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Carbon storage potential in soils of teak and eucalypt plantations

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Abstract

The reservoir of carbon in soil, especial forest soil is both a significant source and sink of atmospheric CO_2 and labile to changes as a result of human activities. The study was carried out in the moist deciduous forest, teak and eucalypt plantations in the Trichur District to examine carbon storage potential in teak and eucalypt plantations. The soils were analyzed for bulk density and organic carbon and the amount of organic carbon per hectare of soil was calculated. It was observed that over a 60 year rotation period, soils under teak stores considerable amount of organic carbon. Eucalypt, being a short rotation crop, is less effective in sequestering carbon. Higher efficiency of replanted eucalypt plantation in storing carbon illustrates the importance of appropriate management practices to improve the carbon storage potential of plantations.

Key words: Carbon storage potential, soil, teak, Eucalypt.

1. Introduction

The reservoir of carbon in soil is both a significant source and sink of atmospheric CO_2. World's soils contain about three times as much carbon as land vegetation and being labile, changes as a result of human activities. In this context, soils in the tropical region are of particular interest, as undergoing major land use changes (Schlesinger, 1983 Trumbore, 1997). Forest plantations, accounting for 130 million ha., is approximately 3% by area of world's forest and play an important role in storing carbon. The balance between clearing and regrowth of plantations is a major factor in determining changes in the net storage of carbon in terrestrial systems. Teak and eucalypt are the major plantation species in Kerala, accounting for than 90% of the land area under plantations. This paper highlights the carbon storage potential of teak and eucalypt plantations in Kerala.

2. Materials and Methods

The study was carried out in the moist deciduous forest, teak and eucalypt plantations in the Trichur District. As teak in Kerala is a long rotation crop and eucalypt a short rotation crop, to study carbon storage potential, age in teak and rotation in eucalypt was selected as the criteria. Teak plantations were selected from Karadipara and Athirapilly and aggregated into four age classes viz., 21-30, 31-40, 41-50 and 51-60 years. Eucalypt plantations selected from Marotichal were second rotation, replanted and coppiced third rotation

plantations. The forest, adjacent to the plantations in all locations was selected as a reference stand to reduce heterogeneity in properties of soil samples. Five sample plots, separated from one another by 200 m, and of size 100m x 100m were laid out at random in natural forest. The number of sample plots in plantations was in accordance with the area. One sample plot was laid out for every 20 ha. with a minimum of five in each age class or rotation. Three soil pits, of 30cm x 60cm x 60cm size, were dug in each plot and soil samples collected from 0-60cm depth for a composite sample. Details of plantations and number of samples are given in Table 1. The soils were analyzed for bulk density and organic carbon (Jackson, 1958). From the bulk density and organic carbon values, the amount of organic carbon per hectare of soil was calculated. From the data, both mean values and relative mean values were calculated.

Relative mean values = (mean values of plantations x 100)/mean values of natural forest

It was thus possible to compare plantations which differed in their reference stand (Mishra *et al.*, 2003).

3. Results and Discussion

The mean values of bulk density, organic carbon and organic carbon per hectare are given in Table 2. Soil organic carbon in plantations of teak was found to initially decrease and then increase with age of the plantation. On the other hand in plantations of eucalypt, it decreased with rotation.

Table 1. Details of plantation selected for study

Study area	Location	Vegetation	Age class (years)	Name of the plantation	Area of plantation (ha.)	No. of samples
Trichur District	Karadipara	Moist deciduous forest	-	-	-	5
		Teak	21 - 30	Karadipara	62	3
				Rapra	41	2
			31 -40	Karadipara	79	4
				Karadipara	61	3
	Athirapilly	Moist deciduous forest	-	-	-	5
		Teak	41-50	Vadamury	53	5
			51-60	Chully	172	9
	Marotichal	Moist deciduous forest	-	-	-	5
		Eucalypt	2nd rotation coppiced	Olakkara	47	5
			3rd rotation replanted	Marotichal	39	5
			3rd rotation coppiced	Chemenkandam	51	5

Compared to natural forest, 20-30Y old teak plantations had lost 43,424 Kg of organic carbon per hectare while the loss from 31-40Y old plantations were still greater – 58,659Kg/ha. That is, over a ten year period 15,235Kg /ha. of organic carbon was lost from plantation soil. In the initial years, organic matter rich top soil is lost by erosion. Organic carbon is also lost by faster decomposition. Plantation operations like mechanical and silvicultural thinnings would be over by 25 years. As the plantation ages, partial canopy closure, establishment of undergrowth and litter cover provide a measure of protection to the soil. Now the net organic carbon added to it is probably greater than its loss from soil resulting in carbon storage. However, as it is a very slow process, the increase would not be apparent immediately but was observed in 41-50y and 51-60Y age class plantation where the amount of organic carbon per hectare of soil was comparable to natural forest. Relative to natural forest, soils in 21-30Y, 31-40Y, 41-50Y and 51-60Y age class teak plantations had 35, 47, 10 and 9 per cent less organic carbon per hectare. The difference from natural forest was significant.

In eucalypt plantations, organic carbon was found to decrease with rotation. Among third rotation plantations, replanted plantations were richer in organic carbon than the corresponding coppiced one. This was probably due to the increased growth of eucalypt as a result of fertilizer application that would translate into higher litter fall and eventually higher organic matter in the soils under it. When compared to adjacent natural forest, second rotation plantations lost 44,281Kg/ha. of organic carbon from soil while, third rotation coppiced plantations lost 83,029Kg/ha. On the other hand, loss of organic carbon from third rotation replanted plantation was comparable to that of second rotation coppiced plantation viz. 48,563Kg/ha. Relative to natural forest, soils in second rotation, third rotation coppiced and third rotation replanted plantations had 76, 54 and 73 per cent less organic carbon per hectare. The difference from natural forest was significant.

When plantations under teak and eucalypts for similar period of time were compared, it was observed that teak lost 35 per cent of organic carbon while eucalypt lost 24 per cent in 20-30Y period. After 30-40Y period, the loss from teak was 47 per cent and in eucalypts it was 46 per cent. The loss from replanted eucalypts during the same period was only 27 per cent. However, soils under teak for more than 40 years show a dramatic increase in organic carbon content with a loss of about 10 percent. From the data available, such an increase in eucalypt cannot be expected.

4. Conclusion

Over a 60 year rotation period, soils under teak stores considerable amount of organic carbon. However, if the rotation period of teak plantation is reduced, a corresponding decrease in carbon storage is to be expected. Eucalypt, being a short rotation crop, is less effective in sequestering carbon. However, the higher efficiency of replanted eucalypt plantation in storing carbon illustrates the importance of appropriate management practices to improve the carbon storage potential of plantations.

Table 2. Mean values of bulk density and organic carbon and relative mean value of organic carbon

Study area	Location	Vegetation types	Age class	Properties			
				B.D	O.C	O.C	O.C
				g/cm^3	--- %---	kg/ha.	Relative mean value
Trichur District	Site I Karadipara	Natural forest	-	1.06	2.0	1,24,774	100
		Teak	21-30Y	1.14	1.2	81,350	65
			31-40Y	1.16	0.9	66,115	53
	Site II Athirapilly	Natural forest	-	1.00	1.91	1,14,779	100
		Teak	41-50Y	1.05	1.6	1,03,253	90
			>50Y	1.04	1.7	1,04,348	91
	Site III Marotichal	Natural forest	-	1.06	2.85	1,81,694	100
		Eucalypt	Rotation 2 coppiced	1. 21	1.90	1,37,413	76
			Rotation 3 coppiced	1.18	1.41	98,665	54
			Rotation 3 replanted	1.14	1.97	1,33,131	73

B.D. = Bulk density; O.C. = Organic carbon

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Study on the phytochemical, antimicrobial and anti-oxidant activities of *Lantana camara*

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Abstract

The present study is done on the hot water and ethanolic extracts of *Lantana camara*, a weed commonly found on the Western Ghats and tropical areas. The study attempts to identify the antimicrobial and antioxidant activities of *Lantana camara.* The antimicrobial activities were checked with optical density and disc diffusion method. The hydroxyl radical scavenging activity and total antioxidant activities were also tested. The present study, of *in vitro* evaluation of plants, forms a primary platform for further phytochemical and pharmacological studies.

Key words: Lantana camara, hot water extract, ethanolic extracts, antimicrobial, anti-oxidant

1. Introduction

For many centuries, it is a known fact that humankind depends on plants as an indirect source of energy. It is been found that near about 80% of all established natural products originate from plants (Phillipson, 1990). These natural products have a significant use in the finding and production of new pharmaceuticals which are then clinically useful. These natural products may contain biologically active compounds, some of which will be valuable may lead for novel drugs. They can be used as primary materials to produce some drugs of synthetic origin or they can be used to make products, which then assist in making fully synthetic drugs (Soejarto & Farnsworth., 1989).

The number of plants that have been extensively studied is relatively few and the vast majorities have not been studied at all. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. Herbalists often reject the notion of a single active ingredient, arguing that the different phytochemicals present in many herbs will interact to enhance the therapeutic effects of the herb and dilute toxicity.

Lantana Camara is a significant weed of which there are some 650 varieties in over 60 countries or island groups. *L. Camara* typically occurs where there is a moderate to high summer rainfall and well drained sloping sites. It is a native to tropical regions and exists as dozens of strains and varieties

that are highly variable in appearance. In recent years, many studies evidenced that plants containing high content of antioxidant phytochemicals can provide protection against various diseases. The free radical scavenging activity of these phytochemicals is predominantly determined by their structures. In recent years, the popularity of complementary medicine has increased. The World Health Organization also recommended the evaluation of plants where safe modern drugs are lacking. Recently, an intensive search for novel types of antioxidants has been carried out from numerous plant materials.

2. Materials and Methods

Lantana camara – Obtained from the suburbs of Thrissur, Kerala, India**.**

Bacterial cultures: *Staphylococcus, Streptococcus, Bacillus, Klebsiella, Escherichia coli and Proteus* sp. were kindly provided by Poly Clinic Pvt Ltd, Thrissur, Kerala, India.

Fungal cultures: *Aspergillus, Penicillium and Mucor* sp. were also kindly provided by Poly Clinic Pvt Ltd, Thrissur, Kerala, India.

Different parts (flower, root, stem, leaf) of the plant *Lantana camara* were collected from the outskirts of Thrissur, Kerala, India. The plant was identified at the Dept. Of Botany, St. Mary's College, Thrissur, Kerala, India. The parts were dried for six hours at 50-60°C. The dried samples were then crushed into powder using mortar and pestle. The powdered samples were stored in bottles at room temperature, prior to analysis.

2.1. Preparation of Extracts

The crude powdered sample of 100 gm was extracted with 70% ethanol in a soxhlet apparatus for 8-10 hours, repeatedly thrice. The hot water extract of the powdered material was also prepared, in a water bath at 80 – 90^0C for 8-10 hrs, repeatedly thrice**.** The extracts were then collected and filtered through Whatmann No. 1 filter paper. The extracts were then concentrated at 40 - 45°C and air-dried. The dried powder samples were then stored in air tight bottles at 4°C (Baku, 2007).

2.2. Phytochemical Screening

Phytochemical analysis were carried out on ethanolic and hot water extracts of *Lantana camara* using standard procedure described by Sofowara(1993), Trease and Evans(1989) and Harborne(1973)

2.3. Antibacterial Activity

2.3.1. Optical Density Method

One percentage of each bacterial culture (mentioned in 2) was inoculated in to test media, and 0.1 percent of each extracts was added to make a final volume of 5ml. 2 percent DMSO was used as the solvent to dissolve the hot water and ethanolic extracts separately which was found to have no adverse effect on the bacteria and fungus. The media were incubated at 37^0 C for 24 hrs bacteria. After incubation samples were measured spectrophotometrically at 600nm .The percentage inhibition was calculated using the following formula.

% inhibition = C-T/C ×100

where C is the absorbance of control and T is the absorbance of test sample.

2.3.2. Disc Diffusion Method

Antibacterial test were carried out by disc diffusion method with some modification. The bacterial cultures (above mentioned) were inoculated

into 250 ml conical flask containing 100ml nutrient broth. From this 0.1 ml culture was uniformly distributed on to Muller Hinton agar plates. Filter paper discs of 3mm diameter were punched out from a Whatmann No: 1 filter paper and sterilized. Then discs were placed on the surface of Muller Hinton agar plates at a distance of 2cm using sterile forceps. Drugs of different concentration [100,250,500µg/ml] were added on each disc with a micropipette. Then the plates were incubated at 37^0 C for 16-18 hrs. After incubation zone diameter was measured.

2.4. Antifungal Activity

2.4.1. Disc diffusion method

Antifungal test were carried out by disc diffusion method with some modification. The fungal cultures were inoculated into 250 ml conical flask containing 100ml sabouraud's dextrose broth. From this 0.1 ml culture was uniformly distributed on to sabouraud's dextrose agar [SDA] plates. Filter paper discs of 6mm diameter were punched out from a Whatmann No: 1 filter paper and sterilized. Then discs were placed on the surface of SDA plates at a distance of 2mm using sterile forceps. Drugs of different concentration [100,250,500µg/ml] were added on each disc with a micropipette. Then the plates were incubated at room temperature for 3-5 days. After incubation zone diameter was measured.

2.4.2. Growth Inhibitory Assay

Antifungal activity was studied using an invitro contact assay which produces hyphal growth inhibition (Cakir *et al*., 2004). Test solution for antifungal assays was prepared. Aliquotes (100µl) of solution were added on each SDA plates. Discs (5mm diameter) of the test organism were cut out from 1 week old culture on SDA plates. The mycelia were placed surface down, on opposite edges of the SDA plates at the center of the dishes. The plates were then incubated in dark at 26°C. The extension diameter (mm) of hyphae from the center to the side of dishes was measured every 24 hrs for 5 days. Mean growth measurement were calculated from replicates of each of fungal species. SDA plates with distilled water without drug used as control. Growth inhibition of treatment against control calculated by

% inhibition = C-T/C× 100

where C is the average of replicates of extension of control and T is the average of replicates of hyphal extension of Test.

2.5. Antioxidant Activity

2.5.1. Total Antioxidant Capacity

The total antioxidant capacity measured according to spectrophotometric method of Preito *et al*., 1999. Each extract (10mg/ml) upto a volume of 0.1 ml was dissolved in water and taken in an eppendorf tube with 1ml of reagent solution (0.6M sulphuric acid, 2.8 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 minute. After cooling to room temperature, the absorbance of the aqueous solution was measured at 695nm against blank. Vitamin C was used as the standards

2.5.2. Hydroxyl Radical Scavenging Activity

Hydroxyl radicals generated from Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton's reaction) was estimated by

its degradation of deoxyribose that resulted in thiobarbituric acid reactive substance (TBARS) (Elizabeth & Rao., 1990). The reaction mixture contained deoxyribose (28 mM); $FeCl_3$ (1 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); EDTA (1 mM); H_2O_2 (1 mM); ascorbic acid (0.1mM) and various concentrations of extract in a final volume of 1.0 ml. The reaction mixture was incubated for 1 hour at 37 ^{0}C. The TBARS formed was estimated by TBA method (Ohkawa *et al*., 1979). The hydroxyl radical scavenging activity was determined by comparing absorbance of control with that of treatments. Vitamin C was used as standard.

3. Results and Discussion

The ethanolic and hot water extracts of *Lantana camara* were prepared with the help of soxhlet apparatus and in water bath respectively. These solvent extracts were analysed for the phytochemical composition by qualitative method (Table 1). The antibacterial activity of *Lantana camara* was tested against three species of each Gram positive and Gram negative bacteria by optical density (Table 2) and disc diffusion method (Table 3).

The hot water extract showed higher activity against *Staphylococcus* sp compared to *Streptococcus* sp and *Bacillus* sp. *Staphylococcus* sp showed a percentage inhibition of 96.5% at a concentration of 500 µg/ml. It showed significant activity against *Klebsiella* sp. Ethanolic extract showed significant activity against Gram positive bacteria, i.e. *Streptococcus* sp, *Staphylococcus* sp and *Bacillus* sp. Ethanolic extract, at a concentration of 500 µg/ml, showed a percentage inhibition of 63.7% against *Bacillus* sp. It also showed activity against *E.coli*, *Klebsiella* sp and *Proteus* sp. Ethanolic extract produced zone of inhibition against all the test organisms. The maximum zone of inhibition was produced by *Bacillus* sp which was around 18mm. In case of Gram negative bacteria the maximum zone of 12mm was produced against *Klebsiella* sp at a drug concentration of 500 µg/ml.

The hot water extract produced appreciable zone of inhibition against the Gram positive test organisms. The highest zone of inhibition was produced against *Streptococcus* sp and *Bacillus* sp, with a zone diameter of 15 mm, in a concentration of 500 µg/ml of the extract. It showed significant activity against *Klebsiella* sp with a zone diameter of 10mm, at a drug concentration of 500 µg/ml. It showed no activity against *E.coli* and *Proteus* sp.

The antifungal activity of ethanolic and hot water extracts of *Lantana camara* was evaluated using disc diffusion method and growth inhibitory assay. The extracts were screened against three fungal species – *Penicillium*, *Aspergillus* sp and *Mucor* sp. From the disc diffusion (Table 4), the ethanolic extract showed activity against *Pencillium* sp. The highest zone of inhibition was seen against *Penicillum* sp with a zone diameter of 20mm at a concentration of 500µg/ml. Hot water extract showed activity against both *Pencillium* sp and *Aspergillus* sp. No zone was produced against *Mucor* sp in both the extracts. Antifungal activity was also evaluated by growth inhibitory assay. In this method, three different concentrations of drug were tested against the three fungal species. After incubation, the growth inhibition was observed and recorded (Table 5) Following this, the percentage inhibition of fungal growth was calculated (Table 6).The ethanolic extract highly inhibited the growth of

Penicillium sp. It did not show activity against *Aspergillus* sp and *Mucor* sp. The hot water extract showed inhibition of *Penicillium* sp as well as *Aspergillus* sp. It did not show activity against *Mucor* sp.

The ethanolic extract of *Lantana camara* showed increasing free radical scavenging activity with increasing concentration of the drug. The highest activity was seen at a concentration of 2000µg/ml which is 74.5% and minimum activity of 33% was seen at 100 µg/ml of the drug. Similarly, the hot water extract showed increasing activity with increasing concentration of the drug. The highest activity was reported at a concentration of 2000 µg/ml which is around 76.5% and minimum activity of 23% at a concentration of 100 µg/ml of drug (Fig 1). IC_{50} for ethanolic extract was found to be 100µg/ml and for the hot water extract was found to be 70µg/ml.

The ethanolic extract of Lantana camara showed increasing hydroxyl radical scavenging activity with increasing concentration of the drug. The highest activity of 96.5% was reported at a concentration of 2000 µg/ml and a minimum of 59% at a drug concentration of 100 µg/ml. Similarly, the hot water extract showed increasing hydroxyl radical scavenging activity with increasing concentration of the drug. The highest activity was reported at a drug concentration of 2000 µg/ml which is about 97% and a minimum of 53.5% at a drug concentration of 100 µg/ml (Fig 2). IC_{50} for ethanolic extract was found to be 600 µg/ml and for the hot water extract was found to be 650 µg/ml.

The successive solvent extraction confirmed the presence of various phytochemical compounds in both the extracts. Phytochemical analysis of ethanolic extract revealed the presence of alkaloid, tannin, phenol, steroids, terpenoids, proteins and sugars, whereas in hot water extracts, alkaloids, saponins, steroids, terpenoids, proteins and sugars were detected. Thus compared to both the solvents extracts, more number of metabolites was detected in ethanolic extract. *Lantana camara* has been studied extensively for their antibacterial properties (Siddiqui *et al*., 1995; Verma & Verma., 2006). From these studies the potent activity was found against *Streptococcus* sp and *Bacillus* sp in ethanolic extract and *Staphylococcus* sp in hot water extract. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structure (Nikaido & Vaara., 1985; Priya & Ganjewala., 2007).

The antifungal studies were also carried out using disc diffusion and growth inhibitory assay. Both the extracts showed potent activity against *Penicillium* sp and the least activity were reported against *Mucor* sp. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction (Nimri *et al*., 1999). The antioxidant activity was observed in a dose dependent manner. The antioxidant property of the plant is well correlated with the concentration of the extracts, which showed the presence of active principals.

Table 1 – Phytochemical screening of *L.camara*

Phyto chemicals	Ethanolic extract of *L.camara*	Hot water extract of *L. camara*
Alkaloids	+	+
Saponin	–	+
Tannin	+	–
Anthraquinone	–	–
Flavanoids	–	–
Phenol	+	–
Steroids	+	+
Terpenoids	+	+
Proteins	+	+
Sugars	+	+

Table 2 – Antibacterial activity of *L.Camara* *by* optical density method

Drug [µg/ml]	Percentage Inhibition (%)					
	Staphylococcus	*Streptococcus*	*Bacillus*	*E. coli*	*Klebsiella*	*Proteuss*
Ethanolic Extract						
100	37.5	29.74	45.0	40.0	30.5	42
250	42.3	44.29	52.1	43.4	41.0	56
500	43.0	54.91	63.7	46.7	53.5	58
Hot water Extract						
100	93.2	21.8	36.5	NA	52.0	NA
250	95.0	27.5	44.9	NA	54.9	NA
500	96.5	30.6	60.0	NA	56.3	NA

NA – No Activity

Table 3 – Antibacterial activity of *L. camara* by disc diffusion method

Drug [µg/ml]	Diameter of zone of inhibition[mm]					
	Staphyl	*Streptoc*	*Bacillus*	*E.coli*	*Klebsie*	*Proteuss*
DMSO	NA	NA	NA	NA	NA	NA
Ethanolic Extract						
100	7	7	7	5	8	5
250	9	10	10	7	9	7
500	12	12	18	8	12	10
Hot water Extract						
100	7	5	9	NA	5	NA
250	10	10	12	NA	7	NA
500	11	15	15	NA	10	NA

NA – No Activity, n=2

Table 4 – Antifungal activity of *L. Camara* by disc diffusion method

Drug [µg/ml]	Diameter of Zone of Inhibition [mm]		
	Penicillum	*Aspergillus*	*Mucor*
DMSO	*NA	*NA	*NA
Ethanolic Extract			
100	10	*NA	*NA
250	14	*NA	*NA
500	20	*NA	*NA
Hot Water Extract			
100	6	7	*NA
250	8	9	*NA
500	12	13	*NA

***NA – No Activity, n=2**

Table 5 - Antifungal activity of *L. camara* by growth inhibitory assay

Drug[µg/ml]	Diameter of Mycelial Mat [mm]		
	Penicillium	*Aspergillus*	*Mucor*
Control	62	58	62
Ethanolic Extract			
100	15	59	60
250	13	58	59
500	10	59	58
Hot water Extract			
100	18	20	51
250	14	18	47
500	11	14	45

n=2

Table 6 – Table showing percent inhibition of fungal growth by *L. Camara*

Drug [µg/ml]	Percentage Inhibition [%]		
	Penicillum	*Aspergillus*	*Mucor*
Ethanolic Extract			
100	**75**	***NA**	***NA**
250	**78.3**	***NA**	***NA**
500	**83.3**	***NA**	***NA**
Hot Water Extract			
100	**67.2**	**63.6**	***NA**
250	**74.5**	**67.2**	***NA**
500	**80**	**74.5**	***NA**

***NA – No Activity, n=2**

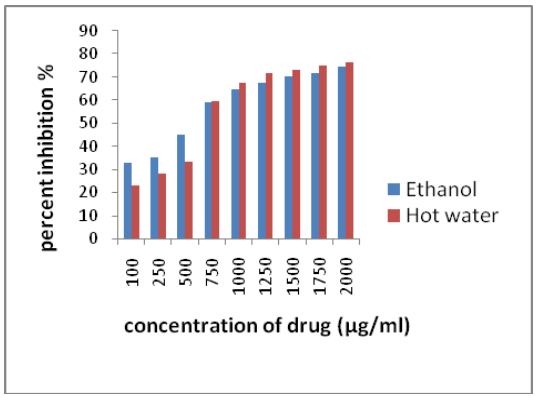

Fig. 1. Total anti-oxidant activity of *L. Camara*

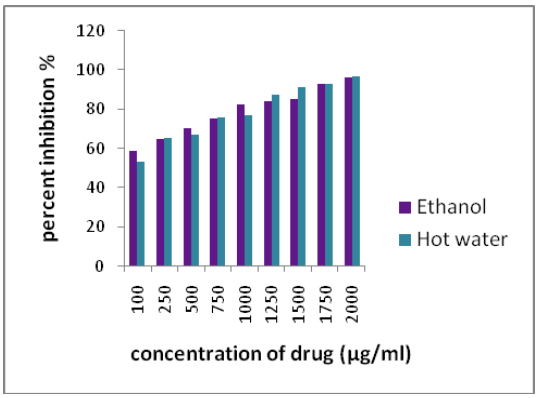

Fig. 2. Hydroxyl radical scavenging activity of *L. camara*

4. Conclusion

The present study suggests that *Lantana camara* has both antimicrobial and antioxidant properties. The antioxidant activity of the extract allows us to investigate the impact of antioxidants in reducing the oxidative stress and thus the implication for disease prevention by further studies. The potential for developing antimicrobial agents from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes with least toxicity to normal cells.

Plant-based antimicrobial agents have enormous therapeutic potential as they can serve the purpose with lesser side effects those are often associated with synthetic antimicrobials. But this drug should be subjected to animal and human studies to determine their effectiveness in whole organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardise methods of extraction and invitro testing so that the search could be more systematic and interpretation of results would be facilitated.

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GA3 ENHANCE SEED GERMINATION AND EARLY SEEDLING GROWTH OF PEA UNDER SALT STRESS

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Abstract

Rapid seed germination and its establishment are critical factors to crop production under salt stress conditions. In this study, application of plant growth hormone, gibberellins are shown to effectively improve germination as well as growth of plants under normal and salt stress. The positive effects of GA3 on some germination traits such as radicle length and shoot length were analyzed. It seems that GA3 could be reduced negative salinity effects on seed germination of pea seeds.

Key words: seed germination, Salinity, abiotic stresses, agriculture

1. Introduction

Salinity is one of the most important stresses limiting agricultural production. The United Nations Environment Program estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed. Excess of ions in root medium exerts effects like osmotic strain, ion specificity/toxicity, and nutritional imbalances, changes in cell

metabolites levels and diminished growth and yield. Both photochemical and biochemical aspects of photosynthesis are affected by salinity, which limit the generation of resources and/or diversion of the available resources towards stress tolerance (Wahid et al., 2007). In saline environments, high salt levels are known to impair seed germination of halophyte species (Debez et al., 2004).

Application of abiotic stresses results in an altered levels of plant growth hormones and decreased plant growth. Plant growth regulators (PGRs) have been found to play a central role in the integration of the responses expressed by plants under stress (Amzallag et al., 1990). Gibberellic acid and kinetin have been reported to increase the percentage of germination and seedling growth (Kaur et al., 1998).

Seed germination is defined as the emergence of the radicle through the seed coat. It is usually the most critical stage in seedling establishment, determining successful crop production. Mahdevi and Modarres Sanavy (2007) reported that seed germination can be initiated by water imbibitions and any shortage in water supply will let seed under stress. Reduction in salt stressed plants can be a result of inorganic ion (Na+, Cl-, K+) and organic solute accumulations. The specific ions likely to be most abundant and to cause the greatest problem are sodium and chloride. Variation in salt levels may restricted seed germination and in some cases resulting the death of seeds. Several investigations of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development.

Little information on the effect of salinity stress on pea germination and seedling establishment is available. The aim of this study was to investigate the influence of salt stress on factors affecting germination of pea seeds. The present work also investigates the role of GA3, during the germination of pea seeds under NaCl salinity in order to get more information on the mechanisms by which salinity may inhibit germination of this species in the natural conditions.

2. Materials and Methods

2.1. Seeds:

Pea is a legume grown as a food for man and both food and forage for domesticated animals. It is extremely drought tolerant capable of yielding well even under the most adverse conditions and well adapted to cool season (winter) production in warm temperate and subtropical areas.

2.2. Gibberellic acid (GA3):

Gibberellin was purchased from Swisco Laboratories, Mumbai. It was dissolved in distilled water by raising the pH to 12.0 with 1 N KOH and then readjusted to 6.0 with 1 N HCI.

2.3.1. Determination of the impact of Sodium chloride (NaCl) on germination of pea seeds

Seeds were disinfected in a 3.5% sodium hypochlorite solution for 5 min before starting the germination tests. Seeds were divided into 4 sets (10seeds/group)

Group I: Distilled water (DW)
Group II: 100mM NaCl
Group III: 200mM NaCl
Group IV: 400mM NaCl

They were placed in 9-cm diameter Petri dishes (5 seeds per each)

containing a Whatman No. 1 filter paper moistened with 2 ml of distilled water or 100, 200 and 400 mM NaCl solution (Li, 2008). The solution and the filter paper were checked everyday. In order to maintain moisture 2ml of the respective solutions were added to each petridish every three days. The following experiments were carried out in the lab at conditions which are suitable for seed germination and were repeated twice.

2.3.2. Determination of the effect of exogenous application of GA3 on seed germination under salinity stress

Seeds were disinfected in a 3.5% sodium hypochlorite solution for 5 min and they were divided into 4 sets (10seeds/group)
Group I: Distilled water (DW)
Group II: 200mM NaCl
Group III: 200mM NaCl+ 20 µM GA3
Group IV: 20 µM GA3

After surface sterilization, group III and IV seeds were soaked for 8h in 20 µM GA3 (Jamil and Rha, 2007). After the pre-treatment of GA3, seed samples were rinsed with distilled water and dried for two hour to eliminate surface moisture. All the seeds were placed in Petri dishes containing a Whatman No. 1 filter paper moistened with 2 ml of distilled water or NaCl solution. The solution and the filter paper were checked everyday. In order to maintain moisture 2ml of the respective solutions were added to each petridish every three days. The following experiments were carried out in the lab at conditions which are suitable for seed germination and repeated twice. This experiment was observed for 9 days.

2.4. Data collection

Germinated seeds were counted at three-day interval, and a seed was considered as germinated at the radicle protrusion (Come, 1982). The germination parameters determined were germination percentage, germination energy, germination index, relative germination rate, relative salt injury rate were determined by the following formula:

Germination percentage = a/b
Germination energy = c/b
Germination index = $\sum Gt/Dt$
Relative germination rate = d/e
Relative salt injury rate = (e-d)/e
where,
a- Germinated seeds total in NaCl concentration
b- Total number of seeds for germination
c- Germinated seeds total in NaCl concentration in three days
Gt- Germinated seeds in t days
Dt- Number of germination days corresponding
d- Germination percentage in NaCl concentration
e- Germination percentage of control
The lengths of roots and shoots of the germinated seeds were measured and recorded after every 3 days.

2.5. Statistical analysis

Data was expressed as mean ± standard deviation (SD). Significance levels were compared by unpaired test and values $p<0.05$ were considered significant using Graphpad Instat 3 software.

3. Results and Discussion

3.1. Effect of salinity on germination of Pea seeds

Salinity caused a significant reduction in germination of pea seeds with increasing salt stress. On 3rd day, out of 10 seeds 8 seeds were found to be germinated in distilled water alone, while in 100 and 200mM NaCl, 7 and 5 seeds were only germinated. No germination was observed in 400mM NaCl solution. At the end of the 12th day 9 seeds were germinated in

distilled water. No further germination was observed in 100 and 200mM NaCl. Even at the end of the 15th day a strong inhibition in germination of seeds was observed in 400mM NaCl solution. There was considerable reduction in the length of the root and shoot was observed with increasing concentration of salts. Based on this we selected 200mM NaCl concentration for further studies (Figure1).

3.2. Effect of GA3 on germination of pea seeds under salinity stress

In distilled water treated group, out of ten seeds, eight seeds were found to be germinated on 3rd day, while in control group 200mM NaCl, only five seeds were found to be germinated (Table 1). In Group III eight seeds were found to be germinated and in group IV only two seeds were germinated. In 200mM NaCl treated group only radicle comes out but not the plumule. Even at the end of the 9th day we can not measure the length of the shoot in the control group. The length of root and shoot was found to be high in distilled water treated group (Figure 2). When compared to the normal group, the length of root was significantly reduced in NaCl treated group. The shoot did not emerge out in salinity induced group. GA3 pretreatment enhanced germination and length of root (Figure 3) and shoot (data not shown) of pea seeds in 200mM NaCl treated group. It can be easily measured when compared to salinity induced group. In 20µM GA3 alone treated group, only two seeds were found to be germinated, but they show stunted growth. Based on all these factors germination percentage, energy and index was calculated (Table 2). With respect to normal group relative germination and salt injury rate was calculated (Table 3). The results showed that an increased salt concentration caused delayed emergence of root and shoot compared to distilled waster. GA3 pretreatment significantly enhanced the length of root and shoot in seeds under salinity conditions. So seed priming with GA3 had positive effects on some growth traits of pea seeds.

4. Discussion

Environmental factors like salinity, temperature, and light greatly affect seed germination. This is particularly due to their impact on seed hormone content (Alboresi et al., 2006).In the present work the effect of NaCl on germination of pea seeds were investigated. The germination pattern varied under salt stress in this experiment. It was indicated that NaCl salinity decreased both germination percentage and germination rate in Pea seeds at 100 and 200mM and fully inhibited these parameters at 400 mM NaCl. These results suggest that seeds of Pea can germinate at lower level of salt but pea seeds couldn't germinate at higher level of salt. The radicle and hypocotyle growth were depressed with increasing salt. Root length was severely influenced by NaCl stress. Shoot growth was more adversely affected by hypocotyle injury due to different levels of NaCl. As a result of decreasing root and shoot lengths, seedling fresh weight decreased under increased NaCl concentrations in pea. This study further showed that NaCl had greater inhibitory effects on early seedling growth rather than on its germination.

NaCl salinity may concomitantly lower the content in germination-stimulating compounds like NO_3 and growth hormones gibberellins, increase ABA levels, and induce changes in membrane permeability, as well as in water relations, so that germination cannot take place. Increased salinity

has multifarious effects on the cell metabolism. Excess ion-induced water deficit is among the other effects of salinity, which is more deleterious to the mobilization of reserves, radicle protrusion and early growth of seedlings. Salinity has a pronounced effect on the peroxidation of membrane lipids, enhances their permeability and modulates the patterns of ions leakage. It is evident that plant growth regulators have been found to play a central role in the integration of the responses expressed by plants under stress conditions.

The root and shoot length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress. It was observed that GA3 treatment improved root and shoot growth as compared to control. Growth parameters are ultimately a function of total photosynthetic capacity and comparison of photosynthetic and growth responses to salinity illustrate that with increasing salinity stress germination of seeds were significantly decreased.

Use of seed pretreatment is advantageous only when it supports the emergence and establishment of seedling. Germination improvement was found when these seeds were treated with GA3. In this study GA3 treated seeds show enhanced performance under stress conditions. It supports that GA3 treatment caused more water uptake than control while salinity influenced germination by decreasing water uptake. Seed treatment with GA3, in this study, may reduce the leakage of ions. The exogenous application of GA3 was found to be effective in mitigating NaCl salinity effect on germination of several halophytes, such as Zygophyllum simplex (Khan and Ungar, 2002), Arthrocnemum indicum L (Khan et al., 1998), and Prosopis juliflora (El-Keblawy et al., 2005). This could be explained by the fact that GA3 may reduce the ABA level in seeds through the activation of their catabolism enzymes or by blocking the biosynthesis pathway ((Xiong and Zhu, 2003).). Expression of stress tolerance may be due to the production of stress proteins which are highly water soluble and heat stable (Dubey, 2005). . It is assumed that reducing cell division and plant growth metabolism induced by accumulation of Na+ ion caused changes in ion balances and the imbalance of mineral nutrients resulted in a reduction or an inhibition of plant growth. Present study indicated that GA3 pretreatment enhanced stress tolerance in pea seeds. Hence we can conclude that treatment of seeds with growth hormone like gibberellic acid is an alternative approach to overcome salinity problems.

Numerous attempt have been made to improve the salt tolerance of crops by traditional breeding programmes, but commercial success has been very limited (Santa Cruz et al., 2002). Pre-sowing seed treatment or seed priming is an easy technique and an alternative approach recently used to overcome salinity problems. Despite mechanisms of this inhibition remain unclear; such an inhibition may partly ascribe to the strong decline in gibberellic acid (GA3) levels during seed imbibition. The exogenous application of GA is known to efficiently alleviate the harmful effect of salinity on halophyte germination.

Figure - 1

Effect of salinity on germination of Pea seeds

Day 0 Day 15 Day 15

Figure - 2

Effect of GA_3 on germination of Pea seeds under salinity stress

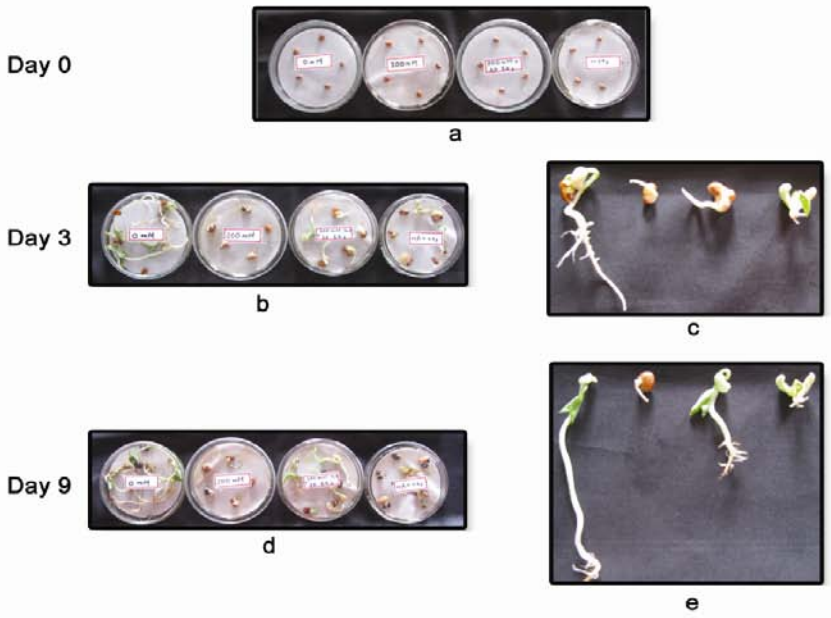

Table 1. Effect of GA3 on germination of Pea Seeds under salinity stress

Group	Day 0	Day 3	Day 6	Day 9
Distilled water	0/10	8/10	8/10	8/10
200mM NaCl	0/10	5/10	5/10	5/10
200mM NaCl + 20µM GA_3	0/10	8/10	8/10	8/10
20µM GA_3	0/10	2/10	2/10	2/10

Table 2. Effect of germination percentage of GA_3 treated seeds under salinity stress

Group	Germination %	Germination energy	Germination index
Distilled water	0.8	0.8	0.88
200mM NaCl	0.5	0.5	0.55
200mM NaCl+ 20µM GA_3	0.8	0.8	0.88
20µM GA_3	0.2	0.2	0.22

Table 3. Effect of GA_3 on relative germination and salt injury rate of Pea Seeds under salinity stress

Group	Relative germination rate	Relative salt injury rate
Distilled water	1.00	0.00
200mM NaCl	0.625	0.375
200mM NaCl +20µM GA_3	1.00	0.00
20µM GA_3	0.25	0.75

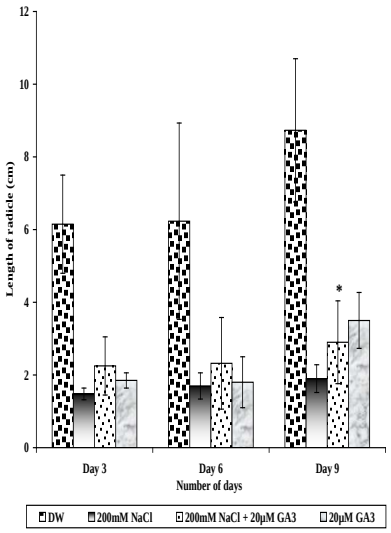

Figure 3. Effect of GA_3 on radicle length of pea seeds under salinity stress. *- $p<0.05$

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Shelf life study with special references to principles of microbiology

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Abstract

This work deals with ensuring quality of 'Kashayam' – a classical ayurvedic product. Some organoleptic characters, microbiological parameters, physico chemical properties were studied for comparing the quality, different batches of three different kashayams that is Maharasnadi Kashayam, Rasneirandadi Kashayam and Gandharva Hasthadi Kashayam were analyzed.

Key words: Kashayam, organoleptic characters, microbiological parameters, physico chemical properties

1. Introduction

Ayurveda (The Science of Life) is the Indian system of natural medicines with a track record of 5000 years of treating millions of patients. Ayurvedic pharmaceutics concerned with the production of medicines in Ayurveda based on classic texts like Charaka Samhita, Sushrutha Samhitha and Ashtagahydya. Other texts are Bhaishajyatnavali, Sarngadhra Samhitha, Bhela Samhitha, Kashayapa Samhitha etc. Pharmaceutical products are generally classified a Aristas, Asavas, Kashayas, Lehyam, Thylas, Choornas etc.

The primary health care of 70 – 80 % of the world's population is based on the use of medicinal plants derived from traditional systems of medicine and local health practices. With an increase in global demand for herbal medicines, it is necessary that the quality and the consistency of these drugs are maintained for their maximum efficacy. Purity and potency of these herbal medicines is only possible under Good manufacturing practice (GMP), quality control and standardization of the ingredients of raw drugs as well as finished products. With the increase in commercial manufacturing there arose a need for increased raw material inputs and mechanization. This led to more and more dependence at business houses and others involved in the manufacturing of ayurvedic drugs (Krishnan Nambiar, 2001). The standardization of finished product becomes more complex as most of the ayurvedic formulations are polyherbal in nature, which constitute different types of alkaloids, flavanoids, phytosterols, glycosides, glucosides etc. All the dosage form of ayurvedic drugs should be subjected to the stability studies in order to find out the shelf life and proper storage conditions (Biswapati Mukherjee, 1993).

Standardization of herbal drugs and raw materials include valuation for authenticity, bioactivity, and stability, and chromatographic profile, toxicity test against microbes, pesticide

residence, heavy metal and radioactive substances. The standardization in herbal drugs is relatively difficult. The activity of a product in modern medical system is based on its bioactive chemical constituents for which instrumental, chemical and bioassay methods are available to dry control laboratories to carry out tests for quality, consistency and batch to batch uniformity (Rajendra Gupta, 1996). Standardization practices suggested for Ayurvedic Medicine has to be aimed at raw material level, determination of active principle in process standardization and at the level of finished product.

Kashayam are the medicaments prepared by boiling the drugs added with water and reduced to the prescribed amount. It is prepared by boiling the mixture of crushed or powdered raw drugs with specified quantity of water and boiled to specific concentration. Medicinal properties are fully preserved. This type of pharmaceutical form is intended to establish the action of water soluble and heat stable extracts of ingredients. Though rather slow in action these have penetrating properties and are useful in chronic disorders.

Basic components are generally raw herbs and water, collected from nature. Ingredients like salts, oleoresins are added after processing, according to the formulations. Preparations are used to make the drug more potent, preserved and clinically effective. Pharmaceutical preparation of 'Oushadhi' are mainly depend upon the principles and practices described in classical texts like Charaka Samhitha, Susrutha Samhitha, Ashtangahridaya, Sahasrayogam.

The current study aims at the assessment of shelf life and standardization of the herbal products and techniques adopted by Oushadhi Pharmaceuticals during the formulation of products. Recently the ancient science 'Ayurveda' is accepted globally. The demand for herbal and ayurvedic preparation has been boosted in recent years to international market. Usually kashaya preparation is instant dosage forms that are to be used within 24 hours. Acceptance of the particular dosage form, lead the pharmacy people to change the method of preparation into high concentration along with preservation. Concentrated kashayam dosage form is an innovate step taken to increase the shelf life of Kashaya dosage form. Hence the standardization of basic and final products is essential for the survival of Ayurvedic products. Standardization and shelf life studies are essential to enter the ancient system to the levels of global market. Interdisciplinary researches are indicated to reform the scientific credibility of the system.

2. Materials and Methods

Different batches of three different kashayams, Maharasnadi Kashayam, Rasneirandadi Kashayam and Gandharva Hasthadi Kashayam were analyzed for organoleptic, physico – chemical and microbiological properties (Tewari and Pandey, 1992). The colour, odour, taste etc of Kashayam were observed. The pH, Specific Gravity and Total solids content were analyzed. The bacterial and fungal count was obtained by spread plating the samples on to nutrient agar plates (for aerobes) or thioglycollate broth tubes (for anaerobes) or Ross Bengal plates (for fungi) and then incubating the plates at 37 ^{o}C for 24-48 hours or at 25 ^{o}C for one week respectively and the colonies developed were further analyzed by staining and biochemical tests.

3. Results and Discussion

The analytical study of stored ayurvedic products was done under three categories, (i) Organoleptic Examination (ii) Physico –Chemical Examination (iii)Microbiological Analysis.

Organoleptic examinations of MaharasnadiKashayam, Rasneirandadi Kashayam, and Gandharvahasthadi Kashayam of all batches showed the standard colour, odour and taste.

Results of Physico-chemical Analysis are presented in Table 1, 2 and 3. Maharasnadi Kashayam packed on 07/09/2004, showed no variation in pH specific gravity at time of study. The TSS% at time of package was 15%. At time of study, it was reduced to 10%. Maharasnadi Kashayam packed on 15/02/2005 showed a small variation in pH and specific gravity. A slight decrease in pH and specific gravity was noted. The TSS% at time of package was 17%. It was reduced to 10% at time of study. Maharasnadi Kashayam selected from a batch packed on 07/07/05. The pH value is 4.40 at time of study. There was a slight increase in pH level at time of analysis. The specific gravity was decreased to 0.9771. The value at the time of package was 1.043. The TSS % value was reduced to 13% at the time of study. The pH value at time of package, of Maharasnadi Kashayam packed on 22/05/06 was 4.41. At the time of study, pH was observed to 4.53. There is a slight variation in specific gravity.TSS % at time of package was 13%. It was reduced to 10% at the time of analysis.

Table – 1: Comparison of Physico - Chemical Analysis of Maharasnadi Kashayam

Date of Package	pH		Specific Gravity		TSS %	
	At time of package	At time of Study	At time of package	At time of study	At time of package	At time of study
07-09-04	4.15	4.15	1.04263	1.0427	15%	10%
15-02-05	4.68	4.30	1.0490	1.0115	17%	10%
07-07-05	4.36	4.40	1.043	0.9771	14%	13%
22-05-06	4.41	4.53	1.040	1.0407	13%	10%

Rasneirandadi Kashyam, packed on 19/10/04 showed a slight variation in pH, TSS % and specific gravity. pH at time of package was 4.25. It was increased at time of analysis. There is a slight variation in specific gravity. TSS % was 14% at the time of package. It was noted that the value was decreased to 10% at time of study. Rasneirandadi Kashayam packed on 01/01/05 showed a slight variation in pH and TSS % pH value was 4.45 at time of package. It was reduced to 4.37 at time of study. TSS % was 10% at time of package and decreased to 9 % at time of study. Rasneirandadi Kashayam packed on 2/08/05 showed 10% TSS at time of package. The value was decreased to 6% at the time of analysis. Specific gravity has no significant variation. Rasneirandadi Kashayam packed on 16/01/06 has no significant variation in pH and specific gravity. TSS % at time of package is 12%. It was reduced to 9% at time of study.

Table – 2: Comparison of Physico – Chemical Analysis of Rasneirandadi Kashayam

Date of Package	pH		Specific Gravity		TSS %	
	At time of package	At time of Study	At time of package	At time of study	At time of package	At time of study
19-10-04	4.25	4.55	1.037	1.03905	14%	10 %
01-01-05	4.45	4.37	1.047	1.0475	10%	9 %
02-08-05	4.31	4.44	1.028	1.01961	10%	6 %
16-01-06	4.36	4.34	1.030	1.04004	12%	9 %

Table – 3: Comparison of Physico – Chemical of Gandharvahasthadi Kashayam

Date of Package	pH		Specific Gravity		TSS %	
	At time of package	At time of study	At time of package	At time of study	At time of package	At time of study
05-03-05	4.08	4.31	1.053	1.0144	17%	13%
08-10-05	3.88	4.03	1.067	1.0316	20%	15%
17-02-06	3.42	3.99	1.0652	1.03248	20%	17%

Gandharvahasthadi Kashyam packed on 5/03/05 showed 17 % TSS at time of package. A reduced value 13% was noted at time of analysis. A significant variation was also noted in specific gravity. 1.05% was the value of specific gravity at time of package. The value was reduced to 1.0144 at time of study. Gandharvahasthadi Kashayam packed on 8/10/05 showed variation in pH, specific gravity and TSS %. The pH was reduced from 4.03 to 3.88. The specific gravity was reduced to 1.0316 to 1.067. TSS % was reduced to 15 % from 20%. There is a slight variation is pH and specific gravity which was packed on 17/02/06. The TSS % was reduced to 17% from 20%. Results of microbiological analysis are shown in Fig.1, 2 and 3. The total viable count of Maharasnadi Kashayam packed on 07/09/2004 was found to TFTC*. The predominant colonies were smooth, circular. On Gram staining Gram positive cocci were observed. Biochemical tests such as catalase, Glucose fermentation were done for the characterization of organism. Both tests showed positive results. Motility of organisms was checked by hanging drop method and the organisms was found to be non motile. On blood agar, smooth circular white, non- hemolytic colonies were observed. From these observations, the typical organism in this kashayam may be *Staphylococcus* species. Slide coagulase test was performed to determine whether the organism is *Staphylococcus aureus*. The negative result of slide coagulase test indicates that the organism obtained may be coagulase negative, non pathogenic species. Fungus and anaerobes were absent in sample (Fig. 1).

Maharasnadi Kashayam packed on 15/02/05 was plated on nutrient agar to determine total viable count. The colonies obtained were TFTC. White, mucoid, non pigment colonies showed Gram negative small rods. Hanging drop motility test of the colonies showed non motile organisms.

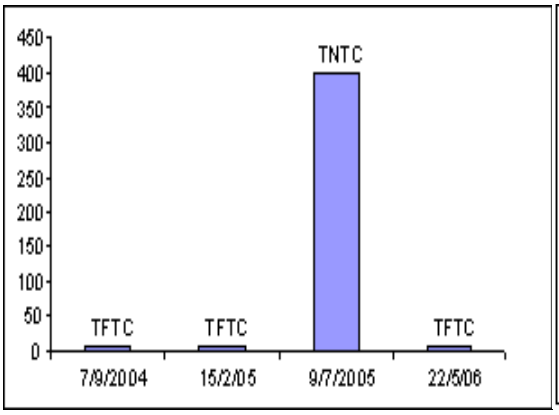

Fig. 1. Total count in Maharasanadi Kashayam during storage period

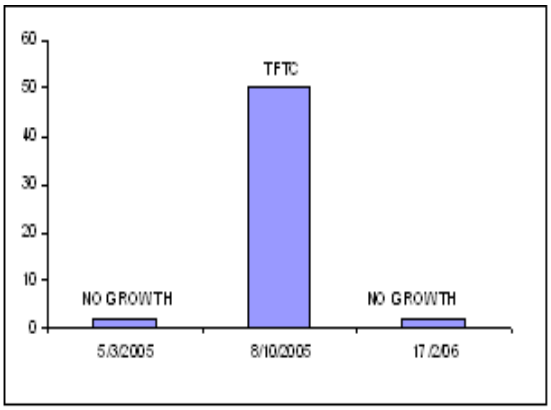

Fig.2. Total count in Gandharvahasthadi Kashayam during storage period

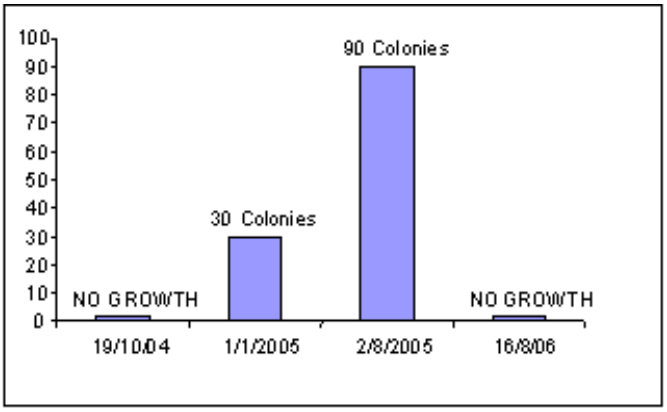

Fig. 3. Total count in Rasneirandadi Kashayam during storage period

Biochemical test such as Indole and carbohydrates test were performed. Indole test was negative, glucose fermentation was positive. From this observation, it was assumed that given organisms may be *Klebsiella species*. On MacConkey agar, Lactose fermenting pink coloured colonies was obtained. Fungi and anaerobes were absent in this batch.

Maharasnadi Kashyam packed on 9/7/05 was plated on nutrient agar to determine total viable count, the number of colonies were TNTC**. On Gram staining, Gram negative rods were found. Hanging drop technique showed the organism was non motile. Characterisation of organism was done by performing glucose fermentation and Indole test. On further culturing of kashayam, on MacConkey agar, lactose fermenting pink coloured colonies was obtained. From the above descriptions, the organisms were assumed to be *Klebsiella* species. Fungus was isolated from Ross Bengal Medium. On lactophenol staining the organism was found to be *Aspergillus* species. In thioglycolate medium, no anaerobes were observed. Total viable count of Maharasnadi kashyam packed on 22/05/06 showed a single colony. Gram staining showed Gram positive cocci it was non motile. On Ross Bengal medium *Aspergillus* species was isolated. Anaerobes were absent in thioglycolate medium.

Result of microbial analysis of gandharvahasthadi kahsyam is presented in Fig. 2. No growth was found in nutrient agar. Fungus and anaerobes were also absent Gandharvahasthadi Kashayam packed on 5/3/06. Total viable count of

Gandharvahasthadi packed on 8/10/05 was found to be TFTC. The predominant colonies were found to be Klebsiella species, Fungus was isolated from kashayam. On lactophenol staining the obtained fungus was found to be Aspergillus species. Anaerobes were absent. No growth was found in nutrient agar anaerobes agar. Anaerobes were absent Fungus was present in Gandhavahasthadi Kashayam packed on 17/2/06. On lactophenol staining, the obtained fungus was *Aspergillus* species.

Result of microbial analysis of Rasneirandadi kashayam is presented in Fig. 3. Only fungus growth was observed in Rasneirandadi Kashayam packed on date 19/10/04. On lactophenol staining, it was found that this particular fungus belongs to *Aspergillus*. Around 30 colonies were found in nutrient agar by culturing Kashayam packed on 01/01/05 agar. The colonies were found to be white, mucoid. On Gram staining, Gram negative short rods were obtained. Then on subsequent biochemical examination and on MacConkey media, it was confirmed that the given organism belongs to *Klebsiella* species. The fungus growth was also seen on the Kashayam. On lactophenol staining, it was found out that his particular fungus belongs to *Aspergillus* species. No anaerobes obtain from thioglycollate media.

Around 90 colonies were found as total viable count of Raneirandadi kahsyam packed on 2/8/05 preliminary examination, the growth of *Klebsiella* speices and S*taphylococcus* species were observed on nutrient agar. Further slide coagulase test was performed to determined wether the culture was Staphylococcus aureus or not. Coagulase test was negative. From this, it was inferred that given culture was not pathogenic *Staphylococcus aureus* species. It was observed that there was no bacterial contamination and no fungus growth in RasneirandadiKashayam manufactured on 16/01/06.

4. Conclusion

The present study was focused on the comparative microbial analysis on stored ayurvedic kashayams. Kashayams from different batches were taken for the experiment. The study was carried out at Oushadhi Research Institute Lab for checking maximum quality of their products. Three decoctions mainly used for study are Maharasnadi, Rasneirandadi, and Gandharvahasthadi kashayam. Various quality parameters such as specific gravity, TSS, pH were also measured. The presence of faecal contaminants as well as food borne pathogens was examined. It is appreciable that given Kashayams do not have faecal contaminants and pathogens. The fungus was observed in certain batched of Kashayam. This is a preliminary evaluation and further analysis must be done to detect the presence of aflatoxin, toxic metals and other contaminants.

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Physicochemical studies on Co(II), Ni(II), Cd(II) complexes of Schiff base, vanillin anthranilic acid

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Abstract

Schiff base derived from vanillin and anthranilic acid (VAA) and it's Co(II), Ni(II) and Cd(II) complexes were synthesized. Various spectroscopic tools such as ^{1}Hnmr, ^{13}Cnmr, Infrared, Mass and Electronic spectroscopy were utilized to establish the structure of the Sciff base ligand. Metal-ligand stoichiometry in the complexes was studied using elemental studies. Molar conductivity data explained the nonelectrolytic behaviour of the complexes. Furthur formulations of the structure of the complexes were done using spectroscopic and magnetic analysis.

Key words: Schiff base, vanillin, anthranilic acid, transition metal complexes

1. Introduction

Chemotherapeutically Schiff base ligands and their transition metal chelates have great importance in the recent years, as they possess antiviral, antibacterial and antitumour activities (Bellaci Ferrari et.al., 1994; Douglas X West et.al., 1993; Pandhye and Koffman, 1985; Joby Thomas and Geetha Parameswaran, 2002; Stanly and Geetha Parameswaran, 2010). The Schiff base complexes derived from anthranilic acid have potential applications in biological systems and chemical fields. In the present course of investigation, a novel potential ligand derived from vanillin and anthranilic acid and it's Co(II), Ni(II) and Cd(II) complexes were synthesized and characterized using various analytical tools and physicochemical studies.

2. Materials and methods

Analar grade samples supplied by E.Merck were used for the preparation

of ligand and complexes. Standard methods like volumetric, gravimetric and pyrolytic methods were adopted for the determination of metal content in the complexes (Vogel, 1978)

Vanillin(1g) was dissolved in minimum amount of ethanol (R.S) and warmed. To this hot solution, equimolar ethanolic solution of anthranilic acid was added, stirred for 1 hour and cooled in ice. The precipitated ligand was filtered at the pump, washed with dil. alcohol (1:1) and dried in descicator over P_2O_5. The purity of the sample was ascertained by TLC technique.

Vanillin (5mmol) and anthranilic acid (5mmol) were dissolved separately and mixed. It is then refluxed for 1 hour in a hot water bath. To this solution added an alcoholic solution of metal acetate (2.5mmol) and further refluxed for 30 minutes, concentrated by evaporation and cooled in ice. The crystals obtained was filtered at the pump, washed with water and dil. alcohol (1:1) and dried in descicator.

3. Results and Discussion

The ^{1}H NMR spectrum of the ligand contains eleven characteristic peaks due to eleven different types of protons present in the ligand. The peak observed at 3.83δ, which is a singlet with intensity corresponds to three protons, can be assigned to methyl protons of methoxy group. This is in very good agreement with the theoretical expectation 3.83δ. Including this, the total proton count of thirteen was observed in the spectrum, as expected. The peak of singlet nature at 7.69δ can be assigned to the azomethine proton, which is very much deshielded due to the presence of two aromatic moieties on both sides of the azomethine group. The carboxylic acid proton gave a singlet signal at 10.3δ. The presence of phenolic OH group in the ligand can be established by the appearence of a peak at 9.77δ, which is very close to the theoretical value 9.83δ. The proton decoupled ^{13}C NMR spectrum of the igand was recorded using DMSO as the solvent and the solvent peak was found at 39.43δ as septet. The fifteen carbon atoms present in the ligand are of different environment and hence showed their own characteristic signals in the ^{13}C NMR spectrum. As expected, the carboxylate carbon of the Schiff base gave its characteristic signal at 191.0ppm. The azomethine carbon exhibited sharp peak at 169.5ppm. The non equivalent carbon atoms on both aromatic rings showed their typical peaks between 109-153ppm. The one and only sp^3 hybridized carbon in the Schiff base, methoxy carbon, showed its own characteristic peak at 55.53ppm with better correlation to the theoretical value (56.3).

The molecular ion peak in the mass spectrum of the ligand, VAA was appeared at m/z value 271. This gives a good correlation with the molecular weight of the compound. Base peak was recorded at m/z value 154 which may be due to $[C_{11}H_8N]^{+\cdot}$ radical ion. The other significant peaks exhibited in spectrum can be explained by the usual fragmentation pattern of carboxylic acids and imines.

A strong band appeared in the infrared spectrum of the ligand at 1586cm^{-1} can be assigned to stretching frequency of azomethine group. The broad peak at 3362cm^{-1} shows the presence of hydroxyl group. The peaks at 1641cm^{-1} and 1512cm^{-1} may be attributed to asymmetric and symmetric stretching frequency of the carboxylate group. In plane and out of plane bending modes exhibited their characteristic peaks at 1018cm^{-1} and

743cm^{-1} respectively. The C-O stretching vibration can be judged by the strong band appeared at 1425 cm^{-1}. The characteristic bands due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions are exhibited by the ligand in its electronic spectrum at 37037 cm^{-1} and 21789cm^{-1} respectively. The spectral studies listed above and the CHN data suggest the following structure for the ligand, VAA (Fig. 1).

All complexes are found to be air and light stable, non-hygroscopic, amorphous powder and melt above 321^0 C. The complexes are coloured and sparingly soluble in common organic solvents but appreciably soluble in more polar aprotic solvents such as dimethyl sulphoxide and dimethyl formamide. The elemental analysis and metal percentage data of the complexes of Co(II), Ni(II) and Cd(II) are presented in Table-1. The analytical data show that there is 1:1 stoichiometry between metal ion and ligand in all the complexes. The molar conductance data of Co(II), Ni(II) and Cd(II) complexes of the Schiff base, VAA in DMSO at a concentration of 10^{-4}mol^{-1} at 28± 2^o C are very low, below 8.0 ohm^{-1}cm^{2}mol^{-1},which indicate their non-electrolytic behaviour.

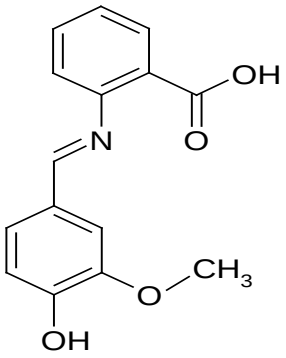


Figure-1: Structure of the ligand, vanillin antranilic acid (VAA)

The Co(II) complex exhibited a magnetic moment value of 4.78 BM. It is reported that an octahedral high spin geometry can be assigned to Co(II) complex, if the measured μ_{eff} value is in the range 4.5-5.1 B.M. Magnetic moment value of Ni(II) complex was 3.12BM, which suggest an octahedral geometry to it. The observed μ_{eff} value is very close to the spin only value of octahedral Ni(II) complexes (2.9-3.4BM), indicating the presence of two unpaired electron with an electronic configuration of $t_2g^6\ eg^2$ ($^3A_2$). Complex of Cd(II) is diamagnetic as expected due to d^{10} configuration and hence tetrahedral structure is proposed with 1:1 stoichiometry between metal and ligand.

Characteristic infrared absorption frequencies of the ligand and the complexes are represented in Table-2. Comparison of the IR spectra of the complexes with that of the ligand shows significant changes in two areas. Firstly, a band of medium intensity at about 1641cm-1 for the ligand, which may be attributed to the carbonyl stretching frequency of the carboxylate group, shows a shift to lower frequencies in the spectra of the complexes indicating the chelation of the ligand to metal through the carboxylate oxygen. The second region showing important changes upon ligand complexation is the 1600-1500cm^{-1} range. Compounds containing >C=N– group have $\upsilon_{C=N}$ in the range 1650-1490cm^{-1}. In this ligand, this band occurred at 1586 cm^{-1} as a strong band and has shifted to lower frequencies in the complexes indicating a reduction of electron density in the azomethine linkage at the nitrogen atom, coordinated to the metal ion (Nakamoto, 1978) in all the complexes the symmetric and asymmetric stretching vibrations of the

Table 1: Elemental analysis, molar conductance and magnetic moment data of the complexes

Compound (Colour)	% Carbon	% Hydrogen	% Nitrogen	% Metal	Molar conductance (ohm^{-1} cm^2mol^{-1})	Magnetic moment (μ_{eff} BM)
LH (Yellow)	67.35 (66.40)	4.49 (4.80)	5.82 (5.17)	--	--	--
$[CoL(Ac)(H_2O)_3]$ (Off-white)	45.59 (46.16)	5.02 (4.75)	2.99 (3.18)	14.83 (13.33)	4.2	4.78
$[NiL(Ac)(H_2O)_3]$ (Pale yellow)	46.87 (46.18)	5.10 (4.75)	3.02 (3.17)	14.48 (13.29)	8.7	3.12
$[CdL(Ac)(H_2O)]$ (Orange)	40.97 (41.18)	3.91 (3.43)	3.10 (2.83)	23.44 (24.46)	7.6	Diamag-netic

Calculated values are in parenthesis

carboxylate groups occur at 1456-1467cm^{-1} and 1586-1619cm^{-1} respectively showing a $\Delta\upsilon$ of about 140cm^{-1}. Monodentate carboxylate group has a $\Delta\upsilon$ value of >130cm^{-1}, but a bidentate carboxylate has a much closer difference (<120cm^{-1}). Therefore monodentate nature of the carboxylate groups is indicated in the present chelates. However conclusive evidence regarding the bonding of nitrogen and oxygen are provided by the occurrence of υ_{M-O} and υ_{M-N} absorptions in the range 510-578cm^{-1} and 652-668cm^{-1} respectively in the metal complexes.

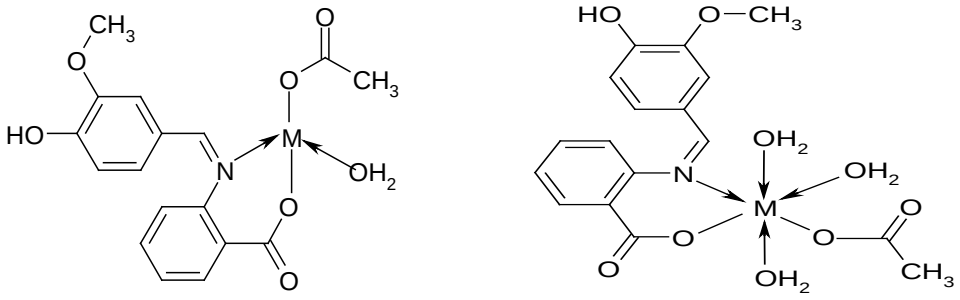
The existence of a strong band at about 3150-3100cm^{-1} and a medium band at 1100cm^{-1} in the infrared spectra of the complexes strongly support the presence of coordinated water molecules in these chelates. The absorption band frequency due to the phenolic C-O stretching vibration of the ligand (1425cm^{-1}) was found to have no considerable shift in the IR spectra of the complexes, which explains the uninvolvement of the phenolic OH group in chelation.

^{1}Hnmr spectrum of ligand shows a characteristic peak at 10.3δ corresponding to carboxylic acid proton. In complexes disappearance of this peak indicates the involvement of the carboxylate group in complexation by the replacement of H-atom. The signal due to phenolic OH proton in the pmr spectrum of the ligand(9.77δ) does not undergo considerable change in the spectra of the complexes (Okafo and Uzoukwu, 1993). This can be attributed to the free nature of this group during chelation.

The characteristic bands due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions are exhibited by the ligand in its electronic spectrum at 37037cm^{-1} and 21789cm^{-1} respectively. During complexation a red shift is detected for these absorption bands which indicate the involvement of Schiff base in coordination (Lever, 1986). Nickel(II) complex shows 3 bands in the spectrum at 35714cm^{-1}, 32258cm^{-1} and 2179cm^{-1}, which can be attributed to following spin allowed d-d transitions.

^3A_2g (F) —> ^3T_2g (F) , ^3A_2g (F) —> ^3T_1g (F), ^3A_2g (F) —> ^3T_1g(P)

Octahedral arrangement around the metal ion in the Ni(II) chelate can be confirmed by these spectral data. Comparatively weak signals are exhibited by cadmium chelate due to its d^{10} configuration and hence a tetrahedral structure is proposed for it. Based upon the above physico-chemical studies, octahedral geometry is suggested for Co(II) and Ni(II)



M = Cd(II) **M = Co(II) and Ni(II)**

Figure-2: Structures of the metal complexes

complexes and a tetrahedral geometry for cadmium complex.

VAA acts as a monovalent bidentate ligand in all these complexes using the donor sites azomethine nitrogen and carboxylate oxygen atoms. The structures of the complexes are given in the Figure 2.

Table -2 Characteristic infrared absorption frequencies (cm^{-1})

Substance	γ_{H_2O}	$\gamma_{CO\ O\ (asy)}$	$\gamma_{C=N}$	$\gamma_{COO\ (sym)}$	γ_{C-O}	In plane bending	Out of plane bending	γ_{M-N}	γ_{M-O}
LH	**-**	**1641**	**1586**	**1512**	**1425**	**1018**	**743**	**-**	**-**
[CoL(Ac)(H_2O)$_3$]	**3102**	**1589**	**1538**	**1456**	**1423**	**1034**	**752,715**	**652**	**510**
[NiL(Ac)(H_2O)$_3$]	**3118**	**1592**	**1541**	**1508**	**1426**	**1067**	**754,715**	**668**	**517**
[CdL(Ac)(H_2O)]	**3158**	**1615**	**1574**	**1497**	**1422**	**1028**	**752,705**	**665**	**578**

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Effect of naturally ocurring antimicrobials and chemical preservatives on the growth of *Aspergillus parasiticus.*

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Abstract

We have chosen to work on the Effect of Natural Antimicrobials on Food Preservatives on the growth of toxigenic strain of *A.parasiticus* at 0.99aw and 4.5pH. To prepare PDA system with different variables, KCl was suitable solute to prepare cost effective media, although the growth in glycerol added plates was equally good. In general, the growth of the fungus was concentration dependent i.e., as the concentration increased the radial growth rate (RGR) decreased. Also, the linear increase in colony diameter is a function of incubation time. As analysis of variance (ANOVA) indicated antimicrobials concentration (150 ppm to 1500 ppm) significantly ($p<0.05$) affected *A.parasiticus* radial growth rate (RGR). Thymol and carvacrol being most effective followed by eugenol, sorbic acid, citral and vanillin than to anethol, potassium sorbate, cineole and guiacol.

Key words: Antimicrobials, A.parasiticus, food preservatives

1. Introduction

Infestation of various food commodities by insects, fungi and rodents causes considerable losses in tropical and sub-tropical countries (Bajaj and Ghosh, 1975). In spite of the use of all available means of plant protection, about one third of the yearly harvest of the world is destroyed. Tropical countries because of their temperature and their particular environment, suffer from losses due to various pests. Synthetic chemicals such as pesticides and fumigants are used extensively to control the pests in stored food grains. These chemicals have contributed in greater extend to manage the pests, but it has also raised a number of ecological and health problems due to residual toxicity and carcinogenicity (Bajaj and Ghosh, 1975). Effective pest control is no longer a matter of heavy application of pesticides, which promotes faster evolution of resistant forms of pest, destroys natural enemies, turns formerly innocuoces species in to pests, harms other non-target species and contaminates food (Jaya Varma and Dubey,1998).

At present phosphine is the only fumigant which is widely used, after phasing out of methyl bromide which has been identified as a major contributor to ozone depletion. There have been repeated indications that some insects have developed resistance to phosphine, therefore its use is now suspected. Fungi cause most of the microbiological problems inherent in cereal production. In cereals these are conveniently divided into two groups, field and storage fungi (Christensen, 1987). Field fungi invade seeds and grains before harvest and are often pathogens or commensals on the grains. For the growth field fungi generally require aw levels greater than 0.09 equivalents to 20-25% moisture on weight basis. In the last two decades problems of toxicity of many filamentous fungi attracted much attention. These problems particularly in agriculture and food industry became at most importance. Most toxigenic fungi belong to the genera *Aspergillus, Penicillium* and *Fusarium.* It is known that these fungi produce toxins only on certain substrates and under specific conditions. In general, the production of mycotoxins is more intense on food rich in carbohydrates. The mycotoxins penetrate into food stuffs with out changing their appearance. It is also problematic that these toxins are mostly heat stable.

It has been recognized that both water activity (aw) and ionic concentration (pH) are important indices in the control of food stability and in the formulation of intermediate moisture foods (Rockland and Beuchat, 1987; Rockland and steward, 1981). The aw values are between 0 and 1, and the desired accuracy of aw measurements and prediction is in the range of ± 0.01 aw. Various sugars, salts and acids may be added to adjust the aw and pH in food product development.

Cornner et. al.,(1984 a,b) suggested that the antimicrobial activity of essential oils of herbs and spices of their constituents such as thymol, carvacrol, vanillin etc., could be the result of the damage to enzymatic cell systems, including those associated with energy production and synthesis of structural compounds. Nychas(1995) indicated that phenolics could denature the enzymes responsible for spore germination or interfere with amino acids involved in germination. Several studies have attempted to determine the efficacy of extracts form selected plants as antimicrobial and antifungal agents.

Some studies have shown that specific essential oils and phenolic compounds control the growth rate and spore germination time of spoilage fungi. Paster et. al.,(1994) found that oregano and thyme essential oils inhibited the growth of *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus flavus*. Growth was fully inhibited with 400ppm of organo and 600 ppm of thyme, these oil concentrations also prevented spore germination. However few studies have taken in account the effect that different environmental factors such as water activity (aw) and pH may have on the effectiveness of phenolic compounds and synthetic antimicrobials in relation to fungal growth. The agents (chemical preservatives and naturally occurring antimicrobial compound) on the growth of *Aspergillus parasiticus* in media formulated with selected pH and aw values.

2. Materials and Methods

2.1.Microorganisms

The toxigenic strain of *Aspergillus parasiticus* (3.18) was kindly supplied by Dr. Vicenete Sanchis, Food Technology Department, Lleida University, Spain. It produces aflatoxin B1, B2, G1, G2.

2.2. Preparation of spore suspension

The pure culture of the organism was cultivated on Potato Dextrose agar slants and incubated for 10 days at 25 degree Celsius. At the end of incubation period, the spores were harvested with 10ml of sterilized 0.1% Tween 80 solution. This suspension was passed through 0.45 microns sterilized membrane fulter to remove debris and other agar particles. The spore suspension was then adjusted with the same solution, to give a final spore concentration of 1X106 spores/ml using haemocytometer and was utilized on the same day.

2.3. *Chemicals*

The chemicals used for the experiment were purchased from different companies. Citral, carvacrol, Eugenol, cineole and thymol were purchased from Sigma-Aldrich, Germany. Guiacol and vanillin were purchased from sisco research laboratories, Mumbai. Anethol was procured from Merk India and Potassuum sorbate and Sorbic acid were procured from Hi-media Ltd, Mumbai.

2.4. Experimental design

Factorial designs were employed to assess the effects of water activity (0.99), pH 4.5 and antimicrobial agents and concentrations (0,150,300, 450, 600, 750, 900, 1050, 1200, 1350, 1500) on mold radial growth rates. Systems prepared with the resulting variable combinations were replicated three times.

2.5. Preparation of the Systems

Initially we have tested the solutes to be used in this study, such as KCl, NaCl, glucose, sucrose and glycerol to know the growth and development of the organism. It was observed that the organism grown in KCl has shown maximum growth. Followitn experimental designs, PDA systems were prepared with the necessary quantity of commercial sucrose to reach aw 0.99, sterilized for 15 minutes at 121degree Celsius, cooled and acidified with hydrochloric acid 0.1N to the desired pH. Sterilized and acidified agar solutions were aseptically divided, and then the necessary amount of every antimicrobial agent was added and mechanically incorporated and dissolved under aseptic conditions.

Agar solutions were poured into sterile Petri dishes.

2.6. Inoculation and Incubation

Triplicate Petri dishes of every system were centrally inoculated by pouring 2 micro litre of the spore suspension to give a circular inocula of 2mm in diameter. For aw and pH measurements, 3 plates of each systems were maintained without inoculation. Growth controls without antimicrobials were prepared and inoculated as above. The inoculated plates and the controls were stored in hermetically closed plastic containers (over sodium chloride solutions of the same aw), to avoid dehydration, for 1 month at 25+_0.5degree Celsius in incubation chambers. Sufficient head space was left in the containers to avoid anoxic conditions. Periodically, inoculated plates were removed briefly to be observed and to measure colony diameter and immediately reincubated.

2.7. Colony radial growth

The inoculated systems were daily examined using a stereoscopic microscope. After the colonies were confluent, their increase in size was followed by measuring the colony diameter. Increase in diameter of each plate was plotted as function of incubation time, and radial growth rate was obtained from the slope by logarithmic regression of the linear phase of growth. Mean radial growth rate for every experiment was calculated and reported.

2.8. Statistical analysis

Analysis of variance (ANOVA) of the effect of the independent variables and their interactions on radial growth rates in different systems were performed. The significant difference between treatments and level of treatments was established by using DMRT at $p < 0.05$.

3. Results and Discussion

The combined effect of aw (0.99), pH (4.5) and antimicrobial agents (citral,anethol, guiacol, cineole, eugenol, thymol, carvacrol, vanillin, potassium sorbate and sorbic acid) on the growth of *A.parasiticus* were evaluated in PDA. Based on the preliminary experiments, we have chosen KCl as a suitable agent to adjust aw in PDA systems, although we have observed almost same growth rate with glycerol. We found that the use of glycerol to adjust the medium is not economical. Therefore, we used KCl as an suitable agent to adjust aw, throughout the experiment.

The increase in colony diameter during the incubation period of *A.parasiticus* inoculated on PDA with 0.99 aw, pH 4.5 and cineole concentrations at 150ppm, 750ppm and 1500ppm was shown in Fig 1, 1A, 1B respectively. The results indicated that the colony diameter exhibited a constant increase with time. Similar results were also obtained for every other evaluated antimicrobial agents. Regression coefficients were obtained for the sigmoid growth phase, with standard deviation/mean values. Several authors reported that a linear increase in colony diameter as a function of incubation time for molds in solid medium and found significant differences among radial growth when different conditions were evaluated.

In general, the growth was directly proportional to the concentration of antimicrobials used. However, the results demonstrate that thymol and carvacrol were more efficient in inhibiting the growth of *A.parasiticus* even at low concentrations (150ppm). Therefore no growth was obtained even after 480 hrs of incubation. In

other antimicrobials tested, eugenol and sorbic acid inhibited the growth of test fungus at 300ppm and 600 ppm, respectively. Similar results were also obtained with citral and vanillin. Citral at 750 ppm and vanillin at 900 ppm, total inhibition was recorded. The least effective antimicrobials being anethol, potassium sorbate, cineole and guiacol.

It has been established that several environmental factors determine and affect mold growth, germination time and radial growth rate in solid media. Mold colony diameter could be used as a growth response to evaluate growth rate of the fungus and compare the influence of several factors on mold response. RGR may be considered as a result of several rates related to fungal physiology, morphology, apical cell division, branch formation and cell enlargement.

In our studies *A.parasiticus* exhibited higher sensitivity to thymol, carvacrol, eugenol, sorbic acid, citral and vanillin pt pH 4.5, than to anethol, potassium sorbate, cineole and guiacol. Plant derived antimicrobials are not yet fully exploited. The major limiting factor for not utilizing spices, herbs, plants and essential oils as antimicrobials is due to the high minimum inhibitory concentration (MIC) required in foods with high protein of fat contents, which also may impart off flavours. These difficulties could be minimized, if natural compounds are used in combination with other stress factors such as reduced aw and pH.

Table 1. Percentage weight concentrations of some solutes for various values of aw at 25 degree Celsius.

Aw	NaCl	KCl	CaCl2	Glucose	Sucrose	
0.995	**0.9**	**1.2**	**1.2**	**5.1**	**9.2**	**2.6**
0.990	**1.8**	**2.3**	**2.4**	**9.3**	**15.9**	**4.9**
0.980	**3.4**	**4.4**	**4.5**	**16.6**	**26.2**	**9.2**
0.960	**6.5**	**8.5**	**8.0**	**27.9**	**39.7**	**16.7**
0.940	**9.3**	**12.2**	**10.8**	**36.4**	**48.4**	**23.1**
0.920	**11.9**	**15.7**	**13.1**	**43.0**	**54.6**	**28.6**
0.900	**14.2**	**18.9**	**15.0**	**48.3**	**59.2**	**33.5**
0.880	**16.3**	**21.7**	**16.75**	**52.7**	**62.9**	**37.7**
0.850	**19.1**	**-**	**19.0**	**-**	**67.2**	**43.3**
0.800	**23.2**	**-**	**22.2**	**-**	**-**	**51.0**
0.750	**-**	**-**	**24.9**	**-**	**-**	**58.61**
0.700	**-**	**-**	**27.35**	**-**	**-**	**64.15**
0.650	**-**	**-**	**29.60**	**-**	**-**	**16.05**
0.600	**-**	**-**	**31.70**	**-**	**-**	**73.40**

4. Conclusion

Several reports revealed that environmental factors such as pH, temperature and moisture availability determine the physiology of mold growth, spore germination and radial growth rate (RGR) in solid media. Mold colony diameter is used as a measure of growth response to evaluate growth rate of fungus. The objective of this study was to evaluate the growth response of different antimictobeal compounds such as citral, anethol, guiacol, cineole, eugenol, thymol, carvacrol, vanillin, potassium sorbate and sorbic acid against a toxigenic strain of *A.parasiticus*. Total ten concentrations of each chemical was employed in PDA systems prepared with 0.99 aw at ph 4.5. The results demonstrated the linear increase in colony diameter as a function of incubation time and the

radial growth rate of fungus was directly proportional to the concentration on antimicrobial tested. For most of the antimicrobials tested, an analysis of variance (ANOVA) demonstrated, antimicrobial concentration (150 ppm to 1500 ppm) significantly ($p<0.05$) affected *A.parasiticus* radial growth rate (RGR). However, in thymol and carvacrol incorporated plates, no growth was observed even after 20 days of incubation. These studies clearly indicate that *A.parasiticus* exhibited higher sensitivity to thymol, carvacrol, eugenol, sorbic acid, citral and vanillin at pH 4.5, than to anethol, potassium sorbate, cineole and guacol. Available literature indicate, the plant derived antimicrobial agents such as herbs spices and essential oils are not fully exploited due to high Minimum Inhibitory Concentration (MIC) required in food with higher protein or fat contents, which may sometimes impart off odours. The results obtained in our studies could help to minimize, if natural compounds are used in combination with other factors such as aw and pH.

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Effect of combined inoculation of *Azospirillum* and Phosphobacteria (Azophos) in Onion (*Allium Cepa*)

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Abstract

Azospirillum inoculants fix and supply atmospheric nitrogen. Phosphobacteria improve the availability of phosphorus to the crop by solubilising the insoluble form of phosphorus there by making it available to the plants. The present study is to find the effect of *Azospirillum* (*A lipoferum*) and Phosphobacteria (*Bacillus megaterium*) singly and in combination (Azophos) on the growth of onion as well as to compare the shelf life of *Azospirillum* and Phosphobacteria in Azophos.

Key words: Azophos, Azospirillum, Phospobacteria

1. Introduction

Biofertilizers are of great agronomic value which improves crop productivity through non-polluting and eco-friendly means. These are microbial inoculants or bioinoculants containing live or latent cells of nitrogen fixing, phosphate solubilising organisms used for application to seed or soil to augument the availability of nutrients to plants. Nitrogen and phosphorus are the two most essential nutrients influencing the crop growth and productivity.

Azospirillum absorbs and fixes atmospheric nitrogen in the root zone and provides 30-50% of nitrogen requirements. The organism is Gram-negative and contains β-hydroxy butyrate granules. On semi solid malate medium, the development of white, dense and undulating fine pellicle is very characteristic of *Azospirillum*. The bacteria, which dissolves the undisolved form of phosphorus is called phosphobacteria. This includes *Bacillus megaterium*, *B subtilis*, *B circulans*, *Pseudomonas rathonis* and *Pseudomonas straita*. Other than bacteria fungi like *Aawamori*, *Pencillium digitatum* and yeast like *Schwanniomyces occidentails* have the ability to dissolve the inorganic phosphorus. The phosphate solubilizers also produce fungistatic and growth promoting substances which influence plant growth.

2. Materials and Method

Azospirillum broth and phosphobroth were obtained from the biofertilizer production unit, Trichy. 35ml of *Azospirillum* broth was mixed well with 165gm of neutralized lignite (carrier) and was packed in a plastic packet. Similarly 35ml of phosphobroth was mixed with 165gm of neutralized lignite and packed. The

Azophos packets were made by mixing equal ratio of *Azospirillum* broth and phosphobroth (20ml) to 160gm of neutralized lignite. The population of *Azospirillum* and phosphobacteria in *Azospirillum*, Phosphobacteria and Azophos packets were seen for a consecutive period of five months by serial dilution and plate count technique using appropriate selective medium in order to compare the shelf life of *Azospirillum* and Phosphobacteria in Azophos.

Onion seeds were treated with *Azospirillum*, phosphobacteria and Azophos 10gm/10 seeds respectively as per treatment individually and in combination with one control. Three replications were maintained for each treatment and 10 seeds were used per replication. Observation on germination, root and leaf length was recorded on 30^{th} day after sowing. Vigour index was calculated using the formula,

Vigour index = (average shoot length + average root length) × germinate

2.1 Pot culture studies

Plastic pots were filled with local soil and flooded with water after seeing the initial population. The treated seeds were sown in the pots, 10 seeds per pot. Non inoculated control was also maintained. Three replications were maintained for each treatment. Rhizosphere soil sample were collected from the pots after 40 days of sowing. The population of *Azospirillum* and phosphobacteria were enumerated by serial dilution and plate count techniques, using appropriate selective medium viz; nitrogen free malic acid medium, Pikovskaya's medium respectively. *Azospirillum* count was determined by the most probable number test (MPN). The chlorophyll content, protein content, phosphatase activity was seen after 45 days of seed inoculation.

2.2 Estimation of chlorophyll

1gm of the sample was weighed and grinded with a clean mortar and pestle using 20ml of 80% acetone. Then it was centrifuged at 5000rpm and the supernatant was transferred to a volumetric flask. The above procedure was repeated until the residue became colourless. The clean washings were collected in the volumetric flask. The volume was made upto 100ml with 80% acetone. The absorbance of the solution was read at 645nm and 663nm against the solvent blank.

2.3 Estimation of protein

2.3.1. Extraction of protein from sample

Extraction is usually carried out with buffers used for enzymes assay. 500gm of the sample was weighed and grinded well with mortar and pestle in 5-10ml buffer. Then it was centrifuged and supernatant was used for protein estimation.

2.3.2. Estimation of protein

The protein present in the sample was estimated by Lowry's method.

2.4 Estimation of phosphatase activity

Enzyme extract: 1gm of fresh tissue was homogenized in 10ml of ice cold 50 mM citrate buffers (pH-5.3) in a prechilled mortar and pestle. Then it was filletered through four layers of whatmann filter papers. The filtrate was centrifuged at 10,000rpm for 10 minutes and the supernatant was used as enzyme source. 3ml of substrate solution (1.4gm of EDTA, 0.84gm citric acid and 0.03gm of P-nitrophenyl phosphate in 100ml water, pH 5.3.) was incubated at 37°C for 5 minutes. 0.5ml of enzyme extract was added

and mixed well. 0.05ml was removed immediately and mixed with 9.5ml of sodium hydroxide (0.085N). This corresponds to zero time assay (blank). The remaining solution was incubated at 37°C for 15 minutes. 0.5ml of the sample was taken and mixed with 9.5ml sodium hydroxide solution.0.1-0.5ml of the standard (69.75mg of P-nitrophenol in 5ml of distilled water) were taken and diluted to 10ml using sodium hydroxide solution.The absorbance of blank and incubated tubes were read at 405nm.

3. Results and Discussion

There was a progressive increase in the population of *Azospirillum* and Phosphobacteria with the advancement of time. The *Azospirillum* population was maintained when *Azospirillum* or Azophos was applied. Mere phosphobacteria application had a lesser population of phospobacteria when compared to coinoculation. Thus, it can be inferred that coinoculation had a synergistic effect on phosphobacteria population. Population count taken upto 150^{th} day after inoculation showed that the phosphobacteria population in Azophos increased massively up to 30^{th} day of preparation and subsequently decreased with the advancement of time, when compared to phosphobacteria biofertilizer preparation.

The reason for this may be phosphobacteria is a fast grower when compared to *Azospirillum*. The phytohormones produced by *Azospirillum* such as IAA, IBA and Cytokinin would have been used by phosphobacteria for its growth and proliferation. After attaining a higher level of population, it would have resulted in the competition for food and the limited nutrient solution applied at the time of preparation would have not been sufficient for its maintainance and resulted in the decline of population, one month after inoculation. But the population is adequate as per quality control works.

Phosphobacteria inoculation resulted in highest root length followed by Azophos and *Azospirillum*. The probable reason for increased root length would have been due to the increased organic acid production by Phosphobacteria resulted in the solubilization of phosphores from unavailable form. Phosphorus uptake is directly correlated with root proliferation. The vigour index was much pronounced in Azophos, followed by Phosphobacteria. In general the coinoculation treatment favours higher vigour index when compared to control. The synergistic effect due to the combination of *Azospirillum* and phosphobacteria than their individual application was much pronounced. Chlorophyll and protein content was high when Azophos was inoculated when compared to control and individual application. The coinoculation had resulted in the highest phosphatase activity than the individual inoculations.

4. Conclusion

A pot culture experiment with onion as the test crop was conducted to find out the effect of individual application of *Azospirillum*, phosphobacteria and their combined effect on different growth parameters, Protein, Chlorophyll content, Phosphatase activity and population load both in soil and in the inoculam preparation. The data revealed that the inoculation of *Azospirillum* or phosphobacteria alone or as coinoculation could not

Table 1. Population of *Azospirillum* and phosphobacteria in single and combined inoculation.

Inoculants	Count taken on (days after inoculation)											
	0		30		60		90		120		150	
	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8
Azospirillum	>16	-	>16	-	>16	-	>16	-	>16	-	>16	-
Phosphobacteria	-	57	-	51	-	47	-	40	-	35	-	28
Azophos	>16	52	>16	60	>16	47	>16	31	>16	30	>16	25

Table 2. Effect of biofertilizer inoculants on various morphological characters of plant.

Inoculants	Germination %	Root length(cm)	Leaf length(cm)	Number of leaves	Vigour index
Control	80	3. 0	27	9	2400
Azospirillum	80	4. 2	33	12	2976
Phosphobacteria	70	6. 0	37	10	3010
Azophos	80	5. 5	35	13	3636

***Mean values of three replications**

Table 3. Effect of seed treatment on Rhizosphere population of bioinoculants.

Inoculants	Initial count		40 days	
	Az x 10^9	P x 10^8	Az x 10^9	P x 10^8
Control	0.14	19	0. 20	24
Azospirillum	-	-	>16	30
Phosphobacteria	-	-	0. 32	70
Azophos	-	-	>16	76

Table 4. Effect of biofertilizer inoculants on various physiological characters of plant.

Inoculants	Phosphatase activity(M)	Protein content(mg/g)	Chlorophyll		
			Chlorophyll A	Chlorophyll B	Total(mg/g)
Control	6M	1.44	0. 205	0. 169	0. 374
Azospirillum	6. 5M	1. 91	0. 21	0. 214	0. 425
Phosphobacteria	7. 2M	2. 01	0. 28	0. 17	0. 451
Azophos	12. 3M	2. 17	0. 353	0. 205	0. 558

favour germination of onion. Increased root and leaf length were observed for phosphobacteria inoculation. The coinoculation increased the number of leaves, the vigour index, chlorophyll and protein content of the leaves. The phosphotase activity was high in the coinoculant and was significantly influenced by phosphobacteria population in the soil.

As far as the shelf life of the biofertilizers preparations are concerned, there was no decline in the population of *Azospirillum* in both *Azospirillum* and Azophos preparations. The phosphobacteria population showed a gradual decrease in both phosphobacteria and Azophos preparation. In the coinoculation preparations, the population of *Azospirillum* was maintained up to 150^{th} day, whereas a decline in phosphobacteria population was observed in the coinoculation 30 days after preparation but it was well within the prescribed works of quality control. Hence it is concluded that coinoculation of *Azospirillum* and phosphobacteria have brought about many beneficial effects. But yet the shelf life of *Azospirillum* and phosphobacteria in the coinoculation has to be studied for further periods to draw any conclusion.

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Cost effective feed formulation for carps

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Abstract

Aquaculture is a booming industry expanding rapidly. Most fish culture operations are facing challenges of economical sustainability because of high price of feeds. The present study investigates the efficiency of Colocasia sp. (chembu) and Amorphophallus sp.(kattuchena) tubers as fish feed ingredients. The study was conducted in Koi carp for a period of 45 days. Colocasia sp. was found to show better growth rate than Amorphophallus sp.

Key words: Aquaculture, Colocasia sp, Amorphophallus sp.

1. Introduction

Carp culture is a fast growing aquaculture industry with huge potential. Feed and feeding management greatly contribute to aquaculture production cost. Farmers are slowly shifting to farm made feed rather than pelleted feed, owing to increased cost of pelleted feeds. Therefore alternatives to combat increasing cost of feed ingredients are a prerequisite of present day aquaculture.

There is no predefined feeding standard available for fish feeds though farmers are following their own feed formulas. There are a variety of plant derivatives that could be used as fish food ingredients. Colocasia commonly called Elephant-ear is a herbaceous perennial plant with large thick tuber containing starch. It is commonly cultivated for food. Amorphopallus commonly called elephant foot yam is used as a staple food and also as a vegetable.Pheng Buntha (2008) investigated the effect of Taro (*Colocasia esculenta*) leaf silage as replacement for fish meal on feed intake and growth performance of crossbred pigs.

Lee Seong Wei (2008) studied the antimicrobial properties of *Colocasia esculenta* against pathogenic bacteria isolated from aquatic organisms. The value of yam peels as energy source in the diet of Oreochromis was studied by Solomon. Chua (2010) investigated the traditional uses of Amorphophallus konjac. The present study investigates the effect of colocasia and Amorphophallus sp. as fish feed.

2. Materials and Methods

The study was conducted in aquaria. The glass aquaria tanks were properly washed, filled with clean water and aerated using air pumps to ensure proper oxygenation. The experimental fish koi carp were collected from the hatchery. They were acclimatized for two weeks. before experiment.

The experimental fish were randomly distributed at a stocking density of ten fish per aquarium tank. Two diets were formulated with various ingredients like rice bran, wheat bran, ground nut oil cake, coconut oil cake, colocassia sp. and Amorphophallus sp. Table I indicates the ingredients and their composition used in the two diets. The fishes were fed at 5% body weight twice daily morning and evening at equal ration. Sampling was done using a sensitive electronic balance (schimadzu) at every 15 days interval to determine the average weight of the fish and adjust the feed accordingly. The study was conducted for 45 days.

3. Result and Discussion

The weight gain in the fish subjected to the two diet formulations are given in the Table 1. The mean weight of the fish after 45 days was higher for fishes that consumed Diet II (5.48g) than that for Diet I (5.18g). The mean weight gain was 1.48g for Diet II and 1.12g for Diet I. Thus the diet with colocasia showed better growth performance than with Diet I (Amorphophallus sp.). The statistical analysis revealed that the effect of the two Diets on body weight of fish during six weeks was insignificant for four weeks but by six weeks it was significant ($P<0.001$).

Similar results were observed by Sankaran et al, (2008) According to him the elephant foot yam plays a vital role in nutrition of tribal people of Tripura. Orire et al, (2010) observed that yam starch could be used as a local alternative binding agent in aquatic feed, Five percent (5%), inclusion level was found to be appropriate in producing desirable water stable pellet. The nutritional quality of colocasia was found to be adequate for meeting nutritional requirement of Juvenile African Catfish (Aderolu, 2009).

Table 1. Percentage composition of different ingredients on dry matter basis (g/100 g diet)

Ingredient	Diet I (Amorphophallus sp.) wt /100g	Diet II (Colocasia sp.) wt /100g
Rice bran	55g	55g
wheat bran	5g	5g
Groundnut oilcake	5g	5g
Coconut oil cake	5g	5g
Amorpho phallus	20g	-
Colocasia	-	20g

Chhay Ty (2011) observed that replacing the mixture of rice bran and duckweed by Taro silage resulted improvements in live weight gain in diets of ducks.

Table 2. Growth performance of Koi carp to Amorphophallus sp. and Colocasia sp. included diets.

Days	Diet I (Amorpho phallus) wt /100g	Diet II (Colacasia) wt /100g
0	4. 06	4. 00
15	4. 42	4. 52
30	5. 0*	5. 37*
45	5. 18*	5. 48*
Mean weight gain(g/fish)	1. 12	1. 48

***Means followed by are significantly different at P<0.0001.**

Mohammed et al (2009) observed that proper processing of cocoyam meal will effectively replace maize at 25% (raw sundried) and 50% (boiled sundried) as a major source of energy in diets of broiler finishers.

4. Conclusion

The result obtained in the present study reveals that colocasia sp. produces better

growth performance in koi carp than Amorphophallus sp. The culture of the plants is easy and thus it can be effectively used as a fish feed.

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Monosodium glutamate (ajinomoto) induced mitotic aberrations in onion root tip

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Abstract

Impact of monosodium glutamate, a commonly used flavouring agent on root mitosis of *Allium cepa* L. has been studied. Root tips were treated with five different concentrations of monosodium glutamate viz. 0.01%, 0.05%, 0.1%, 0.5% and 1% for 48 hrs. The control sample was exposed to distilled water. Various parameters studied were germination percentage, number of roots/bulb, mitotic index, mitotic aberrations and frequency of mitotic aberration. The additive inhibited germination of the bulbs and cell division. The mitotic index was found to be declining with increasing concentrations of the ajinomoto. A wide range of mitotic abnormalities like strap nucleus, tropokinesis, C-mitosis, anaphase bridge and multipolar anaphase with chromosome fragment were observed in the treated cells. Frequency of aberration was increased with increasing concentration of monosodium glutamate. The use of such artificial flavouring agents in the food items warrants serious consideration.

Key words: Monosodium glutamate, Allium cepa L., germination percentage, mitotic index, mitotic aberrations.

1. Introduction

A substance or mixture of substance, other than basic food stuff which is present in food as a result of any aspect of production processing, storage or packaging is known as food additives. Today more than 2500 different additives are intentionally added to foods to produce a desired effect. The use of additives is a well accepted practice but not without controversy. Additives can be divided into six major categories: preservatives, nutritional additives, flavouring agents, colouring agents, texturing agents and miscellaneous additives. Of these, flavouring agents constitute the largest group of additives used in food.

Monosodium glutamate also known as sodium glutamate and MSG is a well known flavouring agent in the trade name Ajinomoto. According to the report of the Federation of American Societies for Experimental Biology (FASB) compiled in 1995, MSG is safe for most people when eaten at customary levels. However, some people may have an MSG intolerance which causes "MSG

symptom complex" or a worsening of asthmatic symptoms (Geha *et al*., 2000).

Animal studies indicate that monosodium glutamate (MSG) can induce hypothalamic lesions and leptin resistance, possibly influencing energy balance, leading to overweight (Ka He *et al*.,2008). According to Gunther (1997), MSG contributes to brain lesions, sterility, obesity, stunted growth, fatty livers, eye problems etc. and that it is toxic to the central nervous system. The current investigation is an attempt to analyze the impact of this food additive at the chromosome level by using a plant material, onion root tip as the experimental material.

2. Materials and Methods

Bulbs of *Allium cepa* was used as the biological material of the experiment. Range finding test was conducted with various concentrations of monosodium glutamate viz. 0.01%, 0.1%, 1% and 10%. From the results obtained concentrations to be used in the experiment were fixed as 0.01%, 0.05%, 0.1%, 0.5% and 1%.

The bulbs of *Allium cepa* L. were placed in the petridishes lined with cotton that was moistened with distilled water (control) or the same volume of test solution. All the petridishes were kept at room temperature for 48 hours. After 48 hours, the germinated bulbs were washed in distilled water for the removal of the test solutions. The root tips from the germinated bulbs were taken and treated with Carnoy's fluid, the fixative. Microscopic preparations for the cytological studies were made by highest value (0.80) in control which decreased by 50% in 0.01%. Least value for mitotic index (0.08) was obtained in 1% MSG treated bulbs (table 1).

Chromosome squash technique by heating the root tips in 10% HCl and staining with 1% acetocarmine.

Parameters studied were:

a. Germination percentage

Germination percentage (GP) was calculated using the equation:

$$GP = \frac{\text{Bulbs germinated}}{\text{Total bulbs}} \times 100$$

b. Mitotic Index was calculated as:

Mitotic Index = Number of dividing cells/ Total number of cells

c. Frequency of mitotic aberrations

Frequency of mitotic aberrations = (Number of cells showing aberrations/ Total number of dividing cells) x 100

d. Statistical analysis

The values obtained were compared using analysis of variance (ANOVA) to confirm the variability of the data and validity of results. Differences between corresponding controls and exposure treatments were considered statistically significant at $p<0.001$.

3. Results and Discussion

Results of the study reveals that monosodium glutamate has adverse effect on germination of *Allium cepa* and the inhibitory effect increased with increasing concentration of test solution. Maximum germination was observed in the control treated with distilled water (100%) with an average of 4 roots /bulb and minimum germination percentage (40%) was noticed in 1% MSG treated bulbs with an average of 1 root/bulb. Monosodium glutamate adversely affected the cell division in *Allium cepa* root cells. Mitotic index registered its

MSG induced several chromosomal abnormalities in root meristem cells like strap nucleus, tropokinesis, C-mitosis, anaphase bridge and multipolar anaphase

with chromosome fragment (fig.1). C-mitosis was the most prominent aberration recorded at all concentrations of MSG. C-mitosis was described by Levan (1938) as an inactivation of the spindle followed by random scattering of the condensed chromosomes. Another major aberration observed was the chromosome stickiness. Stickiness has been attributed to the improper folding of chromosomal fibres, which makes the chromatids connected by means of the sub-chromatid fibres (Badr *et al*., 1987). The chromosome stickiness reflects highly toxic effects usually of an irreversible type probably leading to death. According to Gomurgen (2005), chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be ascribed to unequal translocation or inversion of chromosome segments. Several chemicals have been reported to induce stickiness, and the results of the current investigation are in agreement with previous findings obtained after treating different plant materials with various food additives (Gomurgen, 2005; Pandey *et al*., 2007 and Sifa Turkoglu, 2008). In untreated root cells the great majority of the spindles were oriented in parallel to the longitudinal axis of the root, but during the treatments there was an increased frequency of mitosis orientated in other directions. This deviation is called tropokinesis. The frequency of various aberrations was found to be increasing with increasing concentrations of monosodium glutamate with maximum frequency (91.6%) observed in the 1% MSG treated root tips.

Table 1. Effect of Monosodium glutamate on cell division of *Allium cepa*

Concentration	Bulbs Germinated (%)	Average Number of Roots/Bulb	Mitotic Index	Frequency of Mitotic Aberration (%)
Control	100±0	12.4±0.58	0.80± 0.1	0±0
0.01%	80±5.8	4.75±1	0.55±0.06	22±2.5
0.05%	60±5.8	3±0	0.4±0.06	44.6±7.2
0.1%	60±0	1.3±0.57	0.32±0.1	75±3.6
0.5%	40±5.8	1.1±0.5	0.22±0.6	83.3±5.8
1%	40±0	1±0	0.18±0.6	91.6±1.1

values are mean ± SD of three replicates ; values statistically significant at p<0.001

4. Conclusion

From the findings of the current investigation it can be concluded that monosodium glutamate, which is used frequently in food industry as flavouring agent, has significant mitodepressive effects. It has the capability of producing variety of mutants, chromosomal aberrations and cytotoxic effects in long run, even below the recommended limits. As the potential of higher plants as a first tier assay system for the detection of possible genetic damage by environmental chemicals has been proved beyond doubt (Grant, 1999), the results of the current investigation should be considered as a warning and also an indication that the tested chemical may be a risk to human health and to our environment.

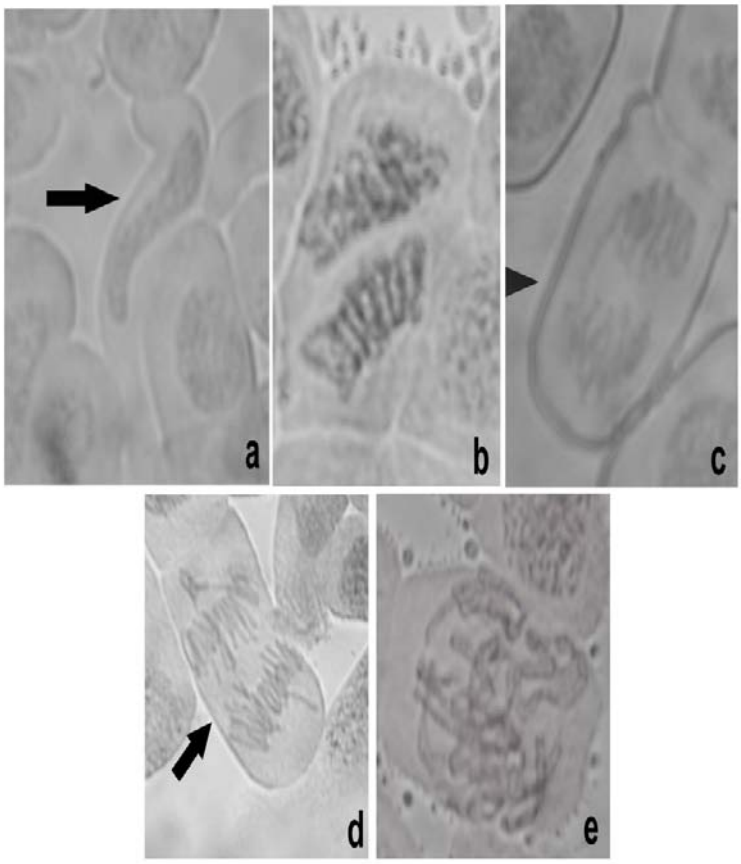

Figure 1. Monosodium Glutamate Induced Mitotic Aberrations in Onion Root Tip Cells; (a) Strap nucleus (b) Tropokinesis (c) Anaphase bridge (d) Multipolar anaphase with chromosome fragment (e) C- mitosis

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Quinoxaline schiff bases: versatile chelators

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1. Introduction

Quinoxalines, also called benzopyrazines, are heterocyclic compounds containing a fused ring made up of a benzene ring and a pyrazine ring. They along with the isomers cinnolenes, phthalazines and quinazolines belong to a class of heterocyclic compounds known as diazanaphthalenes with two heteroatoms in the same or different rings. The fusion of a benzene ring, however, causes decrease in the aromaticity due to the bond alternation (Figure 1). Quinoxalines have 10-π electrons that are located in five bonding molecular orbitals. There are also two non-bonding orbitals that lie in the molecular plane and are confined to the nitrogen atoms. Each of these orbitals contains an electron pair and these electrons are responsible for the basic properties of quinoxalines (Brown., 2004). Quinoxaline can act as Lewis base, form metal complexes, and can participate in hydrogen bonding with hydrogen atoms present in electronegative atoms.

Quinoxaline derivatives have been widely used in dyes, pharmaceuticals, and in electrical/photochemical materials. Quinoxaline ring moiety is a

part of the chemical structures of various antibiotics such as echinomycin, levomycin and actinoleutin, which are known to inhibit growth of gram positive bacteria and are active against various transplantable tumors.

2. Synthesis and structural features

A number of synthetic strategies have been developed for the preparation of substituted quinoxalines. Quinoxalines are readily made from 1,2-dicarbonyl compounds and aromatic 1,2-diamines. A well-known route to quinoxalines is the reaction of o-phenylenediamine with a 1,2 –dicarbonyl compound (Brown., 2004). Heravi *et al*. used Suzuki–Miyaura coupling (Figure 2) reaction for the synthesis of 2,3-disubstituted quinoxalines (Heravi et al., 2006).

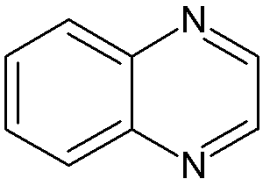

Figure 1: Structure of quinoxaline

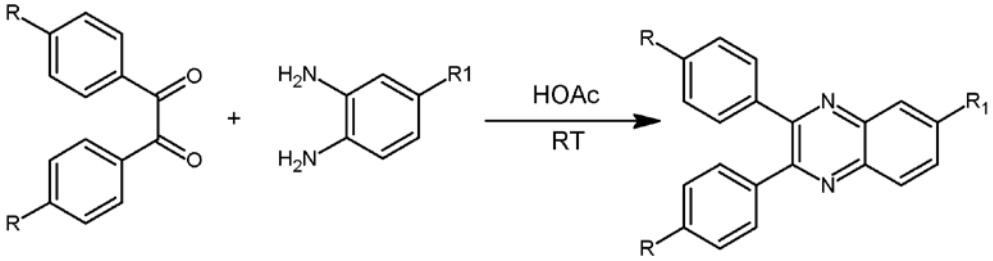

Figure 2: Synthesis of quinoxaline by condensation of 1,2-dicarbonyl compound and ortho phenylenediamines

Quinoxaline compounds show tautomerism if they have a hydroxy, thiolic or amino group at the ortho position. 2-Hydroxy- and 2-mercaptoquinoxalines exist in the quinoxalin-2-one and quinoxaline-2-thione forms, whereas 2-aminoquinoxaline exists as such rather than as an imine (Figure 3) in the solid state (Brown, 2004).

Ovchinnikov and Muèller (1974) reported the possibility of linking of metal ions to heterocycles to form stable complexes, which permits the utilization of heterocyclic systems with pendant arms for modelling cation receptors in proteins. During the past several years, quinoxalines have been used effectively as building blocks for metal-containing two dimensional networks. Metal halides coordinate readily with quinoxalines to form interesting coordination polymers. Substituted quinoxalines, which have the potential to form novel three-dimensional structures upon coordination, have been synthesized. Quinoxaline heterocycles have attracted much attention owing to their natural occurrences and biological activities. Many quinoxaline derivatives display unusual solid-tumor selectivity against multi drug-resistant cancer cells. The synthetic utility and

pharmacological importance of these compounds have prompted many scientists to synthesize and characterize novel quinoxaline derivatives.

3. Transition metal complexes of quinoxaline Schiff bases

Complexes derived from quinoxalines have been reported in the literature Kidani et al, 1974; Inman et al., 1972). The presence of quinoxaline ring helps in the formation of one dimensional structures and may provide potential supramolecular recognition sites for π–π aromatic stacking interactions to form high-dimensional supramolecular networks.

Schiff bases with an electron withdrawing heterocyclic ring system derived from quinoxaline-2-carboxaldehyde would be interesting as their ligand field strengths are expected to be weaker than the Schiff bases containing only aromatic rings, like naphthaldehyde (Mayadevi et al., 2003).

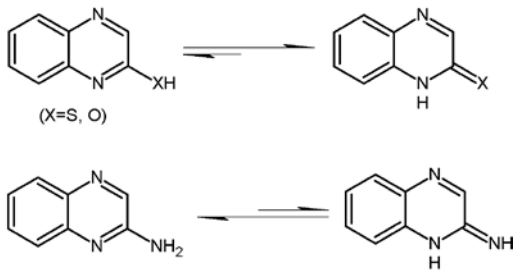

Figure 3: Tautomerism in quinoxaline derivatives

4. Applications of complexes of quinoxaline backbone

Quinoxaline is abundant in chemical and biological systems. Its derivatives appear in proteins and are extensively employed for modelling in biological applications, to electronic devices and materials. Quinoxaline derivatives are also used as analytical reagents in the determination of metal ions.

The quinoxaline-type ligands can act as either neutral or anionic chelators and, in addition, could possibly act as bridging ligands. This leads one to expect that these ligands will exhibit various coordination modes in metal complexes and it is even possible that they can function as controlling ligands in catalytic reaction.

The quinoxaline metal complexes are found to be more active than the free ligand and some side effects may decrease upon complexation. Numerous quinoxaline derivatives are important as biocidal agents, as these compounds have the ability to bind and cleave double stranded DNA under physiological conditions. They are used for genomic research and as diagnostic agents in medicinal applications (Toshima et al., 2002).

Numerous quinoxalinoporphyrins have been electrochemically examined in recent years as models for the development of porphyrin-based molecular wires and devices. Organic light-emitting devices (OLEDs) have received much attention because of their potential applications in flat-panel displays. Doping of a suitable dye into a host layer can improve both the efficiency and the stability significantly. Thus, there is great interest in the synthesis of dyes with

desired properties, such as high emission quantum yield, high thermal and photochemical stability and good colour purity. Fluorescent molecules having quinoxaline building blocks usually display high electron affinities and good thermal stabilities, and also act as electron-transporting materials. Quinoxalines have been successfully incorporated in polymers for use as electron-transport materials in multilayer OLEDs.

5. Conclusions

Quinoxaline Schiff bases are versatile chelating ligands due to the presence of two heterocyclic nitrogen atoms in the ring. Quinoxaline compounds are well known for their good biological activities. The present review mainly deals with quinoxalines, their Schiff bases, transition metal complexes and various applications.

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Nanobiotechnology: Relevance in microbiology

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1. Introduction

Nanotechnology is a multidisciplinary field that involves the design and engineering of materials smaller than 100 nm in diameter level to attain unique properties, which can be suitably manipulated for the desired applications.

Since most of the natural processes also take place in the nanometre scale regime, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine. Nanosized organic and inorganic particles are amenable to biological functionalization and are currently employed as a tool to explore the certain avenues of medicine in several ways including imaging, sensing (Cheng and Van Dyk, 2004), targeted drug/gene delivery systems (Berry et al., 2003, Roy et al., 1999), artificial implants (Sachlos E et al., 2006) etc. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which can combat situations like cancer (Brigger et al., 2002) and fight human pathogens (Stoimenov et al., 2002). This review encompasses a brief account on the diverse areas where the convergence of nanotechnology and microbiology finds its relevance.

2. Applications of nanotechnology in microbiology

The ability of pathogenic bacteria to resist antimicrobial agents has emerged seriously in recent years as a major health problem. Nanoparticles of silver are shown to possess size dependent interaction with bacteria and antibacterial property (Morones et al., 2005). Nanoparticles of silver have been studied as a medium for antibiotic delivery (Li et al., 2005), for use as disinfecting filters and coating materials (Li et al., 2006) and it was also found to be powerful tool to destroy viruses such as HIV (Elechiguerra et al., 2005).

However, the bactericidal property of these nanoparticles depends on their stability and the retention time for bacterium-nanoparticle interaction. The use of antimicrobial enzymes covalently attached to nanoparticles as an antibiotic-free approach to treat microbial infections because of enhanced stability of protein-nanoparticle conjugates and the possibility of targeted delivery also is of great interest.

Gold nanoparticles (GNPs) are particularly of interest for diagnostic and therapeutic applications, since they could be bound to molecules including antibodies, carbohydrates, and

pharmacologic agents, to target specific cells and have been used to detect specific DNA sequences with high sensitivity and selectivity (Daniel, 2004). Gold Nanoparticles are also shown to possess antimicrobial activities (Hyland et al., 2006).

Peptide nanoparticles that can traverse the blood brain barrier offer better treatments for brain infections since most conventional antibiotics cannot cross the impenetrable brain membrane. Further, nanoengineering could prevent bacterial infections using tiny biochemical machines or nanofactories that can confuse bacteria and stop them from spreading, without the use of antibiotics since these nanofactories could trick the bacteria into sensing a quorum too early and to trigger the bacteria to try to form an infection before there are enough bacterial cells to do so. This would prompt a natural immune system response capable of stopping them without the use of drugs.

Another application is the synthesis of nanosensors which find applications in the public health sector in preventing food poisoning and in improving the existing clinical practices by allowing the more rapid quantification and detection of bacteria and viruses (Otles and Yalcin, 2010). A nanoparticle based bioassay is developed which can rapidly detect *E. coli* O157:H7 in food that cause one of the most dangerous and infectious food-borne diseases. In this assay 60 nm-diameter silica nanoparticles are doped with fluorescent dye molecules and antibodies which react with antigens on the bacterial surface so that they will get attached to the particles. The fluorescent signal arising from the dye when the antibodies and antigens react is amplified and the bacterial concentration can be determined readily using fluorescence microscopy or spectrofluorometric analysis. By adding different antibodies to the nanoparticles, different bacteria or bacterial spores also could be detected, allowing the technique be able to check for the presence of multiple contaminants simultaneously. The sensor could be adapted to detect other food borne pathogens as well. Anti- *E. coli* antibody-bound Gold Nanowire Arrays (GNWA) prepared on anodized porous alumina template can be used for the detection of bacteria in a specimen (Seggerson et al., 2004).

Nanotechnology also offers the potential for the treatment of surface water, groundwater and wastewater contaminated by toxic metal ions, organic and inorganic solutes and microorganisms.

3. Applications of microbiology in nanotechnology

For *in vivo* applications such as imaging, sensing, targeted drug delivery, etc nanoparticles are to be internalized and transported through blood stream to the desired area of treatment (Berry et al., 2003) where they could enter into cells via phase endocytosis, receptor-mediated endocytosis or phagocytosis, (Wagner et al., 2004). One of the problems associated with nanoparticles in these applications is that the particles can easily adsorb plasma proteins because of their large surface area that may lead to agglomeration or be cleared by macrophage (Amtenbrink et al., 2009). Functionalized magnetic nanoparticles through surface modification can be utilized to specifically interact with the target molecules on the cell membrane or intracellular ones. Such functionalized surface properties could be achieved in microbially engineered nanoparticles. There are many approaches to the synthesis of nanoparticles such as size reduction

through ball milling, chemical precipitation, and microbial synthesis. A major concern of nanotechnology is the control of the chemical composition, shape and size of nanoparticles in a environmental friendly process which is difficult to attain in a physical or chemical synthesis process (Senapati et al., 2003). Also challenges associated with the synthesis of capped or coated nanoparticles and their stability are tremendous in terms of dispersion and crystallinity of the particles. This has led to the consideration of biological systems, mainly microorganisms for the biosynthesis of nanoparticles. Currently researches now have turned to biological synthesis since biological synthesis gives particles with good control on the size distribution. The nanoparticles could also be stabilized directly in the process by proteins.

The biosynthesis of silver nanoparticles using eukaryotic organisms such as fungi was achieved with the intracellular or extracellular production by *Verticillium* strains and *oxysporum respectively* (Ahmad et al., 2003). Silver particles could be obtained from the bacteria *Bacillus megaterium* and silver nanoparticles generated could be recovered in a process using a biotechnological approach involving *Chromobacterium violaceum*, which is able to metabolize or store metal ions. Nanocrystals of gold, silver and their alloys have been synthesized by the assistance of lactic acid bacterial cells (Nair and Pradeep, 2002). Mukherjee et al., (2001) have successfully synthesized gold nano-clusters using fungus.

Biosynthetic methods can be divided into two categories depending on the place where the nanoparticles or nanostructures are created, intra- or extracellularly (Senapati et al., 2004). For example, the bacteria *Pseudomonas strutzeri* isolated from silver mine materials is able to reduce Ag+ ions and accumulates silver nanoparticles (Klaus-Joerger et al., 2001). The examples also include magnetotactic bacteria which produce magnetite (Fe_3O_4) or greigite (Fe_3S_4) and diatoms which produce siliceous material.

Extracellular synthesis of nanoparticles occurs in alkalothermophilic actinomycete, *Thermomonospora* sp., which reduces gold ions. The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp., *Aspergillus fumigates, Vericillum, Fusarium oxysporum*, [Mandal et al 2006] etc has been reported.

For the purpose of microbial synthesis of metal nanoparticles, fungi possess some advantages over bacteria. The first is that most filamentous fungi have a high tolerance towards metals, and a high wall-binding capacity, as well as intracellular metal uptake capabilities. In addition, they are easy to culture on a large scale, especially by the thin solid fermentation method, thus making it possible to easily obtain enough biomass for processing. The third advantage is that the fungus could grow on the surface of an inorganic vector during culture, which could distribute nanoparticles in a more efficient way as a catalyst.

The iron reducing bacteria produce iron nanoparticles under conditions of relatively low temperature ($<70^oC$), ambient pressure, and pH values near neutral to slightly basic. Precise biological control over activation and regulation of the biosolid-state

processes can produce magnetite particles of well-defined size (typically tens of nanometers) and crystallographic morphology, containing selected dopant metals into the magnetite structure. Examples of bacteria that forms iron nanoparticles are thermophilic *Thermoanaerobacter ethanolicus* and psychrotolerant *Shewanella sp.* (Zhang et al., 1996). The magnetotactic property or the ability to navigate along the Earth's magnetic field by Magnetotactic bacteria is the result of the presence of magnetosomes which are organelles which comprise nanometer-sized intracellular crystals of magnetite (Fe_3O_4) enveloped by a membrane. Magnetite nanoparticles (MNPs) are of biotechnological and biomedical relevance in fields such as magnetic separation of biomolecules, magnetic resonance imaging (MRI), tissue repair, drug delivery, hyperthermia treatment of tumor cells, or research field. Spatial organization in living microbes can be determined using AFM. Over the past decade, there has been tremendous progress in use of AFM to observe membrane proteins and live cells at high resolution. One of the most often cited advantages of the AFM in the study of biological structures is the fact that, unlike electron microscopy, high resolution images can be obtained under physiological conditions and interaction or binding forces between microorganisms and target surfaces can be measured. AFM based nanotechnology could be used to study molecular interaction, molecular and cellular nanostructures and nanoscale *in vivo* immune response of immune molecules (Chen et al., 2004). It is postulated that unique nanoscale proteomic features of immunogen on vaccine particles may determine immunogen-packing density, stability, specificity, and pH-sensitivity on the magnetofection (Gupta and Gupta, 2005). Magnetosomes are enveloped by a biological membrane that contains phospholipids and specific proteins that control the crystal size and morphology and prevents the aggregation of extracted magnetosomes and thus stabilizes magnetosome suspensions and provides a matrix for the functionalization of magnetosomes.

This novel microbial approach to make engineered inorganic nanoparticles is potentially attractive because fermentation and microbial respiration is a well-understood, highly scalable, and environmental friendly process.

4. Nanotechnology to study microbiology:

The nanoscale analysis of microbial cells using atomic force microscopy (AFM) is an exciting, rapidly evolving

vaccine particle surface and thus impact the vaccine-elicited immune responses. AFM-based nanotechnology allowed to elucidate nano-structural and atomic-force binding features underlying highly dense, stable, specific, but immunological-pH-responsive loading of *Y. pestis* V immunogen on the particle vaccine capable of eliciting robust antigen-specific immune response against the bacteria.

5. Applications of nanotechnology and microbiology

Nanoparticles and bacteria simultaneously could be used to deliver DNA-based model drug molecules *in vivo* and *in vitro*. Here the gene (cargo) is loaded onto the nanoparticles, which are carried on the bacterial surface. When incubated with cells, the cargo-carrying bacteria ('microbots') are internalized by the cells, and the genes released from the

nanoparticles are expressed in the cells. Mice injected with microbots successfully expressed the genes as seen by the luminescence in different organs (Akin et al., 2007).

The use of microbes in nanotechnology has brought microbiology to engineering and physics (Mitchell and Kogure, 2006) and bacterial systems are being developed as nano-biosensors (Cheng and Van Dyk, 2004), nano-batteries (Scholz and Schroder, 2003), and nano-computers (Yokobayashi et al., 2002).

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Women and their contribution to science

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1. Introduction

The year 2011 is being celebrated as the international year of chemistry under the auspice of UNESCO and IUPAC. At its General Assembly in Turin, Italy in August 2007, IUPAC unanimously approved a resolution in favor of the proclamation of 2011 as the International Year of Chemistry. Within a year, UNESCO Executive Board recommended the adoption of such a resolution, which subsequently lead to the declaration in December 2008 by the UN General Assembly of 2011 as the International Year of Chemistry.

The objective of this celebration are (1) Increase the public appreciation of chemistry in meeting world needs (2) Increase interest of young people in chemistry (3) Generate enthusiasm for the creative future of chemistry (4) Celebrate the 100th anniversary of the Mme. Curie Nobel Prize and the 100th anniversary of the founding of the International Association of Chemical Societies

In the present context, a look at the role women played in the history of science is appropriate. This also offers us an opportunity to pay our tribute to all the women, known and unknown, who toiled joyfully in the varied branches of science. Women have contributed to science from its earliest days, but as contributors they have generally not been acknowledged. It is for this reason that very few names of women scientist have survived to the present times.

2. Women and science

The earliest woman named in the history of science is an Egyptian, Merit Ptah (2700 BC). Her picture can be seen on a tomb in the necropolis near the step pyramid of Saqqara. Her son, who was a High Priest, described her as "the Chief Physician." By 4th century BC women physician was practicing legally in Athens. Records speak of Agnodike as the first female physician. Aglaonike in 2nd century BC is cited as the first female astronomer in ancient Greece. She was regarded as a sorceress for her ability to make the moon disappear from the sky or in other words predict a lunar eclipse.

Women's contribution to alchemy, the precursor of Chemistry is well known. The most renowned among these is Mary the Jewess. She is believed to have lived around first to fourth century and considered to be the first non fictitious alchemist in the Western world. She is credited with the discovery of hydrochloric acid and with inventing several chemical instruments, including the double boiler which still bears her name *bano-marie.*

Women also made significant contribution to the field of medicine.

The physician, Trotula di Ruggiero, held a chair at the Medical School of Salerno in the 11th century. She is widely believed to be the world's first gynecologist. She taught many noble Italian women, a group sometimes referred to as the "ladies of Salerno". Several influential texts on women's medicine, dealing with obstetrics and gynecology are also often attributed to Trotula. The tradition of allowing women to contribute to science was continued in Italy though in other parts of Europe it declined. Dorotea Bucca was another distinguished Italian physician who held a chair of philosophy and medicine at the University of Bologna for over forty years from 1390.

Astronomy was another area to which women made significant contribution. In the middle ages women were not allowed to attend universities, however much work in astronomy was conducted outside universities. This afforded women an opportunity to both learn and practice astronomy The tradition of female participation in craft production also enabled some women to become involved in observational science, especially astronomy. Between 1650 and 1710, women made up 14% of all German astronomers. However, they weregenerally not acknowledged as independent astronomers but were treated as assistants to men in the field. An illustrative example is Maria Margarethe Kirch**,** an astronomer in her own right, but who in her own life time, was treated only as the assistant of her husband, the renowned astronomer Gottfried Kirch. She was the first women to discover a comet though it was credited to her husband.

Women, especially of the aristocracy, where not entirely excluded from being officially acknowledged by the scientific world. Eva Ekeblad (17^{th} century), a Swedish agronomist, became the first woman inducted in to the Royal Swedish Academy of Science. Her most significant discovery was to make flour and alcohol out of potatoes. She thereby made potatoes, a plant introduced in Sweden in 1658 but until then only cultivated in the greenhouses of the aristocracy, a part of the basic food supply. This greatly improved eating habits and reduced the hunger epidemics.

In the modern times, with the opening of educational opportunity to women, the number of women who have contributed to science have increased manifold. Madame Curie is the most venerated women scientist of our times. She was the first woman to win a Nobel prize in 1903 (physics), went on to become a double Nobel prize winner in 1911 (chemistry). She did pioneering work on radioactivity and discovered the elements radium and polonium.

Not all women were lucky enough to be recognized during their life time. Many were denied the recognition due to them in their life time, often because society could not yet accept the fact that women could also make original contribution in this field. Lise Meitner an Swedish, physicist, along with Otto Hahn gave the theoretical explanation of the fission process. But Otto Hahn alone was conferred with the Noble Prize in Chemistry in 1945 for this work.

As on date 14 women have been awarded Noble prize in Physics, Chemistry and Medicine. Though the total number of Noble Laureate are 545 (189-physics, 160 –Chemistry, 196- medicine). The highest award for science in India –'*Shanti Swarup Bhatnagar Prize for Science and Technology*' is awarded annually by

the Council of Scientific and Industrial Research (CSIR) for notable and outstanding research, in biology, chemistry, environmental science, engineering, mathematics, medicine and Physics. The purpose of the prize is to recognize outstanding Indian work in science and technology. The award is named after the founder Director of the Council of Scientific & Industrial Research, Shanti Swarup Bhatnagar. 454 scientists have been bestowed with the prestigious SSB Prize of which only 14 are women. This is a reflection of the small number of women who are able to work in the field of science.

The first women to win SSB prize was Asima Chatterjee (1917-2006) who was also the first women to win a D.Sc. degree from any Indian university. She was noted for the development of anti-epileptic and anti-malarial drugs. Women have struggled against the prevailing social norms and prejudice of society since the beginning of 19^{th} century. An appreciation of the struggles women had to overcome to pursue a career in science can be gained by examining the life and career of Kamala Sohonie (1911-1996). The daughter of distinguished chemists, Narayanrao Bhagwat, she passed her B.Sc (physics and chemistry) from Bombay University and having stood first (in first class) in that exam, applied to Institute of Science, Bangalore. However her application was rejected as she was a woman. Kamala, a staunch Gandhian, refused to accept this and decided to do Satyagraha in Raman's office, till she was admitted. Prof. Raman granted her admission on condition that for one full year she would be on probation. At the end of a year impressed by her sincerity and caliber, she was allowed to pursue her research. From then on the portals of this illustrious institution were open to women. She went to Cambridge University for her doctoral work and was the first Indian women on whom a Ph. D. degree was conferred. Her discovery that every cell of plant tissue also contains the enzyme "cytochrome C" and that it is involved in oxidation of all plant cells was momentous. She returned to India and continued her research work in various institution and rose to be the Director of Institute of Science, Bombay.

3. Conclusion

In modern times Indian women have made significant contribution to science. For example, Kamal Ranadive (1917-2001) established the first tissue culture laboratory in India at the Indian Cancer Research Centre. She also made significant contribution to the development of leprosy vaccine. At present a large number of women are actively contributing to the science namely, Manju Bansal, Director of the Institute of Bioinformatics and Applied Biotechnology, Bangalore, Archana Bhattacharyya, Director of the Indian Institute of Geomagnetism, Navi Mumbai, Saraswathi Visweshwara, IISc Banglore, Radha Balakrishnan, Institute of Mathematical Sciences, Chennai etc.

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Plant diversity of India with special reference to Kerala

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Study of biodiversity is multidisciplinary and has become an inevitable one. The survival of mankind depends upon the sustenance use of biodiversity. Biological diversity or biodiversity, a term first emerged twenty years ago and is described as the degree of variation of life forms within a given ecosystem, biome, or an entire planet (McNeely, 1990) . It is the measure of the health of ecosystems. World recognized the importance of biodiversity and in the light of the above fact the first International Summit on biodiversity was held at Brazil. More than 150 countries participated in the Summit. The first global agreement to cover all aspects of biological diversity was signed at the Earth Summit in Rio de Janeiro, Brazil, in 1992 and entered into force on 29 December 1993.

Many biologists have tried to define biodiversity. Some defined it as "the totality of genes species and ecosystem of a region". According to some "it is the addition sum of genetic, taxonomic and ecosystem diversity". The most widely accepted definition is "it is the variability among the living organisms from all sources including 'inter alia' terrestrial, marine and aquatic ecosystems and ecological complexes of which they are part" (CBD, 1992).

Importance of biodiversity lies in the fact of understanding the variability of species in a particular ecosystem, role of the particular species in the maintenance of the ecological balance. Food, clothing, housing, energy, medicines are all resources directly or indirectly obtained from the bio - diversity. It forms the rich store house of the raw materials for industrial development, helps in the introduction

of new crop species and in the exploration of bioactive compounds. There is an increasing awareness that biodiversity is intimately interconnected with long term health and vigour of the biosphere, as an indicator of global environment and also as a regulator of ecosystem functioning (Solbrig, 1991). It is very clear that the sustainable use of biological resources provide the very basis for ultimate survival of mankind.

Scientists have estimated that the number of species of plants and animals on Earth could vary from 1.5 to 20 billion. Out of these only 1.8 million species is known and documented (McNeely *et al*., 1990). The Earth's biodiversity is distributed in specific ecological regions. About 1000 eco-regions have been identified in the world. Of these 200 are said to be the richest, rarest and most distinctive natural areas. These areas are referred to as Global 200. Among these ecological regions some regions are identified as "Hotspots of Biodiversity". There are about 34 identified hotspots in the world (Conservation International). The term 'hotspot' was first given by the British Biologist Norman Myres, 1988. It is the biogeographic region characterized by exceptional levels of plant endemism and serious levels of habitat loss. It should contain 1,500 species of vascular plants, >0.5 % of world's total endemics and have lost 70% of its original habitat. It is estimated that by preserving the hotspots of the world, 50% of the global endemic species would be protected (Mittermeier *et al*., 1998). It is estimated that 50,000 endemic plants comprise 20% of the global plant life.

Countries with relatively large number of biodiversity hotspots are referred to as mega diversity nations. India is a megadiverse nation with three globally accepted hotspots namely the north eastern states (1500 endemic species) , the western ghats (1500 endemic species) and Andaman Nicobar Islands (2200 sp of flowering plants0 (Mittermeier *et al*., 1998).

India ranks fourth in asia and tenth in the world in plant diversity. India has 45000 plant species, 17000 flowering plants which accounts for the 6% of total flowering plants. It is estimated that 18% of the Indian plants are endemic to the country and found nowhere else in the world. Out of the 17000 flowering plants, $1/4^{th}$ occurs in the Western Ghats. The Western Ghats which is running parallel to the west coast of Peninsular India is series of Hill Ranges covering a distance of 1600 km from Thapti Valley in Gujarat to Kanya kumari in Tamil Nadu, through the states of Maharastra, Goa, Karnataka and Kerala. Depending upon the rainfall, soil type altitude different vegetation types are formed in Western Ghats (Sasidharan, 2000).

In the Western Ghats it is the Southern parts of Karnataka, Kerala, and South Tamil Nadu which is richest in terms of Biodiversity. Out of the approximate estimate of 4250 sp of flowering plants 3900sp occur in the southern Western Ghats and 3800 in Kerala (Nayar 1997). The 10 dominant families in Kerala are legminosae, Rubiaceae, Orchidaceae, Acanthaceae, Euphorbiaceae, Asteraceae, Cyperacaceae, Lamiaceae, Asclepiadaceae and Poaceae.

There exists a great diversity among the plants of Western Ghats. Habit wise it ranges from tiny *Wolffia arrhiza* (1-2mm) to the Lofty *Antiaris toxicaria* up to 60m ht. There are 740 sp of trees (Sasidharan, 1997), 40 sp of Woody Climbers, 175 sp of orchids, 8 palms 16 sp of Calamus, 12 sp of Balsaminae, 20 species of

Insectivorous plants 3 species of gymnosperms. Nayar, 1997 estimated 3800 species of flowering plants 1272 endemics to the Southern Western Ghats . He has identified three hotspot of endemic centres in Kerala namely Agasthyamala, Anamala High Ranges and Silent Valley Wayanand.

Kerala has unique distinction of contributing to the world- both the King and Queen of spices black pepper and cardamom .Tropical evergreen forests is the centre of origin of ginger and turmeric .Many tree species nutmeg, cinnamon and clove widely grown in Kerala. (Ravindran, 2000). Among 4600 flowering plants in Kerala about 900 possess medicinal values, 540 occur in forest, 150 sp are used in indigenous medicinal preparations. The rural tribal communities use about 2000 sp of lesser known wild plants for various medicinal uses.

Apart from the forest ecosystem the Kerala State is having rich biodiversity with different types of unique ecosystem namely wetland ecosystem, mangrove ecosystem, and marine

Table 1 Comparison of Plant Diversity in Kerala, India and World

Living Organisms	No of Sp. in Kerala	No of Sp. in India	No of Sp. in world
Algae	**231**	**6500**	**4000**
Fungi	**1044**	**16500**	**89000**
Bryophytes	**200**	**2850**	**16000**
Pteridophytes	**200**	**1100**	**13000**
Gymnosperms	**4**	**64**	**750**
Angiosperms	**4000**	**17000**	**2,50,000**

ecosystem etc. The wetlands of kerala are treated as sites of exceptional biodiversity in the country and are characterized by several endemic species. A total of 100 species of phytoplanktons were recorded from the backwaters of Kerala. A compilation all available data revealed the presence of about 90 species of cyanobacteria, 275 species of marine/fresh water algae, 35 species of aquatic fungi. Mangroves are the most important group of plants present in the coastal wetlands of Kerala. The extent and health of mangroves have a marked influence on the migratory species and the abundance of the offshore fisheries in Kerala. There are clear evidences to show that very rich mangrove vegetation existed along the coastal tracts of Kerala and once supported about 700 sq km of mangroves along its Coast and what exist now are only relics of the past. A recent investigation by Anupama & Sivadasan (2004) could identify 15

true Mangroves (belonging to 9 genera and 7 families) and 49 Mangrove associates from Kerala.

In the last few decades we have seen a steady increase in the extinction rate of flora and fauna all over the world. Much conservation methods have been adopted world wide. Conservation can broadly be divided into two types, In-situ**:** Conservation of habitats, species and ecosystems where they naturally occur. This is in-situ conservation and the natural processes and interaction are conserved as well as the elements of biodiversity. Ex-situ: The conservation of elements of biodiversity out of the context of their natural habitats is referred to as ex-situ conservation. Zoos, botanical gardens and seed banks are all example of ex-situ conservation. In-situ conservation is not always possible as habitats may have been degraded and there may be competition for land which means species need to be removed from the area to save them.

The state of Kerala has declared 20 percent of the forests a relatively higher percentage as protected areas (Sasidharan, 2000). This includes Six National Parks and 16 Wild Life Sanctuaries (Table 2). Biosphere reserve such as Nilgiri and Agasthyamalai represent mainly forest areas. Use of forest lands for non-foresting purposes and expansion of area under plantation crops have been regulated by law. Programmes for control of exotic species and regeneration of natural forests and degraded forests would also help in conserving forests biodiversity. The forest biodiversity is less affected in kerala as a result of such activities. Programmes aimed at conserving sacred groves and mangroves would also help in conserving local biodiversity to a certain extent. Wetlands such as Astamudi Lake, Sasthamkotta Lake and Vembanad Lake have been declared as Ramsar Sites or wetlands of International Importance, with the idea to conserve and sustainable manage them.

Further, there are bird sanctuaries at Kumarakam, Thattekkad, Mangalavanam and Choolannur. Coastal Zone Regulation Act and rules to prevent conversion of rice fields provide legal support to protect mangroves and wetlands, including rice fields, contributing towards biodiversity conservation in the State.

6. Relevance of the study

Eventhough stringent laws are there to protect the forest, hotspots etc vast scale man made destruction has already occurred and there is a need for conscious effort for the conservation of these protected areas and the diversity around us. It is the responsibility to maintain and conserve for future generations. More studies have to be conducted to explore the plant diversity of Kerala. Taxonomic identification of many plant species has yet to be done.

7. Conclusion

The plants are the storehouses of alkaloids secondary metabolites, tannins, flavonoids essential oils. Bioprospecting of plants are necessary to explore bioactive compounds in them which will have promising contribution to the pharmaceutical industry. Germplasm conservation, molecular characterization of the crops, spices, vegetables, medicinal resources is our future need.

Table 2. Details of National Parks and Wildlife Sanctuaries in Kerala as on 31. 03. 2004

Name of National Parks/ WLSs	Area (km^2)	Year of formation
Neyyar Wildlife Sanctuary	128.00	1958
Peppara Wildlife Sanctuary	53.00	1983
Shenthuruni Wildlife Sanctuary	171.00	1984
Periyar Wildlife Sanctuary	777.00	dated 27th October 1982)
Chinnar Wildlife Sanctuary	90.44	1984
Idukki Wildlife Sanctuary	70.00	1976
Eravikulam National Park	97.00	1978
Thattekkad Bird Sanctuary	25.00	1983
Chimmony Wildlife Sanctuary	85.00	1984
Peechi - Vazhani Wildlife Sanctuary	125.00	1958
Parambikulam Wildlife Sanctuary	285.00	1973
Silent Valley National Park	89.52	1984
Wayanad Wildlife Sanctuary	344.44	1973
Aralam Wildlifey Sanctuary	55.00	1984
Pampadam Shola National Park	1.32	2003
Mathikettan National Park	12.82	200
Anamudi Shola National Park	7.50	2003
Mangalavanam Bird Sanctuary	0.0274	2004
Kurinjimala Sanctuary	32.00	2006
Choolannur Pea Fowl Sanctuary	3.42	2007
	Total =2452.48	

Note: 148 sq.km Reserve Forest area has been added to Silent Valley national Park as its buffer zone as per G.O (MS) No.36/07/F&WLD dated 11.06.2007

(Source: Forest Department, Govt.of Kerala)

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Osteoporosis

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1. Introduction

Osteoporosis is the thinning and weakening of the bones that leads to their breaking even with minimum force. It is not a disease and you do not get it suddenly. People with osteoporosis may have a fracture (of the arms, legs, wrist, hip and spine) and it heals normally. But then they have more and more fractures and the incidence increases as they get older. Of course, normally bones get weaker as one grows older and the risk of fracture increases. But if one starts off with bones not very strong - that is, osteoporosis - then the risk of fracture gets higher.

For women, there is a particularly important factor - menopause. During this phase, they lose bones rapidly for 5-10 years. The incidence, in general, is higher among women and older people. The reason it is lower among men is that their bones are bigger and hence less likely to break. And also, men tend to live not as long as women. But now as men also tend to live longer, the incidence among them is also increasing.

And, most important, after almost all these fractures, the person's risk of death increases significantly. We do not understand the reason yet, but for almost all osteoporosis fractures the person's risk of death doubles compared to that of a non-osteoporosis person of the same age and similar circumstances.

2. Symptoms of Osteoporosis

Osteoporosis can be present without any symptoms for decades because osteoporosis doesn't cause symptoms until bone fractures. Patients may not

be aware of their osteoporosis until they suffer a painful fracture. The symptom associated with osteoporotic fractures usually is pain. The location of the pain depends on the location of the fracture.

Fractures of the spine (vertebra) can cause severe "band-like" pain that radiates from the back to the sides of the body. Over the years, repeated spinal fractures can lead to chronic lower back pain as well as loss of height or curving of the spine due to collapse of the vertebrae. The collapse gives individuals a hunched-back appearance of the upper back, often called a "dowager hump" because it commonly is seen in elderly women. Hip fractures typically occur as a result of a fall.

3. Preventive measures

Early detection and timely treatment of osteoporosis can substantially decrease the risk of future fractures. None of the available treatments for osteoporosis are complete cures. In other words, it is difficult to completely rebuild bone that has been weakened by osteoporosis. Therefore, prevention of osteoporosis is as important as treatment. Some of the most important treatments for preventing osteoporosis are:

i) Diet, ii) Calcium intake, iii) Vitamin D intake, iv) Protein supplements**,** v) Alcohol, caffeine, and salt intake, vi) Exercise, vii) Stop smoking**,** viii) Preventing falls, ix) Medication monitoring**,** etc.

3.1. Exercise

It is important to have good physical activity, good intake of calcium, enough exposure to sunlight, no smoking and so on. Exercise has a wide variety of beneficial health effects. However, exercise does not bring about substantial increases in bone density. The benefit of exercise for osteoporosis has mostly to do with decreasing the risk of falls, probably because balance is improved and muscle strength is increased. Research has not yet determined what type of exercise is best for osteoporosis or for how long it should be continued. Most doctors recommend weight-bearing exercise, such as walking, preferably daily. An expert will recommend exercising for atleast 30mins three times per week. Avoid activities with high risk of falling as skiing or skating.

3.2. Caution

It is important to avoid exercises that can injure already weakened bones. In patients over 40 and those with heart disease, obesity, diabetes and high blood pressure, exercise should be prescribed and monitored by physicians. Extreme levels of exercise (such as marathon running) may not be healthy for the bones.

Do:

- Pay attention to proper posture. This includes keeping our head erect and eyes forward, keeping your shoulders back, lightly “pinching” your shoulder blades, and tightening your abdominal muscles and buttocks.
- Make sure to use a handrail when climbing stairs.
- Bend from the hips and knees and never from the waist, especially when lifting.

Don't:

- Wear shoes with slippery soles.
- Slouch when standing, walking, or sitting at a desk.

- Move too quickly.

- Engage in sports or activities that require twisting the spine or bending forward from the waist, such as sit-ups, toe touches.

4. How it can be diagnosed

Sometimes it can be seen on an X-ray when there is a lot of bone loss. Then there are techniques such as bone densitometry, with which the density of the bones can be measured. This gives a good index of the risk.

We measure the bone density and find out how far it is away from normal can stop bone loss and even reverse it in part. This can also be maintained if the treatment is continued.

ones. About every 10 per cent away from the normal, the risk of fracture doubles; 20 per cent away, the risk is four times; 30 per cent away, the risk is eight times; and so on. So, the risk increases dramatically as the quality of bones decreases.

5. Treatment

There are lots of treatments now that are very well studied and shown to be effective and largely safe. For example, in the case of women, estrogen replacement therapy

To conclude prevention of osteoporosis is as important as treatment.

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