no attention.

In many developing countries of the world, interest in native plant remedies has continued to increase as a result of the growing awareness of the importance of medicinal plants in health care delivery. This interest now extends to many urban and developed communities including parts of Europe and America. (Gill 1994; Lease and Williams, 1994). In Nigeria and many other poor African countries belief in traditional herbal medicine is strong. In our previous animal experimental studies, we have focused attention on the glycaemic activities of some common Nigerian dietary and medicinal plants with a view to ascertaining their importance in diabetic management (Odeigah et al, 1995; Odeigah et al, 1999; Taiwo et al, 2008). Data from these and other studies (Ogbuokiri and Ekpechi, 1989) suggested that Nigeria and many other African countries are endowed with dietary and medicinal herbs, which may play important roles in the treatment of diabetes and many other diseases. Recent studies in our laboratory have shown that *Vernonia amygdalina* Del. (Astereceae) may be of value in treating diabetes and even cardiovascular diseases such as hypertension (unpublished data).

In our previous experimements particularly in the present study, the control of environmental factors was rigid. In spite of this, it was repeatedly observed that in a given group of animals, considerable variations were always present in the degree of glycaemic response to *V. amygdalina* - the magnitude of glycaemic reduction was high in some animals but low in others. It was therefore thought that such variations in response might represent ordinary differences that occur by chance in a

homogenous group or the consequences of some genetic factors. If the population were purely genetically homogenous and the observed variation was due to chance alone, it would not be possible to separate the group into different strains. By contrast, if sensitivity to hypoglycaemic effect of *V. amygdalina* is, at least, partly controlled by genetic factors, it should be possible to separate, through selective breeding, a given population of animals into two strains that differ significantly in their sensitivity to the hypoglycaemic effect of *V. amygdalina*.

The present paper is a report of a preliminary study which was carried out to test this possibility. It is hoped that the results of this investigation will stimulate further interest and discussions on pharmacogenetic aspects of herbal medicinal products in view of the growing interest in herbal medicine.

2. Materials and methods

2.1 Plant Materials and Extraction

Vernonia amygdalina were obtained from the wild near the University of Lagos main campus. The plant was authenticated at the Forestry Resarch Institute (FRIN), Ibadan, Oyo State, Nigeria. For the extraction, the plant materials were first washed free of sand, cut into pieces and air- dried before grinding into powder. Fifty grams of the powder was extracted with 500ml of distilled water using Soxhlet extraction. The extract was slowly evaporated *in vacuo* to obtain a total yield of 3.5g. weighed sample of the extract was then used to prepare test solutions.

2.2 Animals and their Treatment

An unselected group (U) of 24 Sprague-Dawley (SPD) rats (10 males: 14 females) constituted the parental generation (P) from which 2 strains were developed. The animals were obtained from the Laboratory Animal Center of the Nigerian Institute of Medial Research (NIMR), Lagos, Nigeria. The care of the animals, plant extraction, and the techniques of oral administration of materials and plasma glucose determination were the same as reported earlier (Odeigah et al, 1994). Briefly, the animals were fasted for 24 hours before each day of experiment. Fasting plasma glucose concentration (FPGC) was measured for 180 minutes at 30-minute intervals (0 minutes, 30 minutes.,…,180 minutes) after administration of plant extracts (250mg/kg body weight) by oro-gastric intubation. The control animals (10 rats - 5F:5M) were treated similarly except that distilled water (10.0ml/kg) was administered instead of *V. amygdalina* extract.

2.3 Selective Inbreeding

A modified selection method similar to that of Dahl *et al.*, (1962) was used. From the original unselected stock (U), a mating group of male and females (1 male: 2 females) with the greatest percentage of glycaemia reduction after *V. amygdalina* treatment were regarded as sensitive (S), and were selected for inbreeding; those with the least values regarded as resistant (R) were similarly inbred. The presence of sperm cells observed microscopically on the vaginal smear of the females and the observation of mucus plugs on the floor of the cages were indicative of successful mating. Pregnant females were later separated and caged individually until the time of parturition and weaning of the F_1 offspring. The F_1 offspring from the sensitive line were designated as S_1 while their resistant counterparts were R_1. The procedure of selective inbreeding was repeated in each group to obtain F_2 offspring of sensitive (S_2) and resistant (R_2) animals respectively. Brother-sister mating was ensured, and crossbreeding between sensitive and resistant lines was not permitted except in F_2 generation when reciprocal crosses were carried out between S_2 and R_2 to obtain F_3 offspring. This was done in an attempt to see if sex difference plays a role in sensitivity to the hypoglycaemic effect of the plant extract.

2.4 Data Analysis

The percentage of glycaemia reduction was calculated at the 180th minute using the formula (Gidado et al, 2005):

$$\% \text{ Change of Glycaemia} = \frac{G_X - G_O}{G_O} \times 100$$

where G_O and G_X are the values of 0-minute and 180-minute FPGC respectively. The results were analyzed using a statistical software package – SPSS Version 12. Data were expressed as mean $\pm$ standard error of the mean (mean $\pm$ SEM). Student's t-test was employed for comparison between two sets of data. Differences between means were considered statistically significant when $P<0.05$.

3. Results

After treatment with *V. amygdalina*, the FPGC of the original unselected parents fell steadily during the 180-minute fasting plasma

glucose determination from 6.2±2.1 to 5.3±2.4 mmol/l (P< 0. 05) at the 180th minute giving a percent change of glycaemia of –14.5%. This may be compared to the FPGC of the control animals that fluctuated between 5.4±2.2 and 6.7±2.3 mmol/l during the 180-minute FPGC monitoring period bringing about a percent change of glycaemia of 3.0%. Figure 1 shows the response curves of animals bred for sensitivity and resistance at succeeding generations. It can be seen that there was a development of increasing sensitivity to the hypoglycaemic effect of *V. amygdalina* due to selection in succeeding generations of sensitive animals as the curves obtained from S_1 and S_2 offspring fell below those of the other groups (U, R_1 and R_2). Moreover, *V. amygdalina* caused a greater percentage change of glycaemia reduction in S_1 (-35.7%) and S_2 (-49.1%) generations.

Unlike the pattern indicated above, the hypoglycaemic action of *V. amygdalina* was not pronounced in the animals bred for resistance to the hypoglycaemic effect of the plant. The magnitude of percent change of glycaemia reduced with succeeding generations. The response curves of the resistant animals are located above those of their sensitive counterparts; the gradient of slopes of their glycaemic curves is less when compared to that of the sensitive groups (Figure 1). Moreover, it was noted that the difference in response to *V. amygdalina* between S and R lines as measured by percentage change in glycaemia became more significant with succeeding generations of selective inbreeding.

Reciprocal crossbreeding between S_2 and R_2 were made to produce F_3 Comparison of percentage change in glycaemia between F_3 offspring from different reciprocal crosses of S_2 X R_2 did not indicate any significantly difference (P>0.05). Figure 2 made it clear that the plasma glucose curve of the F_3 offspring falls between those of the R_2 and S_2 animals (Figure 2).

4. Discussion

The results of the present study support earlier reports that *V. amygdalina* has hypoglycaemic effect and may therefore be of value in the treatment of diabetes (Ogbuokiri and Ekpechi, 1989; Gyang et al, 2004). In addition, it indicates the possibility of evolving two strains of rats that differ significantly in their response to the hypoglycaemic action of *V. amygdalina*. The fact that this could be achieved through selective inbreeding implies that genetic factors may play important role in sensitivity to the hypoglycaemic effect of *V. amygdalina* in rats. It will be of interest to know the nature of genetic factors involved. Further genetic and molecular studies are still going on in the laboratory for more insight into the issue raised above.

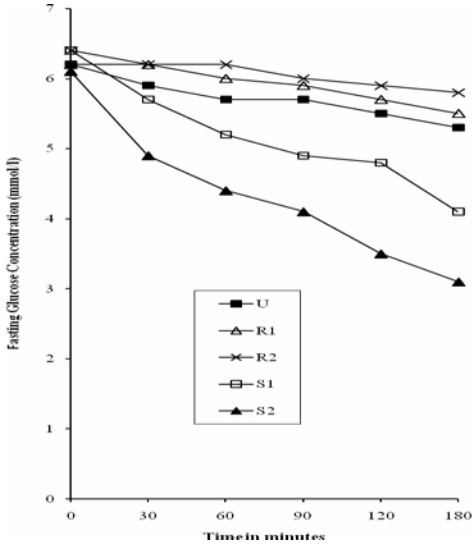

Figure 1. Fasting Glycaemic profiles in Rats in Rats Bred for Sensitivity and Resistance to the hypoglycaemic Action of *V. amygdalin.*
Note: U=unselected stock, R=resistant, S=sensitive, 1=1st generation, 2=2nd generation

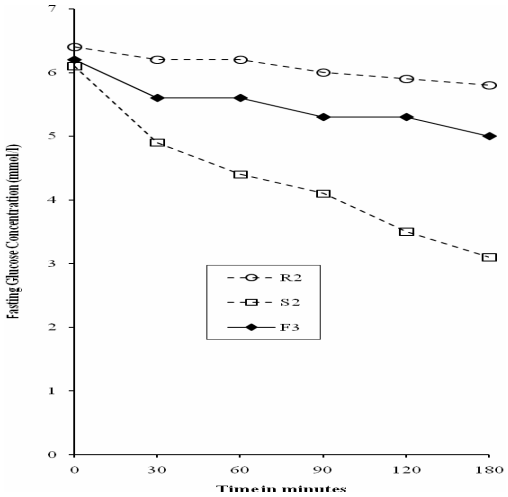

Figure 2. The Result of Cross Mating between Sensitive (S2) and Resistant (R2) to Give F3 Offspring. Note: S=sensitive, R=resistant, F=filial generation, 1,2,3=the level of generation.

In the original unselected parental generation of rats used in this investigation, it was always observed that the variation in sensitivity to *V. amygdalina* was continuous without sharp demarcation into sensitive and resistant animals. Variability in response to drugs may be continuous or discontinuous. If a test is carried out on a large number of subjects and their responses are plotted on a graph, the pattern of distribution may shed some light on the mode of inheritance involved. In the present study, the sample size of the unselected stock (n=24) is too small for any meaningful conclusion to be drawn on the mode of inheritance of glycaemic response to *V. amygdalina*. A much larger population of animals is needed to reveal more clearly the nature of the genetic factors involved. However, the present data, though limited, suggest that variation to glycaemic action of *V. amygdalina* is continuous and may therefore be under a polygenic control.

If the polygenic hypothesis is correct, the S_2 and the R_2 offspring obtained in the study were likely to be more homozygous at many loci than the original unselected stock in view of the well known effects of selective inbreeding on homozygosity. Moreover, the pattern of responses of the S_2 and the R_2 offspring were divergent when compared to their unselected parental stock. In future studies, It will be necessary to further purify these strains through selective inbreeding for more generations with a view to characterizing the genetic components more accurately. In such experiments, it is pertinent to note that albino rats, like other mammals, are outbreeders, and due consideration should be given to the occurrence of inbreeding depression and its associated deleterious consequences. This generally the case with natural outbreeders: Continuous inbreeding in such species increases homozygosity, and many other genes that are slightly or partially deleterious begin to show phenotypically thereby causing reduction in vigour and survival.

An important implication of the results of this study is that different workers studying the hypoglycaemic effect of *V. amygdalina* in animals might arrive at somewhat different conclusions. This will be so if chance selection of animals had led one to study genetically sensitive, and the other a resistant population. It seems reasonable to expect that similar genetic factors regarding sensitivity to the hypoglycaemic action of *V. amygdalina* also operate in man. However, human populations are usually genetically heterogeneous because of outbreeding that is ordinarily enforced in most human societies. It would therefore be illogical to expect a given group of humans to demonstrate uniform sensitivity or resistance to the hypoglycaemic effect of *V. amygdalina* as shown by these selectively inbred rats: Studies of isolated small human populations will, however, be somehow elucidating. Given the heterogeneous nature of human populations and the possible involvement of genetic factors in hypoglycaemic response to *V. amygdalina* as suggested by the results of this study, It is unlikely that diabetic patients on the same *V. amygdalina* treatment schedule would have similar degree of hypoglycaemic response to the plant. On the contrary, one would expect different treatment outcomes despite similar therapeutic regimes.

During our preliminary survey in a study recently concluded (results not yet published), it was discovered that some Nigerian herbalists, especially those in the southwestern part, sometimes secretly add oral hypoglycaemic drugs, particularly tolbutamide, to their concoctions for treating diabetes. The basis of this practice is not yet clear; however, it might be a strategy to increase the potency of antidiabetic herbal preparations as herbalists encounter isolated cases of poor response to herbal treatment in a manner similar to the case of the animals bred for resistance in this study. Previous reports from our laboratory indicating that hypoglycaemic action was enhanced when some plant extracts were administered into experimental animals simultaneously with tolbutamide as combined solutions are pertinent to this practice (Odeigah et al, 1999; Taiwo et al, 2008).

V. amygdalina is a pharmacodynamic plant with wide geographical distribution in Africa. It is commonly used for dietary and medicinal purposes in many parts of Africa especially in Nigeria. Previous reports have indicated that it may be of value in the treatment of diabetes (Ogbuokiri et al, 1989; Taiwo et al, 2008). The data obtained in this preliminary study further suggest that the plant may elicit varying hypoglycaemic responses in animals and, possibly, human subjects as a result of genetic factors. Further selective breeding and molecular studies are now being carried out in the laboratory to determine the heritability of this trait and possible association of various RFLPs and other molecular markers with the different inbred lines. The results of such molecular

studies will help in the easy identification of patients for appropriate treatment programme.

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Influence of Acute intake of Cooking Salt and Laboratory Salt on Glyceamic Response to Glucose Loading in Rats

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Abstract: An investigation on the effect of cooking salt and reagent grade laboratory salt on glyceamic response was carried out in albino rats. Oral glucose load of 3.0 g/kg body weight (b.wt) was given with or without addition of 0.9% cooking or laboratory salt. Both types of salt caused significantly higher ($P<0.05$) peak plasma glucose concentration (PPGC) at 60 minutes after loading (cooking salt: 10.4±2.4 mmol/l; laboratory salt: 10.0±1.2 mmol/l) than the control (PPGC of 7.6±0.3 mmol/l). Moreover, the PPGC of the salt treated groups was not brought down to the normal level at 120 minutes unlike in the control where the level fell to 6.8±0.3 mmol/l at 120 minutes. The glucose tolerance index (GTI), determined as area under the glucose tolerance curve, was higher ($P<0.05$) in the animals treated with laboratory salt (234.0±25.6mmol.min/l) and cooking salt (251.3±21.8 mmol.min/l) when compared to the control (51.0±15.9 mmol.min/l). It was therefore concluded that both types of salt increased glaceamic response to glucose challenge. The results imply a beneficial effect of salt restriction on glycaemic control. [Nature and Science 2009;7(11):70-73]. (ISSN: 1545-0740).

Key words: dietary salt; glycaemic response; oral glucose tolerance; diabetes

1. Introduction

There are considerable human and animal experimental studies implicating excessive dietary salt intake in cardiovascular diseases especially hypertension (Garrett et al, 2006). However, the long standing issue of the effect of salt on carbohydrate metabolism is still unresolved. Thorburn et al (1986) reported that adding salt to two common starchy foods resulted in an increase of the postprandial plasma glucose and insulin responses in human subjects. This agrees with the report of Odeigah et al (1994) that salt caused a higher peak plasma glucose level during oral glucose tolerance test (OGTT) in treated rats when compared with untreated rats. A mechanism that possibly involves influence of salt on digestive, absorptive or/and post-absorptive events was postulated. These authors and many others supported the call urging the diabetics and the general public to reduce their salt intake.

The observations that salt increases glycaemic response attracted considerable attention in the light of the observed association between hypertension and diabetes (Fuller, 1985). More recent reports by Yang et al (2008) and Ma et al (2009) had further highlighted some of the severe complications associated with both diseases. On the contrary, Gans et al (1987) and Foo et al (1998) found no association between salt loading and fasting plasma glucose or insulin levels. Despite the call by other workers urging diabetics and the general population to reduce their salt consumption, Gans et al (1987) and Foo et al (1998) did not support a beneficial role of salt restriction on glycaemic control in diabetes.

Previous studies have considered the effect of reagent grade laboratory salt on carbohydrate metabolism; however, possible glycaemic effects of common cooking salt, the form in which common salt is usually consumed in the general population, has received very little or no attention. Considering the aetiologic role of salt in hypertension, the observed association between hypertension and diabetes, the role of diet in hypertensive and diabetic management, and the fact that in the general population it is common cooking salt that is normally consumed and not the reagent grade laboratory salt, it will be interesting to see the glycaemic effects of this salt as compared to that of the reagent grade laboratory salt used in previous studies. The results of such study may shed more light on the implications of excessive dietary salt consumption in diabetes. We therefore carried out a short-term comparative study on the effects of these two types of salt on glyceamic response after acute glucose loading in rats.

If the observation that salt increases glycaemic response is correct, then both types of salt should reduce oral glucose tolerance in rats. Although sodium chloride NaCl) is the major constituents of both types of salt, It is expected that both salts should influence glycaemic response to different degrees

because of their different composition regarding other components that are present apart from NaCl. The present study was therefore carried out to investigate these possibilities. Possible mechanisms of salt influenced glyceamic response and the implications of salt in diabetic management were discussed.

2. Materials and methods

Twenty adult male Sprague-Dawley (SPD) rats (180-200g) obtained from the Nigerian Institute of Medical Research (NIMR), Lagos, were randomly divided into three groups of 6 – 7 rats per cage for acclimatization in the Animal House of the University of Lagos. They were allowed free access to rat feed and tap water. All the animals were handled following the Guiding Principles in the Care and Use of Laboratory Animals endorsed by the American Physiological Society.

The animals were fasted for 18hours and were given 3.0g/kg body weight (b.wt.) of glucose load as 30% solution with or without 0.9% salt under light ether anaesthesia using oro-gastric intubation. Blood samples (125µl) were taken into heparnised capillary tubes from the tail just before the oral glucose loading (0 minutes) and at 30, 60, 90 and 120 minutes thereafter. Plasma was obtained by centrifugation (3,000 r.p.m.), and plasma glucose determinations were carried out by the glucose oxidase method (Trinder, 1969). The results were expressed as mean ± SEM, and the GTI or glucose tolerance index was taken as the incremental area under the glucose tolerance curve (Lebovitzand Feinglos, 1983). Statistical differences between means were determined by Students t-test and p values less than 0.05 were considered significant. Data analysis was done using a software package: Statistical Package for Social Scientist (SPSS) version 12.

3. Results

The results of the oral glucose tolerance test (OGTT) is presented in Figure 1. The starting fasting plasma glucose concentration (FPGC) of the animals range between 6.8±2.5-7.2±3.0. Thereafter, the plasma glucose concentration rose to different peak levels which were attained at the 60th minute of OGTT. When compared to the peak plasma glucose concentration (PPGC) of the control (7.6±0.3mmol/l), both types of salt caused higher (P<0.05) PPGC (cooking salt:10.4±2.4 mmoll/l; laboratory salt: 10.0mmol/L). Although cooking salt caused slightly higher PPGC than laboratory salt, the difference was not significant (P>0.05). The PPGC dropped to the normal level (6.8±0.3 mmol/l) in the control animal at 120 minutes but remained relatively high (P<0.5) in the salt-treated groups (cooking salt: 8.4±1.4 mmol/l; laboratory salt: 8.0±1.2 mmoll/l). Moreover, the glucose tolerance index (GTI) of the salt-treated groups (cooking salt = 251.3±21.8 mmol.min/l; laboratory salt=234.0±25.6 mmol.min/l) were significantly higher (P<0.05) than that of the control with a mean GTI of 51.0±15.9 mmol.min/l (Figure 2). Animals treated with cooking salt had higher but not significant GTI compared to those treated with laboratory salt; however the difference was not significant (P>0.05).

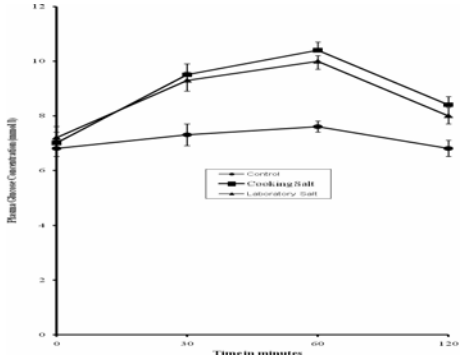

Figure 1. Comparative Influence of Cooking and Laboratory Salt on Glycaemic Response in Rats

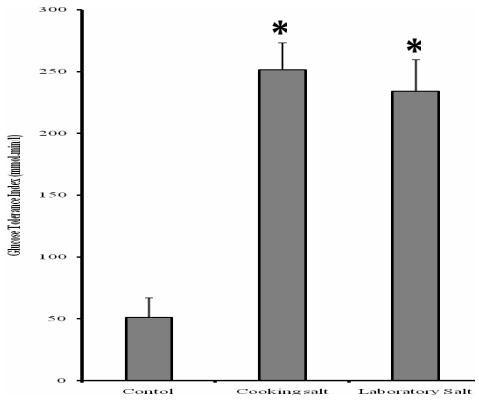

Figure 2. Higher Glucose Tolerance Index in Rats Treated with Cooking Salt and Laboratory Salt. Significant Difference from Control P<0.05) is Indicated by *

4.0 Discussion

Previous studies have shown variable effects of salt on glycaemic response. Our results agree with those of the Thorburn et al (1986) and a later study by Odeigah et al (1998) who reported that salt caused increased glycaemic response to carbohydrate feeding. These reports are discordant with those of Gans et al (1987) and Foo et al (1998) who observed that salt intake had no effect on glucose metabolism. These disparities suggest that other factors possibly

genetic may play important role in glycaemic response to salt.

Findings from these studies have important health implications as regards the effect of dietary salt consumption in man. Considering the fact that human populations are genetically heterogenous as a result of outbreeding that is generally enforced in many societies, different individuals in a population are expected to show different sensitivities to glycaemic effect of salt. In view of this, different results would be obtained if by chance selection a researcher has as subjects, individuals that are sensitive while the other conducts his study in individuals that are resistant. The possibility of such sampling variation is particularly high in human experimental studies because the sample size is usually very small. The work of Gans et al (1987) is interesting in this respect; the results of their work did not support a beneficial effect of salt restriction in glycaemic control; however, they observed a trend toward increase in glycaemic level due to salt. According to these workers, this was not significant because the subjects showed "…considerable(2) variability in glucose response". In our own view, the presence of genetic factors, as the results of the present study suggests, may account for such(3) variability in response. (3) d

Thus, the hyperglycaemic effect may be reduced or even eliminated especially if chance selection had caused the use of animals that were genetically resistant to the glycaemic effect of salt. Compared to(4) (the work of other investigators who used human(4) F subjects, the animals used in our study were likely more isogenic than human subjects in view of some degree of inbreeding that was allowed in the parent(5) (rat colony. This is not the case with humans because inbreeding is usually discouraged in most human populations. Therefore, the differences seen in response to the glycaemic effect of salt may be, at least partly, genetic. Efforts are presently being made in our laboratory to determine the heritability of this trait and its degree of response to selection with a view to creating two strains of rats namely those that are sensitive to the glycaemic effect of salt and those that are not sensitive through selective(5) inbreeding. If sensitivity to the hypoglycaemic effect has a genetic component as suggested in this study, it should be possible to create two strains of animals that differ significantly in their response to the(6) F hypoglycaemic effect of salt through selective[(6) breeding.

In contrast to the views of some workers who do not support a beneficial effect of salt restriction in glycaemic control, the results of this study indicates that the recommendation urging the general population to restrict their salt intake should be upheld. This is particularly so for diabetics since success of diabetic management depends on good glycaemic control.

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Prevalence of Transactional Sex in Selected Fishing Communities of Kainji Lake Basin

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Abstract: The paper examined the prevalence of transactional sex in the fisheries sector of Yauri emirate of the Kainji Lake Basin. A total of 187 questionnaires were administered in ten selected fishing communities and further subjected to descriptive analysis. The study on HIV/AIDS carried out revealed that prevalence of transactional sex is on the increase in selected fishing communities of the Kainji Lake Basin.. 11.8% have accepted sexual various propositions in exchange for work related activities. It is no longer gainsaying that people exchange sex for gift or economic gain therefore; transactional sex activities are gradually being noticed in some of the fishing communities of Kainji Lake Basin. Recommendations were proffered for the study. (Nature and Science. 2009;7(11):74-80]. (ISSN: 1545-0740).

Keywords: Fish, Sex, gift, livelihood and HIV/AIDS

1. Introduction

Nigeria has one of the fastest growing rates of new HIV/AIDS cases in West Africa and an enormous population.(UNAIDS, 2004) Findings from the Ministry of Health's 2003 HIV sero-prevalence survey revealed a national HIV prevalence rate of five percent, similar to the recent UNAIDS estimate of 5.4 percent. Small scale fisheries and related activities such as fish processing and trading have long been recognized to provide safety net for the 'poorest of the poor' in rural communities in developing countries (Panayotou, 1985, Jui Larsen et al 2003, Neiland and Bene, 2004) Food security is one of the major problems facing humanity particularly in the developing countries like Nigeria.

There is a growing consensus in literature that HIV/AIDS increases food insecurity and poverty (Baylies, 2002; DeWaal2002; Du Guerny, 2002; Boudreau and Hollemam, 2002; SADCFANR, 2003). In addition, some scholars note an indirect effect of HIV/AIDS: the early adoption of coping strategies which leave households vulnerable to other "shocks" (Rugalema, 1999; De Waal2002; SADC FANR, 2003). While both men and women are engaged in fish trade, local women are aware that they have a good opportunity to trade fish with full time fishers and other seasonal immigrants in the fishing camp even if they lack initial capital to start with. Women, as local informant put it, "go to the flats with nothing and return with a lot of fish. As a form of networking, some local women have their boyfriends in the fishing camps, which they visit and from whom they get fish usually on a regular basis, in exchange for sexual favour, (Bene and Merten 2008).

As one woman describes it "For those who have a boyfriend there (in the camp) it is easier. They get sometimes for the same price, while others who do not have a boyfriend have to pay more. So, as I am having boyfriend, I get the fish much cheaper so I can also sell it cheaper. So there is no big loss (if prices drop). Nevertheless, many of the fish I was just given because I was staying with the boyfriend. So these I can sell now" (Mbeza, 2002). The objective of the study is to investigate the extent to which fish/gift items are exchange for sex in selected fishing communities of Yauri emirate of Kainji Lake basin.

2. Methodology

Kainji lake basin comprises of Niger and Kebbi States with the following neighbouring emirates Kontagora, Borgu and Yauri . For this study, the sample was taken from Yauri emirates from the following communities: Wara, Wawu, Tunga Mairuwa, Zamare, Rukubalo, Yauri, Rashe Salkawa, Hella, Barashi Tunga Alhaji Sharo. The selections of these communities were based on accessibility, level of fisheries activities and traditional institutions. A total of 187 questionnaires and 20 interview guides for key informants will be administered in the communities and further subjected for statistical analysis.

3. Result and Discussion

On the socio – economic characteristics as shown in Table 1, on sex, 63.6% of the respondents were males while 36.4% were females. The variation may be as a result women restriction to their household that is, they are in Purdah, which buttresses the findings of gender studies carried out by Yahaya, 1999. The higher number of males in the study agrees with findings of experts that

almost twice as many men as women were aware of HIV/AIDS. (UNAIDS, 1998)

76.0% of the respondents were still in their active (reproductive) age, that is, 15 – 45 years. 24% were above 46 years. These ages are the active and they are crucial to agricultural development. It implies that they were in sexually active ages which support the finding of NDHS (2003) that majority of those who contract the HIV/AIDS virus fall under the age of 30 years (NDHS, 2003). Thus, they are the very people who are vital to the economic future of the rural communities where poverty is dominant.

Majority of the respondents (78.1%) were married, 21.4% were single while 0.5% were widow. This is an indication of a tendency for sexual continuation, particularly among the married people of the fishing communities. On religion, the respondents (84.5%) were Muslim faithful, only 15.5% practiced Christianity and 0.5% claimed to be idol worshipper. With this finding the belief of the majority supports more than one wife and encourages multiple relationships. Majority (58.7%) were into polygamy, 2.1% were monogamous and 49.2% could not response. This is not surprising because some of the unmarried respondents may constitute to the high percentage. Only 18.7% had primary education and the same percent for respondents who had secondary school education.

More than half of the respondents (57.2%) had no formal education. Some of the fishing communities are more interested in sending their children to Quranic School within and outside the community than attending western education. This has made them not see the need for at least primary school in their immediate environment. Therefore, the low level of western education may affect the knowledge of devastating HIV/AIDS that is ravaging globally.

The study revealed that 84.5% of the respondents had their primary occupation in fisheries related activities and only 15.5% were into skill labour (such as welding, carpentry) and trading in other products. 27.8% of the respondents had secondary occupation such as firewood cutting, food hawking and haulage. The result corroborates Neiland et al, 2005 that combination of activities ranging from catching, processing, trading and transportation are important occupation in the fishing communities.

Characteristic of Respondent

Variable	**frequency(F)**	**Percent (%)**
Sex		
Male	119	63.6
Female	68	36.4
	187	**100**
Age		
15-25	45	24.1
26-35	55	29.4
36-45	42	22.5
46-55	28	15.5
Above 55	17	9.1
	187	**100**
Marital Status		
Single	40	21.4
Married	146	78.1
Widow	1	0.5
Separated	-	-
Divorced	-	-
	187	**100**
Number of wife		
One	4	2.1
Two	59	31.6
Three	27	14.4
More than three	5	2.7
No response	92	49.2
	187	**100**
Religion		
Islam	157	84.5
Christianity	29	15.5
Idol	1	0.5
	187	**100**
Education		
Primary	35	18.7

Secondary	35	18.7
Tertiary	5	2.7
Adult Education	5	2.7
No formal education	107	57.2
	187	**100**
Primary Occupation		
Fishing	23	12.3
Farming-fishing	23	12.3
Trading in fish	15	8.0
Processing of fish	40	21.4
Boat construction	27	14.4
Craft/gear making	7	3.7
Skilled labour	5	2.7
Others	29	5.5
	187	**100**
Secondary Occupation		
Skilled labour	1	0.5
Firewood cutting	2	1.1
Food vendor	45	24.1
Transporting	4	2.1
No response	135	72.0
Total	**187**	**100**

62.5% of the respondents said they became sexually active between ages 15-20 while 10% were sexually active before age 10. About 35% could not remember the exact age .16.9% have had 1-2 sexual partners since they were 12years old. 11.6% had 3-4 sexual partners since then, 8.3% had above 5 partners, 28.2% had 5 or more sexual partners. Majority (68.1%) live with one partner, 21.9% live with two, and 7.5% live with three and 2.5% live with four. 6.0% of the respondents had over 10 sexual partners before marriage, 7.0%) had between 5-9 partners, 29.9% had between 1 – 4 partners and 57.0% said they had none. 9.3% said they have had extra marital sex while 86.5% claimed that they never did; 3.5% did not response. 1.0% had experienced extra marital sex with over 10 persons in the past 12 months, 4.1% with between 5-9 persons, and 18.4% with between 1-4 persons.

Majority indicated that they have been involved in sexual relationships with more than one partner, suggesting the occurrences of multiple sex partners even among the married. Majority of respondents said that in the last twelve months their involvement in sex with multiple partners had not required the use condom or any other safe sex practices. This is a factor that is capable of spreading of sexually transmitted infections (STIs), HIV and unwanted pregnancies in the communities.

Table 2: showing Sexual behaviour, Common Diseases and Prevention in the Fishing Communities.

Age of first sexual intercourse	**Frequency(F)**	**Percent(%)**
10-15	65	34.8
16-20	74	39.6
21- 25	23	12.3
26-30	10	5.3
Above 30	4	2.1
No response	11	5.8
Total	187	100
Partners since age 12 years		
One	39	20.9
Two	30	16.0
Three	13	7.0
Above three	21	11.2
No response	20	10.7
Total	**187**	**100**
Never had any sexual relation		
Still too young for sex	8	4.3
Too old for sex	5	2.7
A decision to abstain	10	5.3
Don't consider it necessary	2	1.1
No reason	2	1.1
No response	160	85.6
Total	**187**	**100**
If married when did you start		

married life (year)		
1-5	19	10.2
6-10	34	18.2
11-15	50	26.7
Above 15	14	7.5
No response	70	37.4
Total	**187**	**100**
Number of sexual partners before marriage		
Between 1 and 4	60	32.1
Between 5 and 9	17	9.1
Over 10	24	12.8
None	71	38.0
No response	15	8.0
Total	**187**	**100**
Use of condom during sex		
Yes	38	20.3
Never	141	75.4
I can't remember	2	1.1
No response	6	3.2
Total	**187**	**100**

26% of the respondents were involved in extra marital affair while 74 .3% said they are not involved. On reasons for their involvement 17.1% of the respondents said it was a routine as part of life style, 2.7% said long separation from home, 1.6% said because they needed money and 74.3% did not response. The majority did not response it may because they are private or personal matter and such they are willing to discuss especially if someone is not familiar to them. This result may have serious implication on the spread of HIV/AIDS in the selected fishing communities. 36.4% of the respondents said their activities take them away from home which confirmed one of the attributes of fisherfolk as a mobile group, this result also substantiate the reason they are involved in extra marital affairs. The study revealed sexual activity when away from home, 13.4% said they sexual relation and only 10.7% did not response. The information from key informant confirmed that there is prevalence of transactional sex in the study area.

4.8% claims to engage a professional colleague as regular partner among occasional sexual partners while 9.6% said with those individuals who patronized their services. Only 1.1% patronized Commercial Sex Workers (CSWs). The seasonality in the fisheries may encourage the people to succumb to such arrangement to sustain their means of livelihood and most time the fisherfolk have daily cash flow within their reach. 11.8% have accepted sexual various propositions in exchange for work related activities and 41.2% did not response. Although, 2.1% said is what they do often while 15% said sometime which corroborates Awounda (2003) that due to poverty women fishmongers have become victims of fishermen who are now demanding sexual favours on top of supplying fish". It is no longer gainsaying that people exchange sex for gift or economic gain for their up keep, commercial sex activities are thriving in the area which may be one of consequences of effect of global warming on the water bodies which the desired attention has not been proffered. 32.6% said there are commercial sex workers (CSWs) in their communities, 42.2% said there are none, and 17.1% said they do not know if they could be found in the communities. . , 7% said they come from within the community, 11.2% said they come from nearby villages and 15.5% did not know where they come from. The result revealed different ethnic group may be involved in such activity.

5.9% of the respondents said there are just a few CSWs, 19.3% said they are many and majority did not response. The non response of the majority may be to protect the communities from been stigmatized since their religion forbids such activities and this might be that they want to disabuse the mind of the people that their communities are free of HIV/AIDS risks. Most of the CSWs might have been attracted by presence of migrant fishermen who often stay away from their individual families. Similarly, the availability of daily cash income in the hands of young adult fishermen may also attractive ladies/hawkers to the communities. From the study, it was revealed that prevalence of sex for exchange of gift/economic gain. This could be done directly or indirectly in subtle manner among commodity hawkers and their clients which supports the statement of ActionAid Kenya (2003) that "women traders who wants to buy fish are often coerced to offer sex for fish.

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Table 3. Showing the exchange of goods for sex and various transactional sexual activities in the study area

Variable	Frequency (F)	Percent (%)
Involvement in extra-marital sex		
Yes	48	25.7
No	139	74.3
Total	**187**	**100**
Push factors to extra marital affairs		
Vengeance	3	1.6
Routine	32	17.1
Long separation	5	2.7
Meeting old partners	1	0.5
Just a need for change	4	2.1
Need for money	3	1.6
No response	139	74.3
Total	**187**	**100**
Activity cause separation from home		
Yes	68	36.4
No	119	63.6
Total	**187**	**100**
Sexual relation outside home		
Yes	25	13.4
No	142	75.9
No response	20	10.7
Total	**187**	**100**
Category of sexual partners Outside		
Professional colleague	9	4.8
People who use my service	18	9.6
People I met while conducting my activity	5	2.7
Prostitute	2	1.1
No response	153	81.8
Total	**187**	**100**
Acceptance of sexual proposition on work related activities		
Yes	22	11.8
No	88	47.1
No response	77	41.2
Total	**187**	**100**
Sex proposition in exchange for money		
Often	4	2.1
Sometimes	28	15
Never	102	54.5
No response	53	28.3
Total	187	**100**
Commercial sex workers in the locality		
Yes	61	32.6
No	79	42.2
I don't know	32	17.1
No response	15	8.0
Total	187	**100**
Origin of the commercial sex workers		
Indigene	13	7.0
From nearby villages	21	11.2
I don't know	29	15.5
No response	81	20.4
Total	**187**	**100**
Population of commercial sex workers		

Just few	11	5.9
About 10	3	1.6
Many	36	19.3
No response	137	72.7
Total	**187**	**100**
Patronage of commercial sex workers		
Yes	22	11.8
No	1	0.5
I don't know	48	25.7
No response	116	62.0
Total	**187**	**100**
Use the services of the commercial sex workers		
Often	7	3.7
Sometime	6	3.2
Never	92	49. 2
No response	82	43.9
Total	**187**	**100**

Conclusion

The study investigated the exchange of fish and other gift items for sex in fishing communities as observed in a number of countries around the World. It was discovered that the occurrence of transactional sex is gradually setting in some of the fishing communities in the Kainji Lake Basin. These facts were discovered during a study to test the knowledge, attitude and practices of HIV/AIDS in fisheries sector of Yauri emirate of Kainji Lake Basin which is in consonance with the situation found in the Lake Chad Basin. Also discovered was their involvement at one time or the other in fish/gift items for sex brought to mind the relatively high vulnerability of this group to poverty. This is associated with limited access to fishing opportunities, basic infrastructure, social amenities and other livelihood diversifications. The prevalence of fish for sex in fishing communities is gradually been noticed in the study area. Therefore the following are recommended in the Yauri emirate of Kainji Lake Basin.

- Mainstreaming of gender equality in the fishing communities
- Awareness raising, and prevention through condom use campaign
- Empowerment interventions

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Distribution pattern of Oak and Pine along altitudinal gradients in Garhwal Himalaya

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Abstract: The study was carried out in the oak and pine forest for community composition and soil characteristics along altitudinal gradients of Garhwal Himalaya. The soil pH of oak forest was acidic while in pine forest its slightly acidic. The soil organic carbon (SOC) was higher in oak forest. Although between the forests the SOC content decreased with increasing altitudes in both the forest. Nitrogen in both the forest also decreased with increasing altitudes however, the trend was reverse for phosphorus. Among the sites Quercus leucotrichophora was dominant on all the sites. As SOC and nitrogen decreased with increasing altitudes same pattern of species total density and TBC was reported which also decreased with increasing altitudes. Throughout the oak and pine forests *Quercus leucotrichophora* and *Pinus roxburghii* were distributed contagiously. The study can be concluded that SOC and nitrogen availability on the reducing trends in both the forests with altitudes might be due to reducing density and total basal cover of the trees with altitudes. [Nature and Science. 2009;7(11):81-85]. (ISSN 1554-0200)

Keywords: Distribution pattern, oak, pine, altitudinal gradient, Garhwal Himalaya

1. Introduction

The Garhwal Himalayas embodies a number of forest types that are distributed at various altitudes, geological formations and soil types (Champion and Seth, 1968; Saxena and Singh, 1982). The temperate forests of Western and Central Himalaya are usually distributed from 1200 to 3000 m asl, and characterized by extensive oak and coniferous forests (Kumar and Bhatt, 2006). Oak is the most preferred tree species in the temperate region, mainly used for fodder, fuel, and small timber.

Forest soils influence the composition of the forest stand and ground cover, rate of tree growth and other silviculturally important factors. Physiochemical characteristics of forest soils vary in space and time due to variations in topography, climate, physical weathering processes, vegetation cover, microbial activities, and several other biotic and abiotic variables. Vegetation plays an important role in soil formation (Chapman and Reiss, 1992). Plant tissues (from aboveground litter and belowground root detritus) are the main source of soil organic matter (SOM), which influences physiochemical characteristics of soil such as pH, water holding capacity (WHC), texture and nutrient availability (Johnston, 1986). Nutrient supply varies widely among ecosystems (Binkley and Vitousek 1989), resulting in differences in plant community structure and production (Ruess and Innis 1977).

The Himalayan forest vegetation ranges from tropical dry deciduous forests in the foothills to timberline. Forests are the main source of livelihood of the people living in Uttarakhand, Central Himalaya. Forests of this region are mainly dominated by *Pinus roxburghii* and *Quercus leucotrichophora. Pinus roxburghii* is the most common resin producing pine species of India and also provide alternate source of fuelwood and leaves for bedding materials, however *Quercus leucotrichophora* is important source of fuel, fodder and other daily needs of the villagers. Therefore, an attempt was made to analyze the forest community structure in relation to physiochemical properties along altitudinal gradient in both the forests in Garhwal Himalaya.

2. Materials and Methods

2.1 Study site

The present study was carried out in two different regions i.e., temperate oak forest (located 30° 07’ 09.9” to 30 ° 7’ 12.3” N and 78 ° 47’46.5” to 78 ° 47’42.5” E at an elevation range of 1700 to 1900 msl) and sub-tropical pine forest (located 30 ° 12’ 51.2” to 30 ° 12” 51.0” N and 78 ° 48’ 25.2” to 78 ° 49’ 02.2” E at an elevation range of 700 to 900 m asl).

The phytosociological study was carried in the tree layer by using 10 x 10 m quadrats. A total of 10 randomly placed quadrats were used on each site. The size and number of quadrats were determined by the species area curve (Misra 1968) and the running mean methods (Kershaw 1973). In each quadrate >30 cm circumference (at 1.37 m from the ground) were considered tree. The vegetation data were quantitatively analyzed for abundance, density and frequency (Curtis and McIntosh, 1950). The importance value index (IVI)

was determined as the sum of the relative frequency, relative density and relative dominance (Curtis 1959).

For the soil analysis, the samples were mixed well individually before use. Then samples were air dried at 20 to 25°C and 20% to 60% relative humidity (Jackson, 1958). Soil pH was measured with the help of dynamic digital pH meter. Soil organic carbon (SOC) percent was determined by Walkley and Black's rapid titration method (Walkley and Black, 1934).Total nitrogen (%) was measured using the standard kjeldal procedure. Exchangeable phosphorus (P) and available potassium (K) was determined by (Jackson, 1958).

3.2 Results and Discussion

3.1 Soil characteristics

In oak forest, the soil pH (Table 1) was 5.6±0.54 (1700m), 5.8±0.17 (1800m) and 5.5±0.25 (1900m). The SOC decreased with increasing altitude as 0.90±0.10 (1700m), 0.88±0.14 (1800m) and 0.80±0.07 % (1900m). Nitrogen has also remained same trend as SOC. The phosphorous was shown reverse trend with SOC and nitrogen at altitude. The values of phosphorous at altitude were 11.42±0.94 (1700m), 13.02±1.35 (1800m) and 13.45±0.51 kg ha^{-1} (1900m). Potassium at altitude 1700m was 108.2±6.55 kg ha^{-1} and at altitudes 1800m and 1900m 108.90±11.65, 99.02± 29 kg ha^{-1} respectively (Table 1).

In pine forest, the values of soil pH increased with increasing altitudes. The values of SOC decreased with increasing altitude (Table 1). The values of SOC were 0.75±0.05 (700m), 0.63±0.09 (800m) and 0.62±0.10 % (1000m). Nitrogen, potassium decreased with the altitude however, phosphorous increased with increasing altitudes (Table 1).

Table 1: Soil characteristics in oak and pine forests

Site/Altitude	Soil pH (1.2:5)	SOC (%)	Nitrogen (%)	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})
Oak					
Site-I 1700m	5.6±0.54	0.90±0.10	0.045±0.005	11.42±0.94	108.2±6.55
Site-I 1800m	5.8±0.17	0.88±0.14	0.044±0.007	13.02±1.35	108.90±11.65
Site-I 1900m	5.5±0.25	0.80±0.07	0.040±0.003	13.45±0.51	99.02±22.29
Pine					
Site- I 700m	6.51±0.19	0.75±0.05	0.037±0.002	22.1±2.43	116.48±9.81
Site-II 800m	6.75±0.11	0.63±0.09	0.032±0.005	23.80±1.56	107.86±18.0
Site-II 1000m	6.77±0.13	0.62±0.10	0.031±0.005	24.18±0.96	105.15±30.25

3.2 Phytosociological study

3.2.1 Oak Forest

The quantitative information of oak forest is shown in Table 2. On site-I (1700m) the dominant tree was *Q. leucotrichophora* and the least dominant species was *P. roxburghii* which was very low in number at this altitude. The other tree species reported on this site were *Myrica esculenta, Rhododendron arboretum*. The associated ground floras were *Pteris* sp. and *Berberis asiatica*. The distribution pattern of *Q. leucotrichophora* and *M. esculenta* was contagious however, *R. arboreum* and *P. roxburghii* were distributed randomly.

On site-II (1800m), *Q. leucotrichophora* was again dominant with highest value of IVI (159.85), density (620 tree ha^{-1}) and TBC (38.38 m^2 ha^{-1}). Other competing trees were *M. esculenta*, *R. arboreum* and *P. roxburghii*. The distribution pattern of most trees was random except *Q. leucotrichophora* which was distributed contagiously (Table 2). The associated ground floras with trees were *Pteris* sp. *Berberis asiatica, Pyracantha crenulata* and *Eupatorium* sp.

On site-III (1900m), again the dominant tree was *Q leucotrichophora* and least dominant *P. roxburghii* (Table 2). *M. esculenta* and *R. arboreum* were the associated species. The distribution pattern of most species was contagious except *P. roxburghii* was randomly distributed (Table 2).

3.2.2 Pine Forest

The quantitative information of *P. roxburghii* is shown in Table 3. On site-I (700m). The frequency, density and TBC of the tree was 100 (%), 560 (tree ha^{-1}) and 56.94 (m^2 ha^{-1}) respectively. The distribution pattern of *P. roxburghii* was contagious. The shrub species reported on the site were *Asparagus racemoses, Rhus parviflora.*

On site-II (800m), the density of *P. roxburghii* was 540 (tree ha^{-1}) and total basal cover was 53.26 (m^2 ha^{-1}). The distribution pattern of *P. roxburghii* was contagious. Other associated ground floras were *Rhus parviflora, Carrisa spinarum, Asparagus racemoces, Mallotus phillipensis, Nepta*

hindostana, *Artemisia scorpia* and *Colebrookia oppositifolia*.

On site-III (1000m), of this forest, the density and TBC of pine tree was 500 (tree ha^{-1}) and 26.79 (m^2 ha^{-1}) respectively. The distribution pattern of a species was contagious. The associated ground floras were *Sapium insigne*, *Rhus parviflora*, *Lantana camara* and *Carissa spinarum*.

Table 2: Frequency (%), density, TBC, A/F ratio and IVI of oak forest in different altitude

Site/ Altitude	Species	Frequency (%)	Density (Trees ha^{-1})	TBC (m^2 ha^{-1})	IVI	A/F ratio
Site-I (1700m)	*Quercus leucotrichophora*	100	660	41.08	167.26	0.066
	Myrica esculenta	80	320	10.40	77.52	0.051
	Rhododendron arboreum	60	100	4.54	41.88	0.027
	Pinus roxburghii	20	100	2.84	13.34	0.0256
	Total		**1100**	**58.86**		
Site-II (1800m)	*Quercus leucotrichophora*	100	620	38.38	159.85	0.062
	Myrica esculenta	100	200	6.44	62.04	0.026
	Rhododendron arboreum	60	120	5.04	39.35	0.028
	Pinus roxburghii	60	100	5.78	38.76	0.026
	Total		**1040**	**55.64**		
Site-III (19000m)	*Quercus leucotrichophora*	100	520	25.82	155.8	0.052
	Myrica esculenta	60	200	5.62	57.64	0.053
	Rhododendron arboreum	40	120	5.34	40.96	0.075
	Pinus roxburghii	60	120	4.10	45.6	0.031
	Total		**960**	**40.88**		

Table 3: Frequency (%), density, TBC, A/F ratio and IVI of pine forest in different altitude

Site /Altitude	Species	Frequency (%)	Density (Trees ha^{-1})	TBC (m^2 ha^{-1})	IVI	A/F ratio
Site- I (700m)	*Pinus roxiburghii*	100	560	56.94	300	0.056
Site-II (800m)	*Pinus roxiburghii*	100	540	53.26	300	0.054
Site-III (1000m)	*Pinus roxiburghii*	100	500	26.79	300	0.051

4. Discussion

The range values of soil pH, SOC, nitrogen, phosphorus and potassium of oak and pine forests of present study is presented in Table 5. The comparative studies of the related soil parameters is also given by Sharma and Kumar (1991), Bhandari *et al.* (2000), Dhanai *et al.* (2000), Kumar *et al.* (2009) for Garhwal Himalaya forests and Singh and Bhatnagar (1997) and Khera *et al.* (2001) for Kumaun Himalaya (Table 4).

The range values of density, TBC is also present for oak and pine forests in Table 5. The comparative values of density and TBC of other forests is studied by various workers for Garhwal Himalayan forests (Rajwar, 1991; Kusumlata and Bisth, 1991 Sharma *et al.*, 2001) and Kumaun Himalayan forests (Pant, 1987; Nayak *et al.*,1991; Saxena and Singh, 1982).

Among the distribution pattern of the species most of the species in oak forest and pine in all the site was distributed contagiously and few species in oak forest were distributed randomly. Contagious distribution has been reported by several workers Greig-Smith (1957); Kershw (1973); Singh and Yadav (1974). Odum (1971) have emphasized that contagious distribution is the commonest pattern in nature. Kumar and Bhatt (2006) also reported contagious distribution pattern in foot-hills forests of Garhwal Himalaya.

Table 4: Comparative studies of soil of oak and pine forests.

Soil Parameter	Range values	Forest type	Regions	Authors
pH	5.5 to 5.8	Oak (mixed	Garhwal	Present study
SOC (%)	0.80 to 0.90			
N (%)	0.040 to 0.045			
P (kg ha^{-1})	11.42 to 13.45			

K (kg ha^{-1})	99.02 to 108.90			
Soil pH	6.51 to 6.77	Pine (mixed)		Present study
SOC (%)	0.62 to 0.75			
N (%)	0.031 to 0.037			
P (kg ha^{-1})	21.90 to 24.18			
K (%)	89.98 to 116.48			
SOC (%)	1.33 to 1.80	Oak (mixed)	Garhwal	Kumar *et al.*, 2009
pH	5.02 to 5.7	Oak-pine	Garhwal	Sharma and Kumar, 1991
SOC (%)	0.26 to 2.29			
P (kg ha^{-1})	8.47 to 33.88			
K (kg ha^{-1})	15.2 to 35.2			
pH	4.80	Oak	Kumaun	Singh and Bhatnagar 1997
SOC (%)	1.84			
pH	6.20	Pine		
SOC (%)	1.77			
pH	5.1 to 5.9	Oak	Garhwal	Bhandari *et al.*, 2000
SOC (%)	2.10 to 2.5			
N (%)	0.25 to 0.31			
P (kg ha^{-1})	14.40 to 21.60			
K (kg ha^{-1})	170.8 to 295.4			
pH	5.0 to 5.9	Oak (mixed)	Garhwal	Dhanai *et al.*, 2000
SOC (%)	1.12 to 6.80			
P (kg ha^{-1})	296 to 800			
K (kg ha^{-1})	11.82 to 31.32			
pH	7.0 to 8.4	Oak (mixed)	Kumaun	Khera *et al.*, 2001
SOC (%)	0.8 to 2.3			
N (%)	0.04 to 0.11			
P (kg ha^{-1})	13.4 to 24.7			

Table 5: Comparative studies of density and TBC of oak and pine forests

Forest type	**Regions**	**Density (tree/ha)**	**TBC (m^2/ha)**	**Authors**
Q. leucotrichophora	Garhwal (Pauri)	960-1100	40.88-58.86	Present study
Pinus roxburghii		500-560	26.79-56.94	Present study
Q. leucotrichophora	Kumaun	510 to 2060	-	Pant (1987) and Nayak *et al.* (1991
Q. leucotrichophora	Garhwal (Uttarkashi)	1020 to 2460	46.17 to 71.23	Rajwar (1991)
Q. leucotrichophora and *P. roxburghii* (mixed)	Kumaun (Nainital)	540	35.98	Saxena and Singh, 1982
Q. leucotrichophora	Garhwal (Puari)	790	35.39	Kusumlata and Bisth,1991
Q. leucotrichophora	Garhwal (Kinkaleshwar)	1550	57.67	Kusumlata and Bisth,1991
Q. leucotrichophora	Kumaun	940	53.02	Saxena and Singh, 1982
Q. leucotrichophora	Garhwal Mandal -Chopta	100-860	8.42-59.71	Sharma *et al.*, 2001

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Desalination Water with Surfactant a New Method with Clear Vision

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Abstract: Reverse osmosis (RO), multistage flash distillation (MSF), mediated electrochemical oxidation(MEO), electrolysis and ion exchange are in different condition indubitable RO is consider to be the best method (less than 0.8 $ /m3). In this regard for production of desalination water degree of salinity, environmental and economical cost is the determining factors. International Research and Training Institute of Barzegar Zenous have invented a new method of desalination. Desalination of water using surfactant in comparison with other methods in a wide range (700-60,000 ppm) makes it an acceptable method laboratory-scale and pilot plant. It makes sunny horizon for up grading technology and reaching to economical cost for each cubic meter of water. Importance of this technology will be more clear with demand of increase and drinking water. [Nature and Science 2009;7(11):86-90]. (ISSN: 1545-0740).

Key Words- Surfactant-RO-MED-MSF-Electrodialysis-Desalination-Ion exchange

1. Introduction

Energy is one of the most basic and mooted subject in development countries and access to useful water (drinking- industrial- agricultural) from view of economic is one of important developing instrument. In future the method of surfactant desalination of water makes concentrations lower than 700ppm which most rational and economical for ion deleting rather than present conventional methods. But it doesn't work for other impurities. Cost increases with increasing concentration. The electro dialysis and reverse osmosis from view of economical has preferable in concentration more than 5000ppm and distillation is an economical method for elimination of imparities in concentration in range of 100.000 ppm of course, mentioned method has own usage range according to necessity and economical warranty. With growing and developing technologies new methods can reduce cost and increase efficiency. RO has allocated the most efficiency, in high concentration and the cheapest among mentioned methods.

The following conventional methods for desalinate brackish water is investigable:

1) From view of ability and usefulness every method has its portion in desalinate.

2) Economical investigation for every method

3) Environmental investigation for above methods

4) Technological, mechanical and operational technique

Brief description of conventional methods

1. Heating methods

Include multi stages suddenly chummy system methods, distillation with double effect and multi stage distillation,…. basic operation is steam production, exchange steam energy with salinity water and its variation by making vapor causes reducing boiling point. The distillation method produces desalinate water. We can repeat these stages with more efficiency to reduce waste of energy, and reduce cost of exploitation.

In this method if stages increase, cost reduced and according to use of this methods in salinity and sea water below 50 ppm can be prepared achieving drinking water. Usually capacity of motion units diverse and units to capacity under 100000 m_3/ day is useful.

Efficiency of heating unit is increased by:

1) Increasing number of stage
2) Increasing temperature of feed in preheating section
3) Increasing heat transfer
4) Corrosion prevention

Pretreatments for this method include screening (physical) and anti sediment, anti microbe material and remove of gases.

2. Reverse osmosis

In recent years, RO system has been developed with technology expanding and building of membranes with high efficiency to reduce the heating system stages.

RO method removes ions to 95% and microorganisms to 99% and dissolved solid, can replace for heating methods or is competitive technology.

RO operation is to transfer water from diluted media to concentrated media.

With membrane and concentrated media the liquid is raised in concentrated media, if pressure is enforced on concentrated media. By using mechanical instruments , reverse direction of motivation water is possible to pass salt ions by tape of membrane water with rather purity conduct. From salinity water to opposite side and therefore we can get desalinate water from sea water. It's clear that geometrical figure seam and type of membrane and remove pollution and solid ingredient type of membrane can influence on RO method. RO operation in used for desalinate of sea water for drinking, hospital requirement and industrial proportional to achieve quality water.

3. Electrodialysis.

Electrodialysis method is base on using direct electricity that can shift cation and anion opposite direction. According to splice of salt solution and line of demarcation in a process, electrodialysis parts were prepared:

A) Pre treatment part

B) Membranes

C) Circulation pump

D) The source of electricity

E) Perfect treatment part after electrodialysis

4. Ion exchange (DI)

In this unit resin is used to interchange hydrogen with cations such as calcium, magnesium, sodium, salt solutions ,OH with anion can exist in water. Certainly the material used this stage can be anions or cations acting although efficiency of weak resins comparing to strong resin but it is more effective in industries and more expensive resins, because the efficiency of weak resins are better than others. Recycling efficiency is usually less than 40% for strong resins, while it is 100% for weak resins. Weak resins can be recycled by using weak or strong acids or bases. Ion exchange units can be produced water with high purity, and less salt concentration which is preferable to other methods.

Using ion exchange method combined with the following techniques can eliminate pollution from water with higher pollution in water.

1-Floccalation of coagulation

2-Sedimentation

3- Aeration

4-Elimination algae

5-Hardness

6- Filtration

7-Absorbtation

8-Disinfection

9-Micro filter

10-Ultrafiltratin

11-Nano filtration

Different methods for water purification

Desalinated water and increasing pollution of under group water are main sources of desalinated water for creating the desalination unit which has the following costs:

1-Maximum cost of feed (30% the whole cost)

2-Pretreatment cost

3-Desalivation cost

4-Storing cost

5-Operation cost (energy, chemicals, consuming cost)

6-Human cost

According to pattern of sampling (using of sea water) the cost of investiture was estimated to be 1000 euro per m_3/day. This cost can be reduced by changing the geography surrounding, capacity and other parameters. Under the best conditions the cost of produced water can be estimated to be about 50cent/m_3. With RO method and kind of sensitivity, apply consumption ratio to purity and elimination of contamination in water the kind of treatment can be chosen. It's clear that the role of dependent cost plays important role in the choice of other method. Following are principles of environment in desalination of water. In planning & creation of an industrial desalination unit of place plays important role because environment and stable extension source of water consume energy, landfill and nearing to habitable can also play important role in economical & spoiling. One of the main factor that is related to desalination is minimum 20% liquid that should discharge waste in the open place and without planning in a discharging of waste. The pollution can increase in underground and surface water which show in Table 2.

Applying surfactant for desanation of water

According to worldwide requirement to desalination water and it's importance, International Research and Training Institute has defined a project "Desalination of water with surfactant " since 2000 and developed a new method successfully and obtained noticeable results. This method is registered as an innovation on 2008.06.13 in Registration Office for Company and Non Commercial Institute.

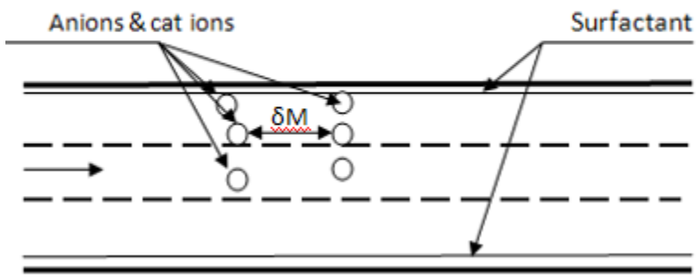
Table 1. Comparing of different desalination method

-	bacteria	suspended solid	range of desalinate	non ion dissolve solid	dissolve solid
distillation	unsuitable	unsuitable	concentrate & salinity 30000	suitable	suitable
ion exchange	unsuitable	unsuitable	very diluted<700ppm	unsuitable	very Suitable
reveres osmosis	very suitable	very suitable	30000<Salinity and brackish water	suitable	suitable
Electro dialysis	unsuitable	unsuitable	-~ 7000	unsuitable	very suitable

Table 2. The method of disposal waste

Surface disposal	Disposal in surface water
	Floating disposal in water
Wastewater treatment plant disposal	-
Waste disposal to end of flow waste water treatment plan	-
Use of water	This method include spray, treatment and leakage lake
Injection to deep water	-
Waste disposal at evaporation lake	-
Disposal by removing total water	Use of evaporation mechanisms in order to change liquid disposal to solid and dry waste

Figure.1.Desalination method using surfactant



In this method there are two pipes with different diameters, the first pipe with fewer diameters is inside the second pipe, internal pipe with membranes is related to external. Internal surface of external pipe is coated by surfactant. Passing water from two pipes attracting anions and cations by surfactant, exhausting water from internal pipe has less salinity and exhausting water from external pipe with more salinity which is the base of desalination in this method. Effective parameters are:

Fluid velocity
Pipes diameters
Rate of salt concentration
Temperature and ….

This Institute has prepared NaCl solutions with different concentrations from 700-60000 ppm and tested them. Electro conductivity is the base of measurement and concentration reduction has been measured in different section of pipe length and figure 2 has been found. As seen in the diagram salt reduction process has reduced with salt concentration reduction during the pipe length.

1) This new method works without using electricity and energy which is the most problem in water desalination. Of course, it is clear that this method needs minimum electricity for water pumping at inside and outside systems.

2) This method doesn't need so much raw materials in contrast to the other methods especially ion exchange.

3) This method does not have any complex technology, doesn't need experts and adversity personal for maintenance and service.

4) According to primaries forecast this method is cheaper than building cost of water desalination unit. According to some calculations, International Research and Training Institute of Barzegar Zenouz has used inexpensive materials for building of a pilot for every cubic m_3 meter which is about 50 percent cheaper than RO equipment cost equivalent to 500 Euro.

5) Achieving cost of a cubic meter of desalinated water with this new method is about 50 cent.

6) This method can be used in small volume and supply water for example for small villages. This method doesn't need energy and heavy and expensive equipments compare to the other methods.

7) This method has remarkably advantages from environmental point of view such as using low of electricity, heating and fixity surrounding temperature since this system doesn't need heating. This system approximately has natural sources, so doesn't have any especial effect on environment. And doesn't have any risk on environment. In case of leakage and disposal, the waste water emission has about 40% desalination water.

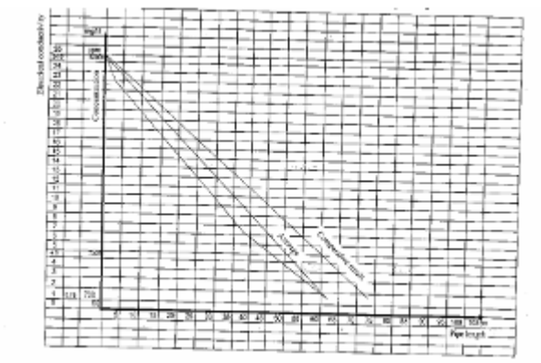


Figure 2. Approximately performance diagram of desalination unit according to testing condition.

Conclusion

Using surfactant is a new method for desalination of water after passing necessary stages for industry. It has ability to enter the competition with ordinary methods. The cost of a cubic meter of desalinated water by RO method is the most desirable and available method in market, arriving to 40-50 cent since its invention in 40 years ago. It is clear that achieving cost of water by this invented method in center after optimization and achieving to product large amount of water will decrease the cost remarkably. Invested cost of this new method in pilot plant and first sample test is about 50 per percent of RO method that is 500 euro. Also achieving cost of water per cubic meter in this stage is about 40 cent that naturally every one of these figures gradually will decrease in mass production with engineering and optimization conditions in system. It should be mentioned that achieving cost of desalinated water per cubic meter by RO in system was more than 2$ using method has decreased the cost to 40-50 cent now.

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PhD of petrochemistry in the specialty of surfactant, he established International Research and Training Institute of Barzegar Zenouz in 1997, the goal is: Developing software and hardware to synthesize surfactant based products, research and innovation in these areas.

Till now the institution has 7 innovations and registered them in Registration Office for Company & Non Commercial Institute and more than 50% of this innovation are applicable and in mass production phase. There are six laboratories in this research institute (water.oil, drug, biotechnology, nano and environment)

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