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CONTENTS

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Rapid *In vitro* Micro propagation of Sugarcane (*Saccharum officinarum* L. cv-Nayana) Through Callus Culture

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ABSTRACT: Standardization of protocol for induction of callus and regeneration of plantlets was established through *in vitro* culture using young meristem of Sugarcane (*Saccharum officinarum* L. cv-Nayana) as an explant. The multiple shoot regeneration at various frequencies was observed by using different concentration and combination of growth regulators. The highest percentage of callus induction was observed in MS medium supplemented with 2.5 mg/l, 2-4 D. The best response in terms of multiple shoot induction was observed on MS medium with BAP 2.0 mg/l + NAA 0.5 mg/l. When *in vitro* shoot lets were inoculated on to the half-strength MS basal media supplemented with 3.0 mg/l NAA, rooting was more profuse. Rooted shoots were transplanted in the green house for hardening and their survival rate was 90% in the field condition. [Nature and Science. 2009;7(4):1-10]. (ISSN: 1545-0740).

Key words: Callus culture, Growth regulators, Micro-propagation, Shoot regeneration, *Saccharum officinarum* L. (cv-Nayana), Young meristems

INTRODUCTION

Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology. During the last thirty years, micro propagation and other *in vitro* techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop plants (George and Sherrington,1984 ; Dodds,1991 ; George,1993 ; Das et al.,1996). Sugarcane (*Saccharum officinarum* L.) is an important agricultural cash crop in tropical and subtropical region of the world and is the major source of sugar with respect to export product in many developing countries that accounts for more than 60% of the world's sugar production (Guimarces and Sobral,1998). It is the only member of the family *Gramineae* belong to genus *Saccharum* in which *in vitro* propagation are standardized and commercially viable. Varieties of sugarcane are highly heterogeneous and generally multiplied vegetatively by stem cutting. In tropical countries nodal sections of sugarcane with 2 or 3 nodes are commonly used as a planting material. Lack of suitable multiplication procedure and contamination by systemic diseases are the serious problem to multiply an elite genotype of sugarcane in the open field (Lal and Singh,1994). *In vitro* multiplication of sugarcane has received considerable research attention because of its economic importance as a cash crop. Micro propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane (Feldmenn et al.,1994 ; Lal and Krishna ,1994 ; Lee ,1987 ; Lorenzo et al., 2001 ; Krisnamurthi and Tlaskal ,1974). As a result of which plant regeneration through tissue culture technique would be a viable alternative for improving the quality and productivity in sugarcane. There are reports on tissue culture of sugarcane from different countries but the first attempts to regenerate plants through *in vitro* technique were made on sugarcane by Naz (2003) and Heniz and Mee (1969). Standardization of protocols for *in vitro* multiplication of sugarcane through callus culture, axillary bud and shoot tip culture have been reported by many authors (Barba et al.,1978 ; Nadar et al.,1978 ; Bhansali and Singh, 1984 ; Nagi ,1998 ; Anita et al., 2000). How ever, reports are scare on young meristem callus culture in sugarcane cultivar, Nayana of Orissa. The present communication demonstrates an effective high frequency regeneration method which allows for expedient multiplication of micro plants that are easily established ex vitro through callus culture of young meristem as an explant.

MATERIALS AND METHODS

Explant source: Healthy young meristems were collected by removing the leaf sheath from field grown plants of sugarcane (*Saccharum officinarum* L. cv- Nayana) maintained in the P.G. Department of Botany Utkal University and brought to the laboratory. These young meristems were cut into thin smaller pieces of 1.0 to1.5 cm length .The explants were washed thoroughly under running tap water for 20-30 minutes followed by bavistin 0.2% for 10 minutes and then washed with sterile distilled water and transferred to laminar air flow cabinet. The young meristem explants were treated with 70 % alcohol for 30 second to one minute, followed by another treatment in 0.1% (w/v) mercuric chloride ($HgCl_2$) for another 5 minutes. Finally, the young meristem cuttings were washed thoroughly 3 to 5 times with sterile distilled water before the inoculation in to sterilized nutrient agar media pre-packed in culture tubes. All the above operations were performed under aseptic conditions in laminar airflow cabinet.

Culture medium and condition: The young meristem cutting explants were inoculated on to sterilized semisolid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations and combinations of different plant growth regulators.

Callus induction: For callus induction different range of concentration of auxins are tried individually such as 2,4-D (0.5,1.0,2.0,2.5,3.0 and 4.0 mg/l), IBA (0.5,1.0,2.0,2.5,3.0 and 4.0 mg/l) and NAA (0.5,1.0,2.0,2.5,3.0 and 4.0 mg/l) with MS basal medium.

Shoot regeneration Medium: White friable calli were cultured on MS medium supplemented with different combinations and concentrations of BAP, Kinetin (0.5- 4.0mg/l) and IBA, NAA (0.1-1.0mg/l) for multiple shoot regeneration either individually or in combination.

Rooting medium: Elongated micro shoots measuring about 5-6 cm in length were excised from culture tube and transferred to half-strength (1/2 MS) MS medium supplemented with different concentrations of IBA,NAA and IAA (0.5-3.0mg/l) either individually or in combinations.

Environmental condition: The pH of the medium was adjusted to 5.8 before gelling with Agar (6g/l bacteriological grade, Qualigens , India) and prior to autoclaving for 20 min at120 ^{o} C and at 15 lbs psi pressure. Sugar was added at the concentration of 30 gm/l. Molten medium of 20 ml was dispensed into the culture tube and plugged with nonabsorbent cotton wrapped in one layer of cheesecloth. All the cultures were incubated in a growth room with a 16h photoperiod except callus culture (cool, white fluorescent light -30µmol $m^{-2}S^{-1}$) and the temperature was maintained at 25 ± 3^{0}C with 70-80% relative humidity in the culture room. Each treatment consisted of 10 replicates and repeated thrice. For better callus induction just after autoclaving the culture tube containing semisolid sterilized media should positioned , such a manner as if the inside semisolid media in the culture tube should spread to maximum to wards one side only. At the time of inoculation 2 to 3 explant per culture tube was used. For callus induction experiment culture tubes were kept under 20, 25 and 30^{o}C in complete dark or light/dark condition with 16/8 h light/dark photoperiod with 140µmol $m^{-2}S^{-1}$ light from cool, white fluorescent lamps.

Acclimatization and transfer of plantlets to soil: Plantlets with well-developed roots were removed from the culture medium. Washed the roots gently under running tap water and were transferred to plastic trays for hardening which contain autoclaved garden soil, farmyard manure and sand (2:1:1). The harden plantlets in the plastic trays were covered with porous polyethylene sheets for maintaining high humidity and were kept under shade in a net house for further growth and development. All were irrigated with 1/8 MS basal salt solution devoid of sucrose and inositol every 4 days for two weeks. After 30 days the plantlets were transplanted in to the soil in field condition.

Statistical Analysis: Experiments were set up in a Randomized Block Design (RBD) and each experiment usually had 10 replicates and was repeated three times. 20 to 30 explants were used per treatment in each replication. Observations were recorded on the percentage of response of callus formation, percentage of response of shoots, number of shoots per callus, shoot length, percentage of response of roots, roots per shoot and root length respectively. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level according to Gomez and Gomez (1976).

RESULTS AND DISCUSSION

Callus Induction: Callus induction was observed within two weeks, after inoculation of the explants on MS medium containing different concentrations of IBA, NAA and 2,4-D (0.5,1.0,2.0,2.5,3.0 and 4.0 mg/l). Although in all concentrations of 2, 4-D the callus induction was triggered, but more profuse callus induction was observed at 2.5 mg/l of 2,4-D with full potential of callus regeneration from the explant of the cultivated varieties, Nayana. On this media composition the explants produced creamy white callus. The percentage of callus induction was 100 % (Table-1). Such type of calli has also been reported by Khan et al., (1998) and Khatri et al., (2002). Begum et al., (1995) found that 3.5 mg/l of 2, 4-D produced highest percentage of callus induction from leaf base explant in Bangladesh Nagabari variety of sugarcane. Islam et al., (1982) also reported that 0.5-5.0 mg/l of 2,4-D showed callus induction from leaf tissue on MS medium. The concentrations NAA at 2.0 and 3.0 mg/l produced small amount (20-30%) of callus with grayish globular and hardy in nature. On second sub-culture the calli were turned into non-regenerable callus in NAA combination media (Fig. B, Table-1) with reduced callus weight where as in IBA+ MS basal media composition explant does not showed any remarkable callusing. The cultivated variety Nayana produced poor callus in lower concentration of 2, 4-D and did not at all in 0.5-1.0 mg/l of hormonal combination with MS basal media. All these studies indicated that the sugarcane variety Nayana require higher concentration of 2, 4-D for callus induction (Fig. A , Table-1).

Regeneration of Micro shoots: Various concentration of cytokinin (BAP and Kinetin) and auxins, (IBA, NAA) were used in different concentration and combinations for shoot regeneration. During this investigation shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment. Among different concentrations and combinations for shoot multiplication , best performance was showed on MS medium supplemented with BAP (2.0 mg/l) + IBA (0.5mg/l) (Table-2).On this combination 92 % of explant produced shoots .The number of usable shoots was 12.4 ± 1.90 with average length of the shoots 6.2 ± 0.37 cm of the cultivated variety Nayana. The second best performance was found on MS medium supplemented with BAP (2.0mg/l) + IBA (1.0mg/l) in which average number of usable shoots was 10.5 ± 1.31 with mean length of the shoot 4.0 ± 0.61cm (Fig.C&D,Table-2).Islam et al., (1982) also reported the positive effects of BAP+IBA combination on shoot formation in sugarcane. It was also observed that BAP+NAA combination showed effective result but not superior than BAP+IBA combination. However, combinations of high level of cytokinin and a low level of auxin were essential for differentiation of adventitious shoot in sugar cane young meristem callus of the variety Nayana than individual concentration of cytokinin. All these studies concluded that regeneration potential of callus was specific and genotype dependent phenomenon and at the same time it parallel with the hormonal concentration and combinations (Maretzki and Nickell,1973 ; Maretzki, 1987). It was also observed that callus derived from different auxins showed different regeneration potential. Callus induction, proliferation and regeneration potential in sugarcane exhibited synchrony to each other (Geetha and Padmanadhan, 2001).

***In vitro* rooting and acclimatization**: Different types of auxins were used at different concentrations and combinations to regenerate adventitious roots. Among them NAA and IBA was found to be comparatively better response than IAA for profuse rooting. NAA+IBA combination showed positive result. Best rooting was observed in 1/2 strength MS medium supplemented with 2.5mg/l NAA(Table-3) and the highest number roots per micro shoots were 13.4 ±1.5 , which take only 8-10 days for initiation of root primordial with average root length 4.0 ± 0.94 cm for the variety Nayana found in Orissa (Fig.E,Table-3).According to Lal and Singh (1994) root can be easily induced on culture shoots by their transfer to another medium with or without NAA ,where optimal growth were observed with 1/2 strength of MS medium . Baksha et al., (2002) used 5.0 mg/l NAA for best rooting response in half strength MS medium. Sabaz et al., (2008) used 1.0 mg/l IBA as the best root initiating growth hormone with highest number of 41 roots per plant. Gosal et al.,(1998) obtained rooting on liquid MS medium containing NAA (5 mg/l) and 70 g/l sucrose. Ali and Afghan (2001) observed only 6 - 7 roots after 3 weeks on MS medium containing 2.0 mg/l IBA and 6% sucrose. Baksha et al., (2002) also got rooting response at 0.1 - 0.5 mg/l IBA along with 0.5 - 2.0 mg/l BAP but these were of poor quality. These findings also agree well with the previous findings of Nadar and Heinz (1977). Alam et al., (2003) reported best rooting response at 2.5 mg/l IBA with 16 number of roots/explant having 1.1 cm root length. Mamun et al., (2004) obtained best results of rooting on MS

medium supplemented with auxins (NAA + IBA) 0.5 mg/l for each one. We also found that 0.5 mg/l NAA+2.5 mg/l IBA was the second best feasible rooting response with 11.3±1.08 number of roots and 3.7 ± 0.47 cm of root length. The plantlets with well developed shoot and roots after acclimatization were successfully transplanted in soil with 85% acclimatization of survivability potential (Table-3, Fig. F). It is difficult to release a new variety of sugarcane by the conventional breeding method for the genetic behavior of sugar cane .More over also it takes long times to release a stable variety. Thus tissue culture technique can plays an important role in this regard for supply of disease free quality planting material in a year round basis and true to true types of the mother plant.

Table.1.The effect of different concentrations of auxin and 2, 4-D on callus induction on young meristem explants of sugarcane varieties Nayana of Orissa.

Treatments	Hormone	Hormonal supplements, mg/l	No. of explant showed callusing	% of explant with callus induction
	IBA			
T1		0.5	0	0
T2		1.0	0	0
T3		2.0	0	0
T4		2.5	0	0
T5		3.0	0	0
T6		4.0	0	0
	NAA			
T7		0.5	0	-
T8		1.0	2	-
T9		2.0	10	20
T10		2.5	30	30
T11		3.0	20	25
T12		4.0	0	-
	2,4-D			
T13		0.5	20	25
T14		1.0	30	30
T15		2.0	50	40
T16		2.5	90	100
T17		3.0	70	60
T18		4.0	30	20

[No callusing : poor callusing =20-50%,considerable callusing =51-85%, Intensive callusing = 86-100%.]

Table.2.Effect of the cytokinin (BAP, Kn) and the auxin(IBA , NAA) at different concentration and combination in MS medium on shoot regeneration from the callus tissue.

Treatments	Hormonal supplements, mg/l	% of explant produced shoots	No.of shoot/ explant	Average length of the shoots
	BAP			
T1	0.5	15	2.8 ± 0.90	3.5 ± 0.84

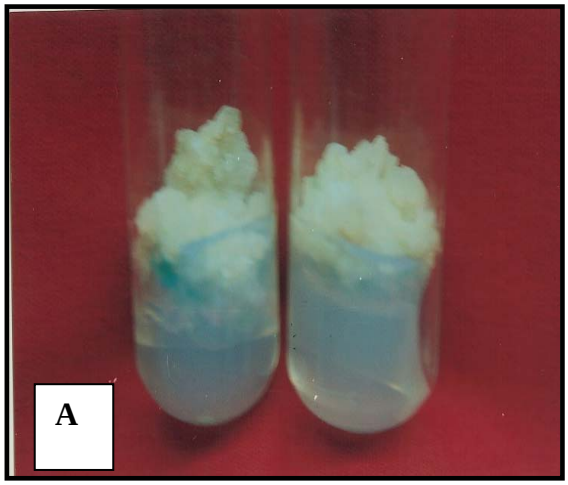
T2	1.0	30	3.1 ± 0.51	3.6 ± 0.77
T3	1.5	40	3.5 ± 0.47	3.7 ± 1.16
T4	2.0	45	4.2 ± 0.65	4.0 ± 0.47
T5	2.5	50	4.5 ± 0.94	5.2 ± 0.24
T6	3.0	30	4.1± 0.51	3.4 ± 0.75
T7	4.0	20	3.2 ± 0.47	2.5 ± 0.47
	Kn			
T8	0.5	12	2.0 ± 0.47	3.1 ± 0.89
T9	1.0	42	3.0 ± 0.81	2.9 ± 0.04
T10	1.5	45	3.2 ± 0.82	3.0 ± 0.47
T11	2.0	48	2 .1 ± 0.44	3.5 ± 0.40
T12	2.5	60	4.5 ± 0.70	4.4 ± 0.28
T13	3.0	40	3.8 ± 0.56	4.0 ± 0.47
T14	4.0	17	1.2 ± 0.32	2.0 ± 0.47
	BAP+IBA			
T15	0.5+0.1	40	3.0 ± 0.81	2.2 ± 0.47
T16	0.5+0.2	65	4.0 ± 0.94	3.1 ± 0.47
T17	0.5+0.5	25	3.2 ± 0.47	3.0 ± 0.94
T18	0.5+1.0	15	3.5 ± 0.62	2.8 ± 0.89
T19	1.0+0.1	50	4.2 ± 0.47	3.2 ± 0.47
T20	1.0+0.2	69	5.2 ± 0.37	3.5 ± 0.16
T21	1.0+0.5	40	3.2 ± 0.43	2.1 ± 0.04
T22	1.0+1.0	30	3.0 ± 0.47	2.0 ± 0.23
T23	2.0+0.1	45	3.3 ± 0.61	3.4 ± 0.29
T24	2.0+0.2	61	3.4 ± 0.65	2.9 ± 0.28
T25	2.0+0.5	92	12.4 ± 1.90	6.2 ± 0.37
T26	2.0+1.0	75	10.5 ± 1.31	4.0 ± 0.61
	BAP+NAA			
T27	0.5+0.1	45	2.0 ± 0.47	2.0 ± 0.47
T28	0.5+0.2	50	3.2 ± 0.29	4.5 ± 0.94
T29	0.5+0.5	57	3.8 ± 0.82	4.2 ± 0.8
T30	0.5+1.0	60	4.1 ± 0.73	3.0 ± 0.84
T31	1.0+0.1	67	5.3 ± 0.53	4.3 ± 0.32
T32	1.0+0.2	36	5.0 ± 0.94	4.0 ± 0.94
T33	1.0+0.5	25	2.2 ± 0.74	2.0 ± 0.23
T34	1.0+1.0	40	3.2 ± 0.74	2.5 ± 4.0
T35	2.0+0.1	35	3.4 ± 0.61	2.3 ± 0.16
T36	2.0+0.2	33	3.2 ± 0.47	3.0 ± 0.23
T37	2.0+0.5	90	8.2 ± 0.95	7.5 ± 1.02
T38	2.0+1.0	75	4.6 ± 0.24	5.5 ± 0.47

BAP = 6-Benzyal amino purine : Kn= Kinetin : IBA= Indole3-butyric acid : NAA = α-naphthalene acetic acid : IAA= Indole 3-acetic acid.
[10 replicates/treatment; repeated thrice. Means are calculated by Duncan's multiple range tests at the significance level of 5%]

Table.3. Effect of different auxins on root formation of the *invitro* grown micro-shoots cultured on ½ strength MS medium.

Treatments	Auxin supplements, (mg/l)	% of micro shoots rooted	No of roots/micro shoots	Average length of roots(cm)	Days to emergence of roots
	IBA				
T1	0.5	20	3.2 ± 0.47	1.9 ± 0.45	15-20
T2	1.0	25	3.5 ± 0.61	2.1 ± 0.41	15-20
T3	1.5	60	5.3 ± 0.32	2.3± 0.32	11-14
T4	2.0	72	8.2 ± 0.84	2.5 ± 0.23	10-12
T5	2.5	82	10.5 ± 0.70	3.4 ± 0.65	10-12
T6	3.0	46	4.6 ± 0.65	1.8 ± 0.09	10-15
	NAA				
T7	0.5	20	3.2 ± 0.65	0.9 ± 0.45	12-15
T8	1.0	40	3.8 ± 0.47	1.0 ± 0.29	12-15
T9	1.5	65	5.2 ± 0.74	1.5 ± 0.23	10-12
T10	2.0	79	8.3 ± 0.28	3.4 ±0.47	10-12
T11	2.5	85	11.2 ± 1.5	4.0 ± 0.94	8-10
T12	3.0	55	5.1 ± 0.47	2.0 ± 0.47	10-15
	IAA				
T13	0.5	0	0	0	0
T14	1.0	15	2.2 ± 0.33	0.75 ± 0.04	10-18
T15	1.5	20	3.2 ± 0.65	0.8 ± 0.12	10-17
T16	2.0	25	1.5 ± 0.23	1.0 ± 0.43	10-15
T17	2.5	30	2.2 ± 0.16	2.5 ± 0.47	10-15
T18	3.0	50	5.6 ± 0.57	1.5 ± 0.23	12-15
	NAA+IBA				
T19	0.5+0.5	0	0	0	0
T20	0.5+1.0	40	5.2 ± 0.61	2.3 ± 0.37	10-17
T21	1.0+1.0	52	5.8 ± 0.61	3.2 ± 0.89	10-15
T22	1.5+0.5	60	6.4 ± 0.71	1.4 ± 0.28	15-17
T23	0.5+1.5	48	5.3 ± 0.74	1.2 ± 0.33	10-17
T24	2.0+0.5	50	6.4 ± 0.92	1.9 ± 0.14	10-12
T25	0.5+2.0	75	10.4 ± 0.67	3.5 ± 0.47	12-14
T26	2.5+0.5	60	6.7 ± 0.96	2.5 ± 0.89	10-12
T27	0.5+2.5	82	11.3 ± 1.08	3.9 ± 0.47	10-15
T28	3.0+0.5	40	5.2 ± 1.01	3.2 ± 0.61	15-17
T29	3.0+1.0	35	4.2 ± 0.37	3.0 ± 0.80	15-17
T30	1.0+3.0	30	3.3 ± 0.47	2.8 ± 0.49	15-17

[10 replicates/treatment; repeated thrice. Means are calculated by Duncan's multiple range test at the significance level of 5%]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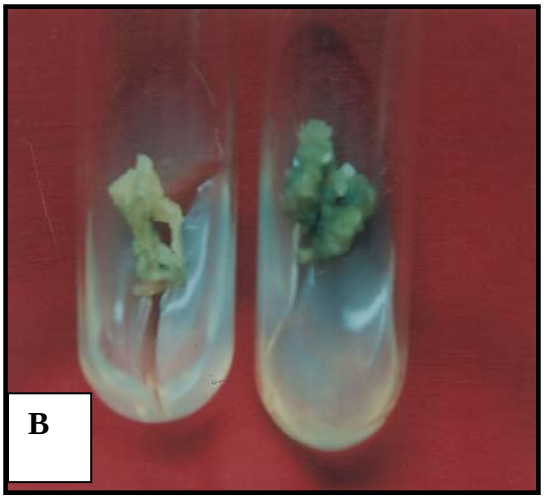
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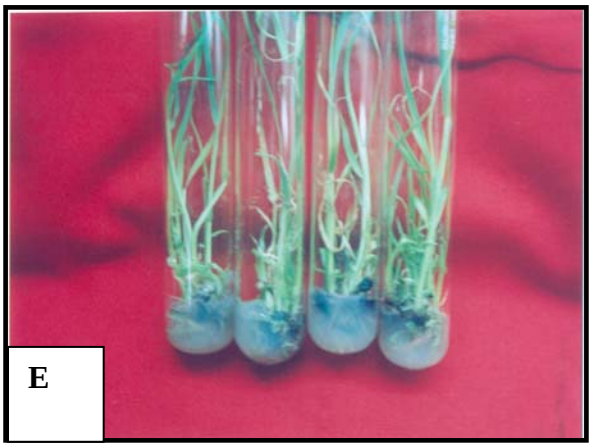

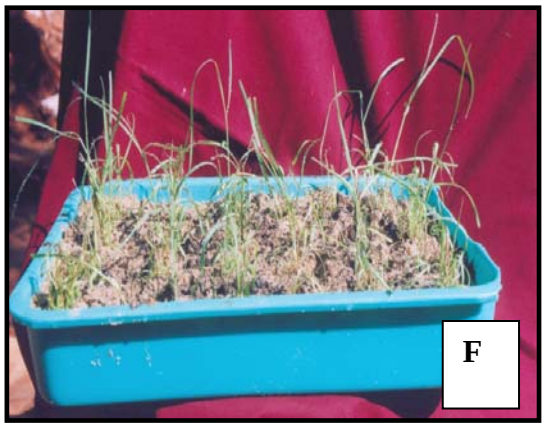

Fig.(A-F) In vitro callus regeneration and plant establishment of *Saccharum officinarum* L.(cv-Local, Nayana) (A) Callus regeneration in MS+2.5mg/l 2,4-D (B) Callus regeneration in MS+2.5mg/l NAA (C&D) Multiple shoot emergence from callus tissue in MS+2.0 mg/l BAP+0.5mg/l IBA (E) Micro shoots rooted in 1/2MS+NAA(2.5 mg/l).(F)Hardening of rooted plant lets in plastic trays.

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Acacia *nilotica*: a multipurpose leguminous plant

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Abstract: *Acacia nilotica* is multipurpose nitrogen fixing tree legume that is widespread in Africa and Asia, and occurs in Australia. It is a complex species with nine subspecies, of which six are native to the African tropics and three others are native to the Indian subcontinent. It occurs from sea level to over 2000m and can withstand extremes of temperature (>50^{o}C) and air dryness but is frost sensitive when young. It is considered as a very important economic plant since early times as a source of tannins, gums, timber, fuel, fodder and medicine. The main advantage of this genus is its fast biological nitrogen fixation, ability to establish on nitrogen- deficient and drought prone soils and suitability for agro forestry systems and thus can be used in rehabilitation of dry lands. This article briefly reviews the botany, distribution, ecology, uses of the plant and its effect on soil and crops. This is an attempt to compile and document information on different aspect of *A. nilotica* and its potential use in land reclamation. [Nature and Science. 2009;7(4):11-19]. (ISSN: 1545-0740).

Key words: Legume, nitrogen-fixation, agroforestry, afforestation, nutrients.

Introduction

Acacia nilotica (L.) Willd. ex Del commonly known as babul, kikar or Indian gum Arabic tree, has been recognized worldwide as a multipurpose tree (National Academy of Sciences 1980). In Australia it is regarded as one of the worst weeds because of its invasiveness, potential for spread, and economic and environmental impacts. It is widely distributed throughout arid and semi-arid zones of the world. Presently about 20% of the total geographical area of India is wasteland. Growing demand for fuel, fodder, wood and food has extensively depleted or eliminated protective plant cover and exposed soils to processes of degradation resulting in partial to complete loss of soil productivity. Since nitrogen is generally deficient in such lands, there is a great need for the identification of suitable nitrogen fixing plants; those can thrive well during the process of stabilization and recovery of degraded sites. In such conditions *A. nilotica* can play an important role. It is a relatively fast growing, drought resistant multipurpose legume with the ability of biological nitrogen fixation. In addition, its strong tap root system (Toky and Bisht1992), long growing period of more than 300 days with four peaks of leaf flush (Beniwal et al 1992), it can intensively exploit soil column for nutrients and moisture. This species has high potential for nitrogen fixation(Toky et al 1994), and has been considered as one of the fast growing species of the wastelands, and agro forestry systems throughout India providing strong timber, fodder for goats and sheep, and high quality fuel wood apart from enriching the soil with nitrogen. In the present article information on various aspects of *A. nilotica* and its role in recovery of wastelands/degraded lands was reviewed.

Description

Acacia nilotica (family Leguminosae, subfamily Mimosoideae) grows to 15-18 m in height and 2-3 m in diameter. The bark is generally slaty green in young trees or nearly black in mature trees with deep longitudinal fissures exposing the inner grey-pinkish slash, exuding a reddish low quality gum. The leaves are bipinnate, pinnae 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long. Thin, straight,

light grey spines present in axillary pairs, usually 3-12 pairs, 5-7.5 cm long in young trees, and mature trees commonly without thorns. Flowers in globulous heads, 1.2-1.5 cm in diameter of a bright golden yellow colour, born either axillary or whorly on peduncles 2-3 cm long located at the end of branches (Fig. 1). Pods7-15 cm long, green and tomentose when immature and greenish black when mature, indehiscent, deeply constricted between the seed giving a necklace appearance (Fig. 2). Seeds 8-12 per pod, compressed, ovoid, dark brown shining with hard testa.

Growth pattern

A. nilotica germinates following rainfall in the wet season. Although 95% of seed become dead after two years, some seeds may still germinate up to 15 years after seed drop. Germination is aided when seeds are disturbed, e.g. by fire or by passing through the digestive system of animals. Seedlings grow rapidly near water but more slowly in open grasslands. Trees can flower and fruit two to three years after germination, and more quickly after high rainfall years. It flowers between March and June, with pods forming between July and December. Most leaf fall corresponds to this dry period between June and November. Seedpods drop from October to January (Table 1).

Distribution

A. nilotica is naturally widespread in the drier areas of Africa, from Senegal to Egypt and down to South Africa, and in Asia from Arabia eastward to India, Burma and Sri Lanka. The largest tracts are found in Sind. It is distributed throughout the greater part of India in forest areas, roadsides, farmlands, tank foreshores, agricultural fields, village grazing lands, wastelands, bunds, along the national highways and railway lines. Mostly it occurs as an isolated tree and rarely found in patches to a limited extent in forests. It has been widely planted on farms throughout the plains of the Indian subcontinent It is a species of Southern Tropical dry deciduous forests and Southern Tropical thorn forests as distinguished by Champion and Seth (1968).

Ecology

There is some evidence that *A. nilotica* is a weed in its native habitat e.g. South Africa (Holm et al 1979), but in other areas it is planted for forestry or reclamation of degraded land (Puri and Khybri 1975, Shetty 1979). The ecological implication of using *A. nilotica* as a browse source while maintaining in appropriate stocking rates is land degradation. It grows well in two types of soils i.e. riverian alluvial soil and black cotton soil. This species grow on saline, alkaline soils and those with calcareous pans. *A. nilotica* grows under climatic conditions ranging from sub-tropical to tropical. It can withstand extremes of temperature (> 50 ^{o} C) and conditions of drought however; adequate moisture is needed for full growth and development. It is frost tender when young and trees of all age classes are adversely affected by conditions of severe frost. It is fire tender and both seedlings and saplings are aversely affected by fire. The average annual rainfall varies from 250-1500 mm.

Economic importance

Acacias are established as very important economic plants since early times as source of tannins, gums, timber, fuel and fodder. They have significant pharmacological and toxicological effects In Africa and the Indian subcontinent; *A. nilotica* is extensively used as a browse, timber and firewood species (Gupta 1970, Mahgoub 1979, New 1980). The bark and seeds are used as a source of tannins(Shetty 1979, New 1980) The species is also used for medicinal purposes. Bark of *A. nilotica* has been used for treating hemorrhages, colds, diarrhea tuberculosis and leprosy while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions (New 1980). The gum of *A. nilotica* is sometimes used as a

substitute for gum Arabic (obtained from *A. senegal*) although the quality is inferior (Gupta 1970). Indian Gum is sweeter in taste than that of the other varieties and is used in paints and medicine. The species is suitable for the production of paper and has similar pulping properties to a range of other tropical timbers (Nasroun 1979). The dark brown wood is strong, durable, nearly twice as hard as teak, very shock resistant and is used for construction, tool handles and carts. It has a high calorific value of 4950 kcal/kg, making excellent fuel wood and quality charcoal. It burns slow with little smoke when dry. It has a25% more shock resisting ability than teak. At the time of tree felling total wood production was estimated 167 Mg ha^{-1} that included 45 m^3 marketable timbers(Pandey and Sharma 2005). Survey of local timber market revealed that farmers fetch Rs 1000 from one tree (> 15 years) and Rs 30 to 90 thousand from 1 ha land, depending upon the stocking rate that makes the system economically viable. *Acacia nilotica* leaf is very digestible and has high levels of protein (Table 2). Micronutrients, with the exception of sodium, are adequate for animal requirements Leaves and pods contain 8% digestive protein (12.4% crude protein), 7.2 MJ/ kg energy and are rich in minerals and generally used for feeding sheep and goats in certain parts of India and also very popular with cattle. Pods are best fed dry as a supplement not as a green fodder. The bark contains high levels of tannin (12-20%) that is used for tanning leathers. Deseeded pods from ssp. *indica* have 18-27 tannin levels, whereas ssp. *nilotica* reached up to 50%. The relative tannin levels in *A. nilotica* from least to most are pods (5.4%), leaves (7.6%), bark (13.5%) and twigs (15.8%). The tannin also contributes to its medicinal use as a powerful astringent. It is also a powerful molluscicide and algaecide. Sub species *indica* and *cupressiformis* are commonly used in agroforestry. These subspecies makes an ideal windbreak surrounding fields. In India this species is used extensively on degraded saline/alkaline soils, growing on soils up to pH 9, with a soluble salt content below3%. It also grows well when irrigated with tannery effluent; and colonizes waste heaps from coalmines. Over 50, 000 hectares of the Indian Chambal ravines have been rehabilitated with *A. nilotica*.

Effect of *A. nilotica* on soil Characteristics

It was reported that the tree of *A. nilotica* improves soil fertility under its canopy by reducing proportion of sand with simultaneous increase in clay particles, mainly due to protection of soil from the impact of raindrops. Higher nutrient concentration under canopy compared to canopy gap (Table 3) is mainly a consequence of increased above and belowground organic matter input , nutrient cycling through leaf litter and protection of soil from erosion (Pandey et al.2000, Nair 1993 Palm 1995).The decrease in nutrient concentration towards the canopy edge compared to mid canopy position is mainly due to relatively low inputs of leaf litter as the canopy of *A. nilotica* is thin towards canopy edge(Pandey et al 1999).

A. nilotica is reported to be well nodulated with *Rhizobium* species (Dreyfus and Dommergues1981).This nodulation behaviour help in biological nitrogen fixation which help to meet the nitrogen requirement in nutrient-poor soils. In addition, this species form symbiotic associations with naturally occurring soil fungi called vesicular arbuscular mycorrhizae (VAM) (Kaushik and Mandal 2005). This association assists the roots to exploit more soil volume and to gain improved access to available nutrients especially phosphorus under stress and also makes the unavailable forms of nutrients into utilizable forms(Bowen 1973).

Effect of *A. nilotica* on crop yield

In the Central plain of Indian subcontinent *A.nilotica* grow naturally in the agricultural fields and forms an important agroforestry system (Pandey et al. 1999). It was reported that *A. nilotica* generally reduced crop yield under its canopy and this reduction varies with distance from the tree trunk (Pandey et al. 1999, Bargali et al.2004). In an experiment Bargali et al (2004) reported that gram yield increased with increasing the distance from the tree trunk and decreased with increasing the age of the tree (Fig. 3). In the Mitchell grasslands of northwest Queensland Australia, *A. nilotica* suppresses pasture production by 50% at 25-30% tree canopy cover or 2 m^2 basal area per hectare (Fig 4). It dramatically alters the ecological balance of grasslands and thereby threatens biodiversity. Pandey and Sharma (2005) reported that crop

production depend upon distance from tree trunk and tree canopy size. Reduction in grain yield was maximum (30%) under the large tree canopy and lowest (12%) under the small tree canopy due to decreased availability of light by 44 to 62% under the canopy that resulted in slow photosynthetic rates and growth (Pandey et al.2000). Pandey et al (1999) suggested that the gradient of the incident light was the principal factor governing the gradient of grass biomass under developing canopies of all tree plantations. However, when the tree is felled after the completion of rotation cycle (>12 years) grain yield increased 126% for the first cropping season and 30 % for the fifth cropping season at 1 m distance from the tree stump and declined with distance (Pandey and Sharma 2005). These results suggest that the crop may exploit the greater amount of nutrients to increase productivity, if the tree canopy is open to facilitate greater light availability.

Allelopathic effect of *A. nilotica*

El-khawas and Shehata(2005) reported that the leaf leachates of *A. nilotica* inhibited the germination and growth of *Zea mays* and *Phaseolus vulgaris.* Duhan and Lakshinarayana (1995) found that the growth of *Cyamopsis tetragonoloba* and *Pennisetum* growing at distance of 1-2 and 7.5 m from tree of *A. nilotica* was inhibited. Velu et al (1999) reported that the *Acacia* spp. Have phytotoxic effects on the tree crops of legumes. These results suggested that the inhibitory effect of *A. nilotica* on seed germination and seedling growth is related to the presence of allelochemicals including tannins, flavonoids and phenolic acids. Moreover, the toxicity is caused due to synergistic effect rather than single one (Fag and Stewart 1994). According to Stratmann and Ryan (1997) and El- Khawas (2004) allelopathic effect of *Acacia* spp. induced the formation of stress proteins. These proteins are responsible for folding, assembling, translocation and degradation in a broad array of normal cellular processes such as improvement of plant growth, physiological and molecular characteristics (Wang et al. 2004). This allelopathic ability of *A. nilotica* may have the potential as herbicide and can be used in biological control of weeds (Li And Wang 1998).

Table 1. General growth pattern of *A. nilotica*.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Flowering												
Pod formation												
Seed drop												
Germination												
Leaf fall												

Table 2. Nutrient levels in *A. nilotica* leaf and fruit[30].

	Leaves	**Fruit (pod and seed)**
Parameter	Mean ± SD	Mean ± SD
Protein (%)	13.92 ± 2.53	12.30 ± 2.03
Fat (%)	6.63 ± 3.41	1.93 ± 1.14
NFE (%)	60.99 ± 3.41	63.68 ± 7.35
CF (%)	10.35 ± 2.85	15.36 ± 5.85
ADF (%)	20.38 ± 6.35	25.44 ± 4.16
Ash (%)	9.29 ± 2.95	5.26 ± 1.29
Tannin (%)	7.62 ± 1.00	5.45 ± 1.48
Lignin (%)	6.95 ± 2.17	-
P (%)	0.23 ± 0.22	0.26 ± 0.21
Ca (%)	2.53 ± 1.13	0.64 ± 0.19
Mg (%)	0.18 ± 0.08	0.13 ± 0.02
Na* (%)	<0.32	<0.01
K (%)	1.25 ± 0.79	1.28 ± 0.22
Si (%)	0.45 ± 0.47	0.24 ± 0.21
S (%)	0.26 ± 0.03	0.59 ± 0.11
Cl (%)	0.70 ± 0.26	0.36 ± 0.04
Cu (mg/kg)	-	6.43 ± 0.90
Zn (mg/kg)	25.63 ± 9.20	28.50 ± 9.76
Mn (mg/kg)	90.25 ± 19.00	2650 ± 0.71
Fe (mg/kg)	428 ± 205	100.00 ± 86.27
ME (mg/kg)	8.69 ± 1.09	10.19 ± 0.16
OMD (%)	69.9 ± 5.20	67.2

*Some values below limit of detection (0.05%)

NFE = Nitrogen free extract; ADF = Acid detergent fibre; CF= Crude fibre;

OMD= Organic matter digestibility; ME = Metabolisable energy

Table 3. Soil pH, Organic Carbon, Total N and Total P concentration under mid-canopy, canopy edge and open field of *Acacia nilotica* tree.

Soil depth (cm)	**Soil pH**			**Organic C (%)**			**Total N (%)**			**Total P (%)**		
	MC	CE	CG	MC	CE	CG	MC	CE	CG	MC	CE	CG
0-10	6.64	6.75	6.98	1.18	1.04	0.82	0.118	0.091	0.059	0.066	0.066	0.062
10-20	6.52	6.97	7.15	1.09	0.91	0.58	0.102	0.077	0.040	0.065	0.064	0.064
20-30	6.59	7.17	7.25	0.66	0.55	0.44	0.035	0.026	0.016	0.064	0.061	0.061
Mean	6.58	6.98	7.13	0.98	0.83	0.61	0.085	0.045	0.038	0.065	0.064	0.062

MC= Mid- canopy; CE = Canopy edge; CG = Canopy gap

Fig. 1. A twig of *A. nilotica* showing flowers.

Fig. 2. Pods of *A. nilotica.*

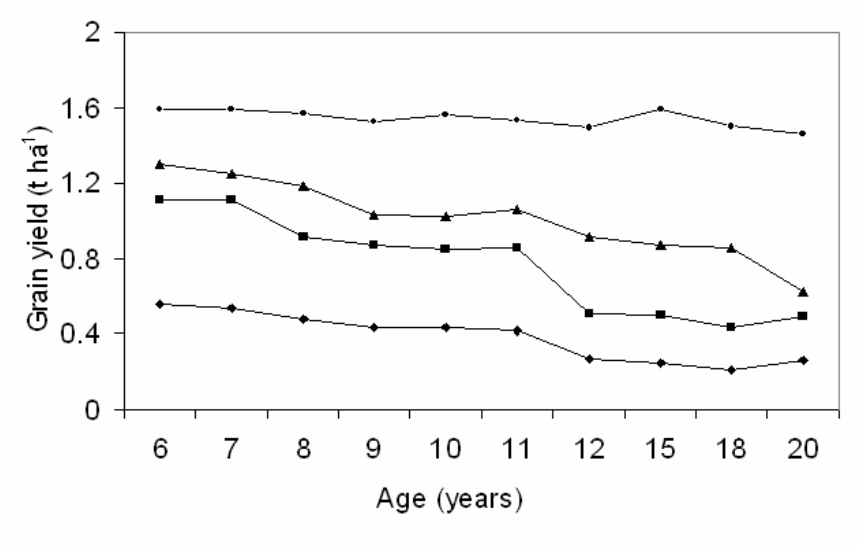

Fig. 3 Effect of *A. nilotica* on gram crop measured at different distances from tree. ◊--◊= 1m distance; ■-■= 3m distance; ▲-- ▲=5m distance and ●--● = open field

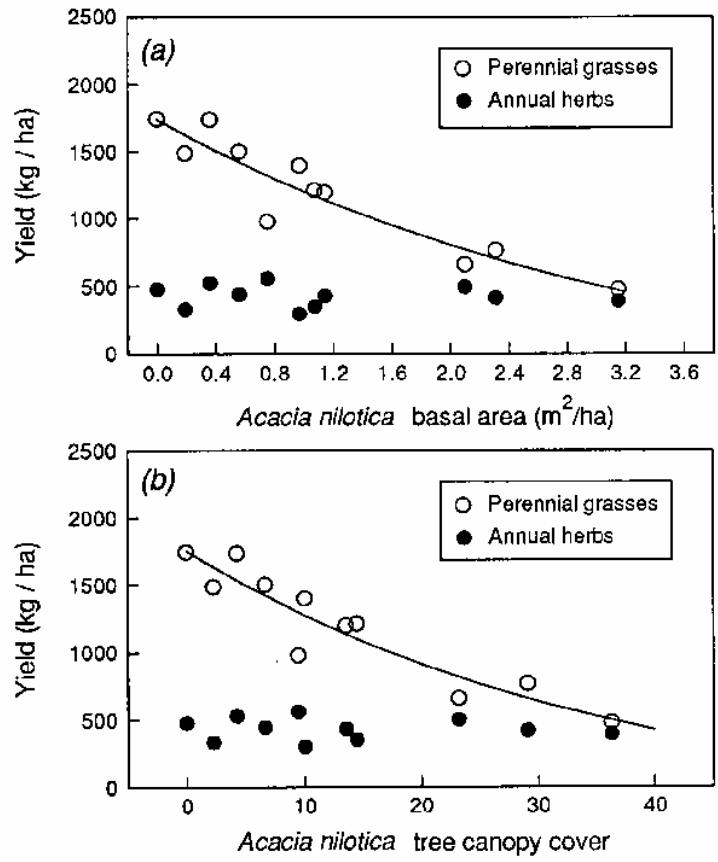

Fig 4. Effect of *A.nilotica* on pasture production (kg ha^{-1})

CONCLUSIONS

Being a drought resistance species *A. nilotica*; a multipurpose legume can be used for rehabilitation of dry lands. It increases soil organic carbon, total and available forms of N and P under its canopy so it can be used in soil amelioration. The chances of nitrogenous fertilizer use in various afforestation programmes are very bleak in the near future. The only alternative is to select such species that can meet their nitrogen requirements from soil as well as atmosphere. The nutrient generated by *A. nilotica* tree by biological nitrogen fixation, can be exploited within production system, either simultaneously as an intercropping plant or sequentially as in rotational fallow systems.

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Occurrence of Water Borne Conidial Fungi in Relation to Some Physico-Chemical Parameters in a Fresh Water Stream

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ABSTRACT: Relative effect of some physico-chemical parameters of water to the occurrence of water borne conidial fungi was studied in a high altitudinal fresh water stream. A total of 30 species belonging to 21 genera were isolated. The number of conidial fungal species was enumerated in water samples collected monthly during the period of March 2007-February 2008. There was marked seasonal fluctuation in the occurrence of the species. The maximum number of species was found during spring to early summer and winter, while there was a decline in the number of species during late summer, rainy and early autumn seasons. Species richness was correlated with seven different water quality variables measured for each sample period, viz., temperature, pH, dissolved oxygen, organic and inorganic content, phosphate and sulphate concentration. The data were analyzed statistically for correlation and these factors were found to be significant for the occurrence of water borne conidial fungi. [Nature and Science. 2009;7(4):20-28]. (ISSN: 1545-0740).

Key words: aquatic hyphomycetes, occurrence, physico-chemical parameters

INTRODUCTION

The aquatic ecosystem comprises of variety of biota. Fungal community is one of them. Several physico-chemical factors of aquatic ecosystem influence the composition and activity of the fungal community. Of these, fluctuation in temperature, hydrogen – ion concentration, oxygen content, dissolved organic and inorganic matter, phosphate and sulphate concentration have been found to be important factors for the occurrence and distribution of individual species of water borne conidial fungi in the fresh water stream (Ingold, 1975; Nilsson, 1964). Within a stream site differences are closely correlated with altitude or other factors associated with it, which may include differences in water chemistry (Fabre, 1998; Raviraja et al., 1998).

Species assemblage of aquatic hyphomycetes varies seasonally and site to site and these changes are thought to occur mainly due to differences in water temperature (Barlocher & Kendrick, 1974; Suberkropp, 1992). Temperature range of 10-25°C favors the growth and multiplication of conidial fungi in water bodies. The optimum temperature for the growth of *Tricladium chaetocladium* and *Lunulospora curvula* was within the range of 15-20°C (Iqbal & Webster, 1973). Ingold (1975) and others from temperate countries have found abundant conidia in autumn, winter and early spring and abundance decreased in the late spring and summer.

pH greatly affects the decompositional activities of aquatic hyphomycetes in running fresh water bodies. The occurrence and degradative ability of water borne conidial fungi colonizing on submerged leaf litter is influenced by the hydrogen ion concentration (pH) of water (McKinley & Vestal, 1982). While working in an arctic lake they found a progressive decline of fungi with increasing acidity and their almost complete absence at pH 4.0-3.0.

These fungi require a fresh oxygenated environment for their occurrence (Webster & Towfic, 1972). Increase in fungal species number is related with increasing dissolve oxygen and dissolve organic matter of the stream (Kaushik & Hynes, 1971).

Several studies indicate that in lotic ecosystems, leaf litter decomposition and fungal activity can be affected by the concentration of nutrients (e.g. nitrogen and phosphorus) in the water (Suberkropp & Chauvet, 1995; Sridhar & Barlocher, 2000; Grattan & Suberkropp, 2001; Rosemond et al., 2002). Aquatic hyphomycetes might obtain inorganic nutrients (nitrogen and phosphorus) not only from their organic substrate (leaf litter, wood debris etc.) but also directly from water passing by ravine areas (Suberkropp, 1995; Suberkropp & Chauvet, 1995).

The presence of these fungi in the sulphide containing water bodies indicates the importance of these salts for the growth of aquatic hyphomycetes (Field & Webster, 1985). Recently (Gulis & Suberkropp, 2003, 2004; & Gulis et al., 2004) have initiated some studies on the effect of nutrients on aquatic hyphomycetes in the American streams.

The present work was carried out to study the relation of physico-chemical factors to the occurrence and distribution of water borne conidial fungi in a fresh water high altitudinal stream of Kumaun Himalaya.

METHODOLOGY

Study Area

Vinayak stream (1200 m asl), of Kumaun Himalaya was selected for the present work. It is nearly 25 Km away from Nainital (Fig. 1). This stream passes through well-canopied oak and pine trees forest area.

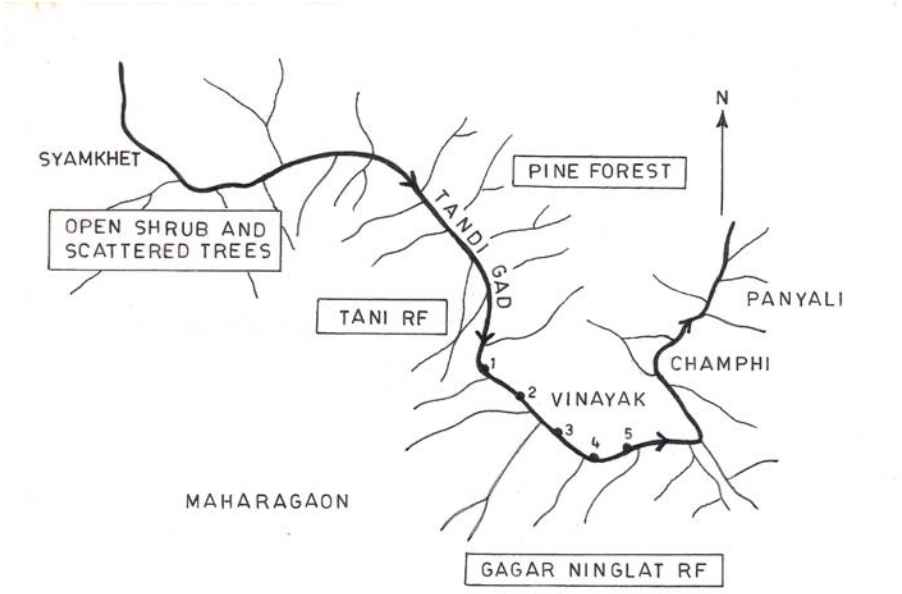

Figure 1. Location map with the sampling sites along the Vinayak stream (29° 22′ N latitude and 79° 33′ E longitude).

Sampling

Monthly five samples were made from the five selected sites (Fig. 1) in the study area for a period of one year (March 2007 to February 2008). Samples of five sites were taken as five replicates and collected in sterilized bags and were processed as described by Nilsson (1964). Species were identified using relevant monographs and literature. Conidia of species were taken as an index of occurrence. Stream water was analyzed to determine the seasonal variation, frequency of occurrence (%), and relative frequency of water borne conidial fungi by using following formula:

$$\% \text{ Frequency} = \frac{\text{Occurrence of Conidia in samples}}{\text{Total no. of samples analyzed}} \times 100,$$

$$\text{Relative Frequency} = \frac{\text{Frequency of Occurrence}}{\text{Total of Frequency}} \times 100$$

Analysis of water quality

The physico-chemical parameters of water viz., stream temperature (° C), water pH, dissolve oxygen (mg l^{-1}), dissolve organic matter (mg l^{-1}), dissolve inorganic matter (mg l^{-1}), sulphate (mg l^{-1}) and phosphate (mg l^{-1}) were analyzed following the methods of Trivedi and Goel (1986) and A.P.H.A. (1989).

Temperature was measured by using a centigrade thermometer by dipping it at a depth of 5-8 cm in water for 5 minutes, at the time of sample collection. pH was recorded on spot with the help of a digital portable pH meter (*Hanna*) periodically at the time of collection of samples.

Dissolve oxygen content (mg l^{-1}) was determined on the spot by making a composite sampling of water at each month following the Winkler method (Welch, 1948). Dissolve organic and inorganic matter (mg l^{-1}), phosphate and sulphate content (mg l^{-1}) were analyzed by following A.P.H.A. (1989).

Statistical analysis

Each physico-chemical factors of water were analyzed statistically for the relationship with the occurrence of water borne conidial fungi on the basis of coefficient of correlation (r).

RESULTS

A total of 30 species belonging to 21 genera of water borne conidial fungi viz., *Alatospora*, *Anguillospora*, *Camposporium*, *Campylospora*, *Clavariopsis*, *Dimorphospora*, *Diplocladiella*, *Flagellospora*, *Heliscella*, *Heliscus*, *Lemonniera*, *Lunulospora*, *Pestalotiopsis*, *Pleurophragmium*, *Setosynnema*, *Speiropsis*, *Tetrachaetum*, *Tetracladium*, *Tricladium*, *Tripospermum* and *Triscelophorus* were isolated from Vinayak stream (Table 1). These species were isolated from different decomposed leaf litter of known and unknown species and foam.

Seasonal variation in species composition

During the course of present study a seasonal fluctuation in the occurrence of water borne conidial fungi was observed. The total fungal population was higher during late spring and winter seasons (16-24 species), than early summer season (with 20 species). However, the decline in their occurrence was found during rainy to autumn seasons (10-15 species).

It was interesting to note that some species appeared in particular seasons, *Camposporium pellucidum* occurred only in late spring to summer. *Campylospora parvula* and *Heliscella stellatacula* appeared only in spring. *Pleurophragmium sonam* occurred only in summer. *Tricladium chaetocladium* occurred during rainy and late winter seasons. *Tripospermum myrti* appeared from late rainy to early winter seasons. *Campylospora chaetocladia*, *Clavariopsis aquatica*, *Lunulospora cymbiformis*, *Tetracladium marchalianum* and *Triscelophorus monosporus* appeared throughout the year in every season and can be regarded as temperature tolerant species, while other species were found during maximum seasons.

Frequency of occurrence

Frequency of occurrence and relative frequency of occurrence is summarized in table 1. As evident from this table *Triscelophorus monosporus* occurred throughout the year and showed maximum frequency of occurrence (100 %) with 6.32 % relative frequency followed by *Tetracladium marchalianum* with 91.66 % frequency of occurrence (5.79 % relative frequency) and *Campylospora chaetocladia*, *Clavariopsis aquatica* and *Lunulospora cymbiformis* with 83.33 % frequency of occurrence (5.26 % relative frequency) each. 14 species showed 41-80 % of frequency of occurrence which appeared to be moderate species in occurrence, these species were viz., *Alatospora acuminata*, *Anguillospora crassa*, *A. longissima*, *Flagellospora penicillioides*, *Lemonniera terrestris*, *L. cornuta*, *Lunulospora curvula*, *Pestalotiopsis submersus*, *Setosynnema isthmosporum*, *Speiropsis scopiformis*, *Tetrachaetum elegans*, *Tetracladium apiense*, *T. setigerum* and *Triscelophorus acuminatus*. 9 species viz., *Alatospora pulchella*, *Camposporium pellucidum*, *Campylospora parvula*, *Dimorphospora foliicola*, *Heliscella stellatacula*, *Heliscus lugdunensis*, *Lemonniera pseudofloscula*, *Tricladium chaetocladium* and *Tripospermum myrti* showed low frequency of occurrence i.e. < 40 %. *Diplocladiella longibrachiata* and *Pleurophragmium sonam* represented less than 20 % frequency of occurrence and were regarded as rare species with 0.53 -1.05 % of relative frequency.

The average value of temperature, pH, dissolve oxygen, organic matter, inorganic matter, phosphate and sulphate of five samples collected from five different sites is given in table 2.

The results of different physico-chemical water parameters of species are summarized as below:

i. Temperature: Water temperature of stream recorded during the study period indicates a marked seasonal variation (Table 2). The temperature of the stream water ranges between 8-24.3 ° C. Fungal species were found temperature dependent, as fluctuation in the temperature also change the species composition of water borne conidial fungi. Statistical analysis indicated a negative correlation (r = - 0.06454) of the fungal species with temperature. It was noted that species number declines with the rising of water temperature (Fig. 2).

ii. Hydrogen- ion concentration (pH): pH of water ranged between 7.6- 8.8 (Table 2), with minimum in March and maximum in May (Fig. 2). Water pH had a close relationship with the occurrence of water borne conidial fungi. The number of fungal species had a negative correlation with pH, having values of (r = - 0.26817).

iii. Dissolved Oxygen (DO): Dissolved oxygen content of water ranged between 4.3 – 12.5 mg/l (Table 2), with maximum in December and minimum in September. A negative correlation (r = - 0.22985) was found between the dissolved oxygen content of water and occurrence of species.

iv. Organic Matter: Organic matter ranged between 57 – 200 mg/l (Table 2.). The maximum species flourished during winter to late spring when the organic matter ranges between 100 – 200 mg l^{-1}. A positive correlation (r = 0.66198) was obtained between organic matter and fungal species during the coarse of present study.

v. Inorganic Matter: Inorganic content of water ranged between 60 – 200 mg/l (Table 2.). The observation of the present study indicate occurrence of greater number of species in winter and spring seasons coincided with the higher values of inorganic matter, i.e., 107 – 200 mg l^{-1}, while species number decreased with a fall in the inorganic content. A positive correlation (r = 0.78148) was found between the occurrence of species and inorganic matter of water (Fig. 2).

vi. Phosphate: The Phosphate content of the water varied between 0.200 – 0.966 mg/l (Table 2.). The minimum value was recorded in November and January (Fig. 2). Statistical analysis showed a positive correlation (r = 0.77061) with the occurrence of species. It was noted that the maximum number of species occurred in winter, spring and early summer seasons when phosphate content of water was higher (0.415 – 0.966 mg l^{-1}).

vii. Sulphate: The values of Sulphate content of water ranges between 1.200 – 16.10 mg/l (Table 2.). It was observed that the number of species increase with the increase in the sulphate content. A positive correlation (r = 0.41001) was obtained between number of species and sulphate content of water (Fig. 2).

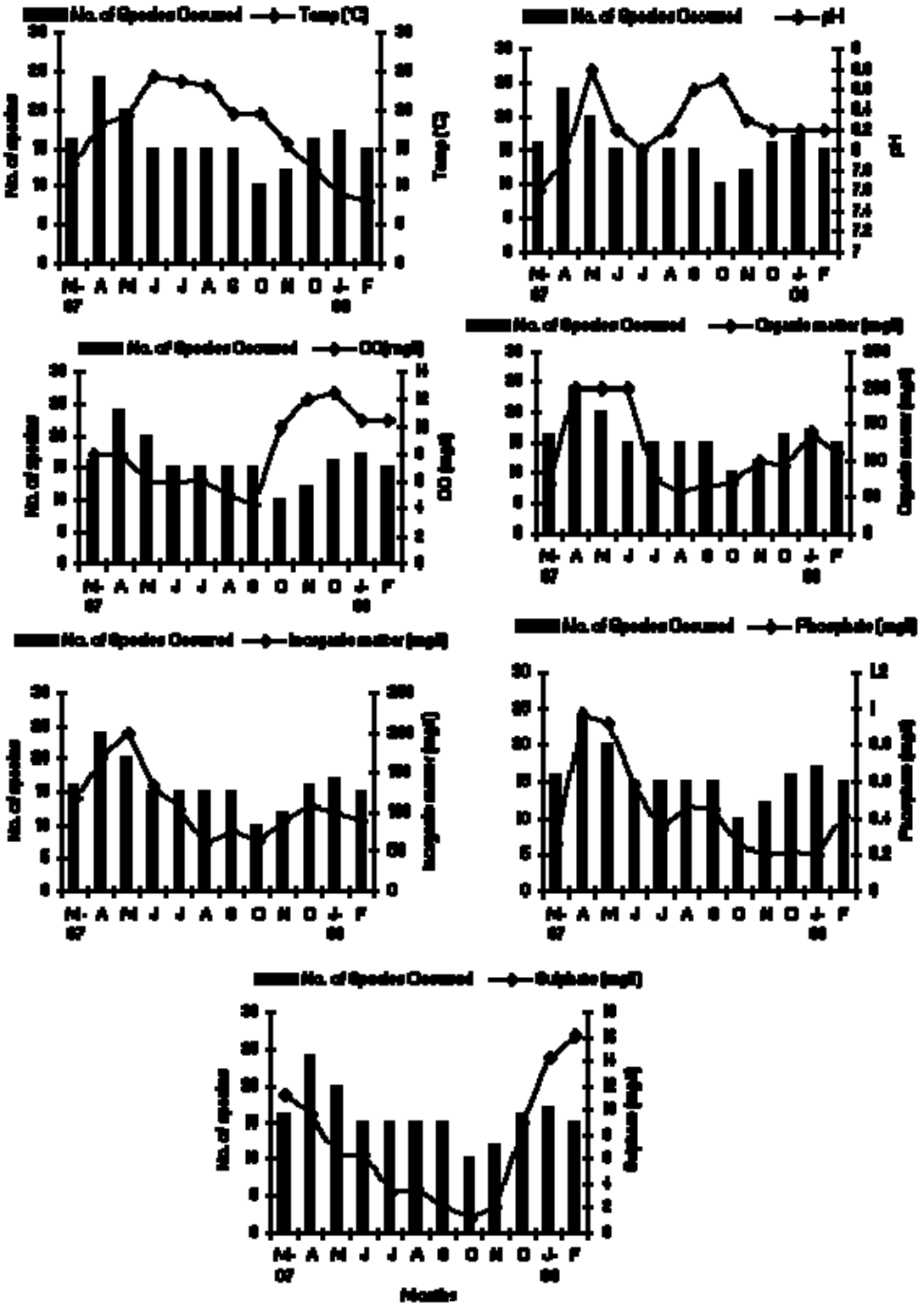

Figure 2. Relationship between number of fungal species and different physico-chemical parameters. (Temperature, pH, Dissolved Oxygen, Organic, Inorganic, Phosphate and Sulphate matter)

Table 1. Seasonal fluctuation in species composition, % frequency and % relative frequency of occurrence of water borne conidial fungi of a fresh water stream Vinayak, Kumaun Himalaya during March 2007 to February 2008

		Spring		**Summer**		**Rainy**			**Autumn**		**Winter**					
		M-07	Ap	My	Jn	Jul	A	S	O	N	D	J-08	F	Occurrence	% Frequency	% Relative Frequency
	Temp.	13.0	17.9	19.3	24.3	23.7	23.0	19.5	19.5	15.5	12.0	9.0	8.0			
	pH	7.6	7.9	8.8	8.2	8.0	8.2	8.6	8.7	8.3	8.2	8.2	8.2			
S.No.	Name of fungi															
1.	*Alatospora acuminata* Ingold	+	+	+	+	+	-	-	+	-	+	+	+	9	75.00	4.74
2.	*Alatospora pulchella* Marvanova	-	+	+	-	-	+	-	-	-	-	-	-	3	25.00	1.58
3.	*Anguillospora crassa* Ingold	+	-	+	+	-	-	+	+	-	+	+	+	8	66.66	4.21
4.	*Anguillospora longissima* (Sacc. & Therry) Ingold	+	+	+	-	-	-	-	-	+	+	+	+	7	58.33	3.68
5.	*Camposporium pellucidum* (Grove) Hughes	-	+	+	+	-	-	-	-	-	-	-	-	3	25.00	1.58
6.	*Campylospora chaetocladia* Ranzoni	-	+	+	+	+	+	+	+	+	+	+	-	10	83.33	5.26
7.	*Campylospora parvula* Kuzaha	+	+	+	-	-	-	-	-	-	-	-	-	3	25.00	1.58
8.	*Clavariopsis aquatica* de Wildeman	+	+	+	+	+	+	-	-	+	+	+	+	10	83.33	5.26
9.	*Dimorphospora foliicola* Tubaki	+	+	-	-	-	-	-	-	-	-	+	+	4	33.33	2.11
10.	*Diplocladiella longibrachiata* Nawawi & Kuthub.	-	+	+	-	-	-	-	-	-	-	-	-	2	16.66	1.05
11.	*Flagellospora penicillioides* Ingold	-	+	-	+	+	+	+	-	-	-	-	+	6	50.00	3.16
12.	*Heliscella stellatacula* Marvanova	+	+	+	-	-	-	-	-	-	-	-	-	3	25.00	1.58
13.	*Heliscus lugdunensis* Sacc. & Therry	+	+	+	-	+	-	-	-	-	-	-	-	4	33.33	2.11
14.	*Lemonniera terrestris* Tubaki	+	-	-	-	-	-	-	-	+	+	+	+	5	41.66	2.63
15.	*Lemonniera cornuta* Ranzoni	+	+	-	-	-	+	-	-	+	+	+	+	7	58.33	3.68
16.	*Lemonniera pseudofloscula* Dyko	+	+	-	-	-	-	-	-	-	+	+	-	4	33.33	2.11
17.	*Lunulospora curvula* Ingold	+	+	-	-	+	-	+	+	+	+	+	+	9	75.00	4.74
18.	*Lunulospora cymbiformis* Miura	+	+	-	+	+	+	+	+	+	+	+	-	10	83.33	5.26
19.	*Pestalotiopsis submersus* Sati & Tiwari	-	-	+	+	+	+	+	-	-	+	+	-	7	58.33	3.68
20.	*Pleurophragmium sonam* Sati & Tiwari	-	-	+	-	-	-	-	-	-	-	-	-	1	08.33	0.53
21.	*Setosynnema isthmosporum* Shaw & Sutton	-	+	+	+	+	+	+	+	-	-	+	-	8	66.66	4.21
22.	*Speiropsis scopiformis* Kuthub.& Nawawi	-	+	+	+	+	+	+	-	-	-	-	+	7	58.33	3.68
23.	*Tetrachaetum elegans* Ingold	+	+	+	+	-	-	-	+	+	+	+	+	9	75.00	4.74
24.	*Tetracladium apiense* Sinclair & Eicker	-	+	+	+	+	+	+	-	-	-	-	-	6	50.00	3.16
25.	*Tetracladium marchalianum* de Wildman	+	+	+	+	-	+	+	+	+	+	+	+	11	91.66	5.79
26.	*Tetracladium setigerum* (Grove) Ingold	-	+	+	-	+	+	+	-	-	-	-	-	5	41.66	2.63
27.	*Tricladium chaetocladium* Ingold	-	-	-	-	+	+	+	-	-	-	-	+	4	33.33	2.11
28.	*Tripospermum myrti* (Lind)Hughes	-	-	-	-	-	-	+	+	+	+	-	-	4	33.33	2.11
29.	*Triscelophorus acuminatus* Nawawi	-	+	-	+	+	+	+	-	+	+	+	+	9	75.00	4.74
30.	*Triscelophorus monosporus* Ingold	+	+	+	+	+	+	+	+	+	+	+	+	12	100	6.32
	Total	16	24	20	15	15	15	15	10	12	16	17	15			

M= March, Ap= April, My= May, Jn= June, Jul= July, A= August, S= September, O= October, N= November
D= December, J= January, F= February, + = Species present, - = Species absent

Table 2. Occurrence of water borne conidial fungi and water physiochemical characters of Vinayak Stream of Kumaun Himalaya during March 2007 to February 2008 *

S. No.	Months Name	No. of Species Occurred	Temp(° C)	pH	DO (mg/l)	Organic matter (mg/l)	Inorganic matter (mg/l)	Phosphate (mg/l)	Sulphate (mg/l)
1.	March-2007	16	13.0	7.6	8.0	70	117	0.262	11.214
2.	April	24	17.9	7.9	8.0	200	167	0.966	9.605
3.	May	20	19.3	8.8	6.0	200	200	0.927	6.385
4.	June	15	24.3	8.2	6.0	200	133	0.580	6.385
5.	July	15	23.7	8.0	6.2	73	103	0.346	3.340
6	August	15	23.0	8.2	5.0	57	60	0.473	3.580
7.	September	15	19.5	8.6	4.3	67	77	0.452	2.000
8.	October	10	19.5	8.7	10.0	70	63	0.251	1.200
9.	November	12	15.5	8.3	12.0	100	90	0.200	2.100
10.	December	16	12.0	8.2	12.5	93	107	0.226	9.000
11.	January-2008	17	09.0	8.2	10.5	140	100	0.200	14.30
12.	February	15	08.0	8.2	10.5	110	90	0.415	16.10

DO = Dissolved Oxygen

* Average values of five samples studied at five sites

DISCUSSION

The result obtained during present investigation revealed that the species composition of the water borne conidial fungi varied considerably from seasons to seasons, that would be attributed to the variation in physico-chemical characteristics of the habitat which have profound influence on the occurrence and distribution of water borne conidial fungi.

A perusal of seasonal occurrence of different species in the habitat indicates that the water borne conidial fungi show a marked seasonal fluctuation in their occurrence. A maximum number of the fungal species was found during spring to early summer and winter seasons, while number of species decline in summer to early autumn seasons (May - Oct). Sridhar and Kaveriappa (1989) also observed that the total number of aquatic hyphomycetes was lowest during summer season. Occurrence of maximum number of species during winter and spring seasons in the present study might be due to moderate temperature and slightly higher percentage of organic and inorganic matter. Many investigators have observed similar maxima during post monsoon periods (Sridhar & Kaveriappa, 1984) and suggested that after rainfall the large amounts of various leaf detritus get transferred into the stream through rain wash from distant places and stream gets greater abundance of these fungi. Figure 2 summarizes the relationship between number of fungal species and different physico-chemical parameters (Temperature, pH, Dissolved Oxygen, Organic, Inorganic, Phosphate and Sulphate) of Vinayak stream.

There was a negative correlation with species number and pH within certain range (r = - 0.26817). This indicates that both high and low pH might not be suitable for these fungi. Barlocher and Rosset (1981) suggested that pH close to 7.0 favour higher numbers of fungal species. The occurrences of water borne conidial fungi also show negative correlation with temperature (r = -0.06454). This finding was found to be in support of Mer and Sati (1989) and Raviraja et al., (1998).

Water borne conidial fungi obtain phosphate and sulphate not only from the leaf litter, wood debris but also directly from water passing by ravine areas (Suberkropp, 1995; Suberkropp & Chauvet, 1995). During the observation a positive correlation was found between the occurrence of aquatic hyphomycetes and the sulphate concentration of water (r = 0.41001)). However, the previous work of Field and Webster (1985) shows reduction in the survival of Ingoldian aquatic hyphomycetes with increasing concentration of sulphide.

The result of the investigation shows a positive correlation between phosphate concentration and species occurrence species (r = 0.77061). It justifies the findings of Krauss et al., (2001), who also reported the stimulation of fungus activity at high P concentrations.

The physico – chemical characters of the water were found much influenced to the occurrence and distribution of water borne conidial fungi in a fast flowing stream (Field & Webster, 1985; Sridhar & Kaveriappa, 1989; Sridhar et al., 2001; Webster & Descals, 1981).

Out of the 30 species isolated from the fast flowing stream of Vinayak, *Campylospora chaetocladia, Clavariopsis aquatica, Lunulospora cymbiformis, Tetracladium marchalianum* and *Triscelophorus monosporus* occur throughout the year, having maximum abundance. They may be regarded as temperature tolerant species and common species of the stream and can be concluded, as their appearance does not seem to be affected by different physico-chemical factors.

In the present study, the impact of temperature, pH, dissolved oxygen, organic and inorganic matter, phosphate and sulphate content showed a marked influence on the occurrence and distribution of the water borne conidial fungi. Relying upon the data observed it can be conclude that occurrence and distribution of the water borne conidial fungi is governed by interaction of temperature, pH, dissolved oxygen, organic, inorganic, phosphate and sulphate matter of the stream water.

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Chemical Examination Of Bergenia Stracheyi (Hk.) For Antioxidative Flavonoids

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Key Words: *Bergenia stracheyi* (HK), Antioxidative flavonoids.

ABSTRACT： *Bergenia Stracheyi* (HK.), a perennial rhizometic herb of family Saxifragaceae, has frequently been reported from Afganistan to U.P. between 3300 – 4500m in alpine slopes. This is a common alpine species of Western Himalaya. It is found in the form of vast patches in sub-alpine and alpine areas. Its rootstock is thick, dark – brown with short stem, thick and fleshy. Leaves are stalked and sheathing at base, ovate or orbicular, cordate, margin fringed with short and stiff hairs. Flowers are white, pink or purplish in terminal corymbose cymes. Fruit is a conical capsule having minute seeds. It is a traditional medicinal plant and the local tribal inhabitants of the region use the rhizome of plant for curing kidney and gall bladder stone, cough and cold, heeling old wounds, cuts and burns, inflammation etc. Looking on the various biomedicinal uses of the plant in the various systems of medicines the objectives of the present chemical investigation of the plant are: Evaluation of antioxidative activity from flavonoidal positive fraction. Structural elucidation of active flavonol glycosides by chromatographic, hydrolytic and spectral methods. Three flavonol glycosides were isolated from the antioxidative activity guided fraction by PPC and these structures are identified as: Quercetin-3-O-α-L-rhamnoside, Kaempferol-3-O-α-L-rhamnoside and Kaempferol-3-O-rhamnosyl (1- 6) glucoside. Among these three flavonol glycosides, the compound (1) and (2) gave promising antioxidative activity while the compound (3) did not show effective activity. [Nature and Science. 2009;7(4):29-34]. (ISSN: 1545-0740).

Keywords: Chemical Examination; Bergenia Stracheyi; Antioxidative; Flavonoids

INTRODUCTION

Bergenia stracheyi (HK.) is a rhizometic herb species belonging to Saxifragaceae and is mainly found in Afghanistan to Uttarakhand, between 3300-4500 m in alpine slopes (Chauhan, 2000). The rhizome of *Bergenia stracheyi* is used as a folk medicine for its antiscorbutic, astringent, dirrretic, febrifuge and opthalmic properties (Pandey, 1995) and previous chemical and pharmacological studies on his species reported the occurrence of glycosides, gallic acid, tannic acid, mucilage, wax, albumens, starch etc. (Sharma, 2003). Flavonoids are also found in other species of *Bergenia* as glycosides (Farooq, 2005). Flavonoids have widely been associated with various biological activities such as antimicrobial, antioxidant, anti inflammation and anticancerogenic (Havsteen, 2002).

Antioxidants play a role in the maintenance of the pro/antioxidant balance by neutralizing the radicalar oxygen and nitrogen species which are responsible for deleterious processes in biological system (Ribeiro, 2002).

This paper deals with the detection of antioxidant compound from the aqueous- ethanolic extract of *Bergenia shracheyi* leaves. The fractionation of the extract through chromatography in order to isolate and elucidate the molecular structure of the compounds responsible for the antioxidant activity.

MATERIALS AND METHODS

Plant material and authentification:

Leaves of wild growing plants of *Bergenia stracheyi* (HK.) were collected in September 2006 from alpine slopes of Kumaun Himalaya. Voucher specimen of the collected plant (*Bergenia stracheyi* No. H.02) were confirmed and deposited at the herbarium of the Department of Botany, Kumaun University S.S.J.Campus, Almora, Uttarakhand.

Method of extraction:

About 2.5 kg air dried and powdered leaves of *B. stracheyi* were extracted with 50% aqueous ethanol for five days by cold percolation method. The extract was decanted and concentrated under reduced pressure until only a small layer of H_2O (approx. 50 ml) remained. It was partitioned with CH_2Cl_2 and BuOH. The BuOH fraction was examined for antioxidative flavonoidal compounds.

Isolation of flavonoidal positive fraction from BuOH soluble:

The BuOH soluble was evaporated to dryness under reduced pressenre at 55^0 C in Rota evaporator. The residue of BuOH soluble was dissolve in 70% EtOH and it was banded in Whatman No. 3 PC using 20% HOAc as an eluent. A broad dark purple fluorescent band was observed on PC with UV light. It was cut and eluted with 70% aq - MeOH. The aqueous methanolic elute was evaporated to dryness and residue was chromatographed on cellulose (Merck grade) cc and eluted initially with H2O then increasing polarity with acetic acid. On eluting column with 10% HOAc two dark purple fluorescent bands were observed on column each band was eluted and collected separately by monitoring with UV light. The aqueous-acetic acid elute of two UV fluorescent bands, faster moving and slower moving, representing FRAC-01 and FRAC-02 respectively.

Evaluation of anti-oxidative activity of above CC eluted fraction:

Each fraction was examined in 2DPC for total flavonoidal constituents using BAW (n-BuOH-AcOH-H2O, 4: 1: 5, V\V, upper layer) and 30% HOAc as a developing solvents. 2DPC examination of FRAC-01 and FRAC-02 afforded flavonoidal compounds 5 and 3 respectively. Each flavonoidal positive fraction was screened for antioxidative activity by thin layer autochromatographic method.

The residue of FRAC-01 and FRAC-02 were dissolve in MeOH and methanolic soluble of each fraction was spotled on silica gel coated TLC. The TLC plate was developed with CHCl3 : MeOH (3 : 1) and after development the dried plate was sprayed with methanolic solution of DPPH (Aldrict chem.) free radical. FRAC- 01 and FRAC-02 gave positive antioxidative spots on TLC as they produced light yellow spots on purple back ground, while FRAC-01 did not give any antioxidative positive spots on TLC. Thus FRAC-01 was discarded and FRAC-02 was used for the isolation of antioxidative positive flavonoidal positive compounds.

Isolation of flavonoidal compounds from antoxidative positive fraction, FRAC-02:

The residue of FRAC-02 was chromatographed on Whatman No. 3 PC using BAW (4: 1: 5, V/V, upper layer) as an eluent. The dried and developed PC was inspected with UV light. Two major broad dark purple UV fluorescent bands were observed on PC were cut and eluted separately with 70% EtOH. The faster moving band, representing FRAC-02 (a) gave two dark purple UV fluorescent bands after its re-chromatography with 30% HOAc. The faster moving band, representing compounds (C) and slower moving compound, representing compound (B) were cut and eluted from PC by monitoring with UV light. Each compound was finally purified on Sephadex LH-20 cc using 50% aq. MeOH. The slower moving fraction, FRAC-02 (b) was further purified on Whatman No. 3 PC using BAW (4 : 1 : 5, V/V, upper layer) as an eluent, A broad dark purple UV fluorescent band was observed an PC was cut and eluted with 70% aq, EtOH separately. It was finally purified on sephadex LH-20 cc using 60% HOAc as an eluent. The isolated compound representing structure (A). (Structure 1).

RESULTS and DISCUSSION

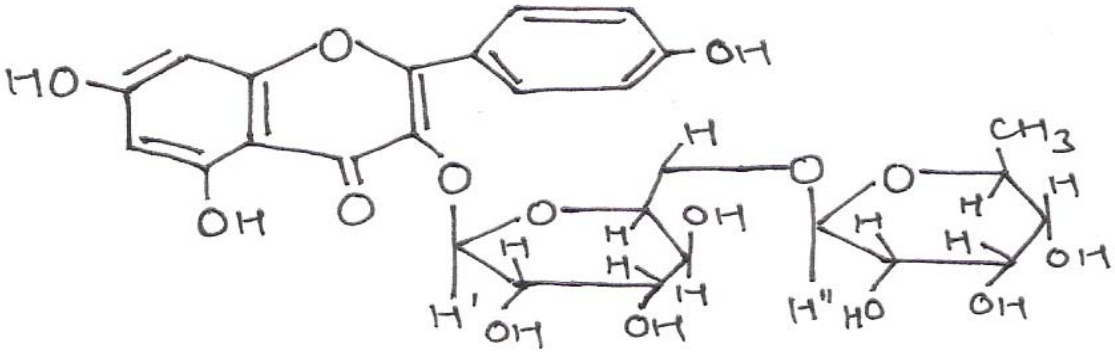
Compound (A) appeared dark purple under UV light changing to fluorescent yellow with NH3, indicating a flavone with free hydroxyls at C-4' and C-5 (Mabry et al., 1970). When cellulose TLC of Compound (A) was sprayed with NA reagent, it turned to yellow, indicating presence of p-hydroxy substituted B-ring and absence of orthodehydroxyl group in the B-ring (Homberg and Geiger, 1980; Geiger and Homberg, 1983). Compund (A) gave positive Feigl spot test for suger. Total acid hydrolysis of (A) with 2N HCL yielded rhamnose and glucose sugar and kaempferol, all identified by chromatographic comparison and spectral method. The aglycone, kaempferol was identified by 'HNMR (DMSO - d6, 400 MHz). `HNMR spectrum of aglycone showed the expected signals in the aromatic region, i.e. two meta coupled doublets each with J=2.0 H2 at δ 6.20 and 6.44, for the A-ring H-6 and H-8 protons, and two ortho coupled symmetrical doublets each with J=8.8 H2, at δ 6.94 and 8.02. Which correspond to the protons of H-3', H-5' and H-2', H-6' of B-ring. A low field broad singlet of δ 12.5, represent chelated 5-OH group of flavonoid nucleus.

Compound (A) appeared dark purple under UV light while its acid hydrolysed aglycone, kaempferol exhibited dull yellow colour on PC with UV light, indicating a release of sugar moieties from the 3-position (Sayed et.al., 1999). The `HNMR (DMSO - d6, 400 MHz) of (A) showed two ortho coupled symmetrical doublets each with J=8.5 H2 at δ 6.82 and 8.03 which correspond to the H-3', H-5' and H-2' and H-6' of the B-ring and the two meta coupled doublets each with J=2.0 H2 at 6.21 and 6.45, for H-6 and H-8, of the A-ring. Two anomeric protons at δ 5.28 (d, J=7.5 H2) and δ 4.38 (d, J=2H2) were attributed to glucose moiety (B- configuration) directly linked to the aromatic ring at the 3-position and a rhamnose (δ-configuration) linked to the 3-O-glucosyl moiety. The rhamnosyl methyl appeared as doublet at δ 110 (J=5.0 H2). The remaining sugar protons were observed in the range δ 2.98 - 4.02. The appearance of anomeric proton of rhamnose terminal sugar at δ 4.38, indicated it is attached with C-6 of primary glucose sugar moiety.

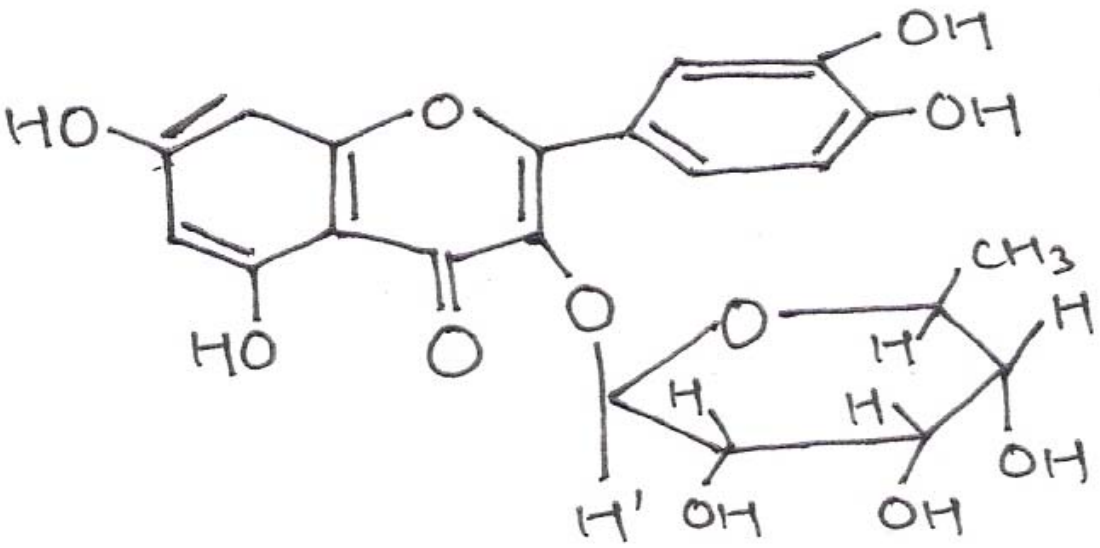
H_2O_2 oxidation of compound (A) with H_2O_2 and reacting mixture was evaporated to dryness and residue was dissolved in isopropanol. Isopropanol soluble was chromatographed on Whatman No. 1 PC using BAW (n-BuOH-AcOH-H2O, 4 : 1 : 5, V/V, upper layer) as a developing solvent. The PC was inspected with UV light. A dull yellow UV fluorescent spot on PC appeared at Rf 60 under UV light was identified as kaempferol. The chromatographic properties of it were found similar to Kaempferol, which was isolated as an aglycone from the complete acid hydrolyzed mixture of compound (A). After identification of UV fluorescent spot as kaempferol, on PC of isopopanol soluble from H2O2 oxidised mixture, the chromatogram was sprayed with benzidine reagent. The PC was dried in oven at 110^{o}C for 10 minutes. A brown spot was visualized at Rf, 13 in BAW and it was identified as rutinose sugar by CoPC with its standard. Thus, compound (A) was identified as Kaempferol-3-O-rhamnosyl (1-6) glucoside or kaempferol-3-O-rutinoside. (Structure 1).

Compound (B) a dark purple UV fluorescent on PC with UV light, was isolated from FRAC-02, a fraction derived from 10% HOAc cellulose cc fractionation of BuOH soluble. On the basis of chromatographic properties (Rf values and colour reactions with UV/NH3, UV/NA and UV/ZrOCl2 it was identified as a Quercetion-3-O-mono glycoside (Mabry *et al*., 1970; Mabry and Markham, 1975). Complete acid hydrolysis of the compound with 2NHCl gave Quercetin (COPC) and rhamnose (COPC). Thus the compound (B) was identified as Quecetein-3-O-α-L-rhamnoside. The `HNMR (DMSO-d6; 400 MH2), of compound (B), gave two meta coupled doublets each with J=2.0 H2, at 6.20 and 6.40, representing H-6 and H-8 of the A-ring. Three proton signals at 7.32 (1H,d, J=2.0 H2), 6.88 (1H,d,J=8.5) and 7.20 (1H, d,d, J=2.0 H2 and 8.5 H2), correspond to the H-2', H-5' and H-6' protons of B-ring. The anomeric proton signal appeared as doublet at 5.56 (J= 1.15) assigned to a α- rhamnose moiety directly linked to aromatic ring at 3 position. Thus, the compound (B) was identified as Quercetin-3-O-α-L rhamnoside. (Structure 2).

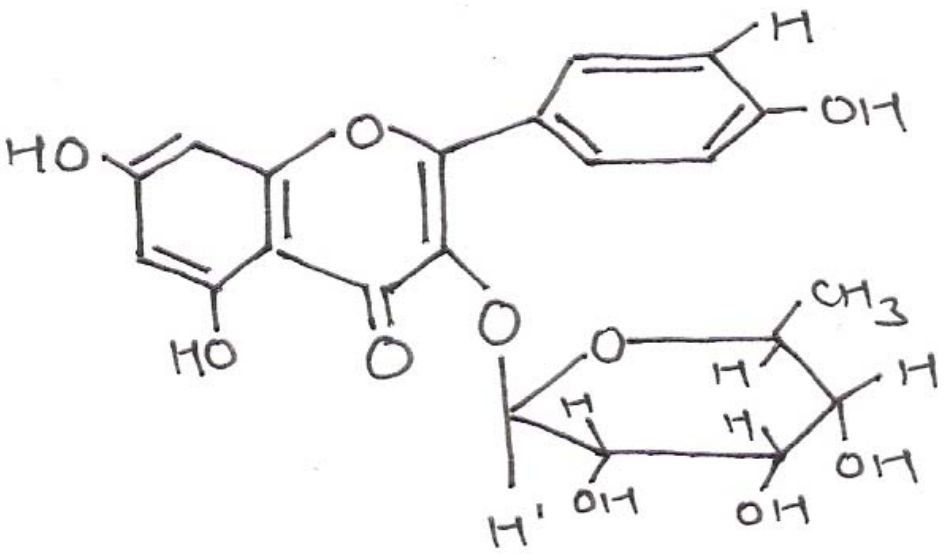
Compound (C), a dark purple UV fluorescent compound, was isolated from FRAC-02, a fraction derived from 10% HOAc fractionation of BuOH soluble on cellulose cc. On the basis of chromatographic properties (RF values and colour reations, UV, UV/NH3, UV/NA, UV/ZrOCl2), it was identified as a flavonol-monoglycoside (Mabry *et al*., 1970; Markham, 1982; Yahara *et.al*., 2000). Complete hydrolysis of compound with 2NHCl, gave a flavonol aglycone, kaempferol. It was identified by `HNMR spectra. In `HNMR spectra four doublets were observed in aromatic region at δ 6.20, 6.40, 6.94 and 8.02, which were assignable to H-6, H-8, H-3', 5' and H-2', 6' respectively. The sugar released after the acid hydrolysis of the compound was identified as rhamnose (CoPC). The compound (C) gave violet spot on PC with UV light while its aglycone, gave dull yellow UV fluorescence, indicating release of sugar moiety from 3-position (Mabry *et al*., 1970). The `HNMR of the compound (C) was found similar to the compound (B) in sugar region. Thus, the compound (C) was identified as Kaempferol-3-O-α-L- rhamnoside. (Structure 3).



Structure 1. Kaempferol-3-O-rutinoside



Structure 2. Quercetin-3-O-α-L-rhamnoside



Structure 3. Kaempferol-3-O-α-L-rhamnoside

CONCLUSIONS

The pursue for antioxidant compounds from the ethanol extract of *Bergenia stracheyi* (HK.) leaves led to the isolation and identification of two flavonol glycosides. Flavonoids are secondary metabolities widespread in plant kingdom and their occurrence in leaves has been related to the need of protection from the oxidative processes (Ribeiro *et al*., 2002). Thus flavonol glycosides isolated in this work might play a role in the protecting system in various oxidative processes system in various oxidation processes occurs in human beings.

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Responses of nurse plants on the growth of forest tree species along nutrient gradients

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Abstract: The present study deals with the influence of 'nursing effects' of actinorrhizal plants on the growth of some important Central Himalayan species. The studied species were: *Pinus roxburghii* Sarg. (chir-pine), *Quercus floribunda* Lindl. (tilonj-oak), *Cupressus torulosa* D. Don (cypress), *Coriaria nepalensis* Wall. (makoi), and *Alnus nepalensis* D. Don (alder). For this study seedling of both nitrogen fixing and target species were grown in pure culture and mixed cultures in polyethylene bags containing sand and soil mixture in 3:1 ration. The gradients of nutrient availability were developed by adding 0, 80, 160 and 240 mg /kg ammonium nitrate to the bags (as two equal doses, one at start and other during growing season). The gradients are referred to as N1, N2, N3 and N4, respectively. In all target species, seedling growth and seedling dry mass increased with increasing nutrient levels. Our experiments indicated that when individuals of different species grow together, more than one interaction may be involved and their effects change with changing environments. [Nature and Science. 2009;7(4):35-50]. (ISSN: 1545-0740).

Keywords: Nitrogen fixing, nutrient gradients, Central Himalaya, target species, biomass

INTRODUCTION

Nutrients are one of the important environmental resources for which species occurring together in a community compete with one another. Nutrient gradients are complex indirect effect if only one nutrient is used. Artificial conditions imposed in a study effects the interpretations of results and their application to field conditions. The difference between the gradient suggests neighbors affect differences in resource availability (Parrish and Bazzaz, 1982a). However, plant species do show marked differences in physiological and ecological responses along nutrient gradients, and these differences can offer valuable insights into plant community organization and competitive relations. In theory, competition should be a stronger selective factor in more persistent communities than in short lived ones, resulting in more divergence of resource use patterns in the persistent communities (Parrish and Bazzaz, 1982b). Soil nutrients are markedly higher in the oak forest (0.33 to 0.58% total nitrogen, Singh and Singh, 1986), than in the pine forest 0.15 to 0.18% total N, Singh and Singh, 1987). Singh *et al.,* (1984) suggested that the slow decomposing pine litter by promoting N-immobilization in microbes renders site nitrogen poor, and that shortage of nitrogen in combination with frequent fires has helped in expansion of pine in oak areas. The former is less nutrient demanding than the latter. Where nitrogen in soil is low in earlier stage and increase with progress of succession, the early successional (*Alnus nepalensis* and *Cupressus torulosa* D. Don.*)* species should grow more rapidly at low nutrient level than latter successional species (*Quercus floribunda* Lindl. and *Pinus roxburghii* Sarg.) (Tilman, 1982). A study on the Sierra Medre Mexico suggested that the evergreen species occurring in nutrient poor sites is out competed by the deciduous species of nutrient rich sites by competition when nutrient availability is increased (Goldberg, 1982).

The main objectives of this investigation are to (i) examine the facilitative effect of the nitrogen fixing actinorrhizal plants on important forest species of central Himalaya, and the way it is modified with changes in soil resources, nutrients and (ii) to discuss whether the species mixtures involving a facilitator species bring about enhancement in productivity.

Materials and Methods

Seedlings of *Pinus roxburghii, Quercus floribunda, Cupressus torulosa, Alnus nepalensis* and *Coriaria nepalensis* were raised from the seeds of current crop (1998) and transferred to polyethylene bags (containing 8 kg of sand: soil mixture in 3:1 ratio) of 60 x 30 cm size. It was considered that this sand soil mixture had negligible concentration of nutrients, and the amount of soil was sufficient for an unrestricted root growth of seedlings for the duration of experiment.

To analyze the nursing effect of nitrogen fixing species on growth performance of selected non-nitrogen fixing species, seedlings of both (N-fixing and non-N-fixing) were maintained in following combinations each with six replicates:

Non-N_2-fixing N$_2$-fixing	**Non-N_2-fixing N$_2$-fixing**
1. *P. roxburghii* with *A. nepalensis*	2. *P. roxburghii* with *C. nepalensis*
3. *C. torulosa* with *A. nepalensis*	4. *C. torulosa* with *C. nepalensis*
5. *Q. floribunda* with *A. nepalensis*	6. *Q. floribunda* with *C. nepalensis*
7. *Q. floribunda* with *C. torulosa*	8. *Q. floribunda* with *Q. floribunda*
9. *C. torulosa* with *C. torulosa*	10. *P. roxburghii* with *P. roxburghii*
11. *A. nepalensis*. with *A. nepalensis*	12. *C. nepalensis* with *C. nepalensis*

The polyethylene bags were moved from one place to another to avoid the self-shading of the plants. The bags were filled, each with 8 kg of sand and soil mixture in 3:1 ratio.

Ten individuals of each species were separated into their component parts and oven dried at 60°C to obtain the initial dry weight of all the six species. The experiments were performed under greenhouse conditions from October 1998 to November 2000 with temperature ranging from 5°C (minimum) in December- January to 30°C (maximum) in June.

Observations for height growth (up to shoot apex) recorded at monthly intervals. For dry mass, the plants were harvested at six months interval in the first year and finally at the end of second year. The roots were washed and the different parts of the plant (i.e. leaves, stem, roots and nodules) were separated and dried at 40°c in an oven for one week and weighed. Statistical analysis was done following Snedecor & Cocharan (1968).

After establishment, seedlings of *P. roxburghii, C. torulosa, Q. floribunda, A. nepalensis* and *C. nepalensis* were provided with four nutrient gradients in each combination. The gradient of nutrient availability was developed by adding 0, 80, 160 and 240 mg kg^{-1} NH_4NO_3 to the bags (in the form of two doses) once each growing season and referred to as N1, N2, N3, N4 nutrient gradient, respectively. Other macro and micronutrients were provided in the form of Arnon nutrient solution (Tables 1 a & b). All the nutrient levels were subjected to equal, adequate and regular water supply.

Table 1(a): Composition of Nitrogen Free Nutrient Solution (in 500 ml of distilled water each time of irrigation):

COMPOUND	**STRENGTH**
$Ca(OH)_2$	0.1 mM
$MgSO_4$	0.7 mM
MoO_3	1.88 µM
$CuSO_4$	37.5 µM
$MnSO_4$	1.0 mM
H_3BO_3	0.7 mM
K_2SO_4	0.25 mM
$ZnSO_4$	0.25 mM
Ferric citrate	12.5 µM

Table 1(b): Composition of Complete Nutrient Solution (in 500 ml of distilled water each time of irrigation).

COMPOUND	STRENGTH
$Ca(OH)_2$	0.1 mM
$MgSO_4$	0.7 mM
MoO_3	1.88 µM
$CuSO_4$	37.5 µM
$MnSO_4$	1.0 mM
H_3BO_3	0.7 mM
K_2SO_4	0.25 mM
ZnSO4	0.25 mM
Ferric citrate	12.5 µM
NH_4NO_3	18.75 µM

Results

Height growth

Height growth of 2-yr-old seedlings under different nutrient levels is presented in Fig. 2.1. In general, the height of all species tended to increase with increasing nutrient level both in pure culture and mixed culture. Across the nutrient levels, the height increment in the study period for *Pinus* was 173-313 % in pure culture, 304-411% in combination with *A. nepalensis* (hereafter referred to as *Alnus*) and 287-421% with *C. nepalensis* (hereafter referred to as *Coriaria*) (Table 2.1). The facilitative effect of symbiotic plants was measured as growth of target species individuals (oak, pine, *Cupressus*) in the presence of nurse species individuals relative to that when grown in presence of conspecific individuals. Facilitative effect of *Alnus* was maximum at the lowest nutrient level, and then tended to decrease with increasing nutrient level up to N4. Facilitative effect of *Coriaria* on *P. roxburghii* (hereafter referred to as *Pinus*) was about 30% at N1 level and then continuously decreased up to N4 nutrient level (16%) (Table 2.2). Height of *C. torulosa* (hereafter referred to as *Cupressus*) both in pure and mixed culture followed the same pattern as that of *Pinus*. Across the nutrient levels, the height increment in one-year was 866-1119% in pure culture and 986-1214% in combination with *Alnus* and 1056-1250% with *Coriaria.* Facilitative effect of *Alnus* on *Cupressus* was comparatively greater (33% at N1 level) than of *Coriaria* (25% at N1 level), and tended to decrease with increasing nutrient level (at N4 level 16% and 11% by *Alnus* and *Coriaria*, respectively). Height of *Quercus* followed the same pattern as that of *Pinus* and *Cupressus* in pure culture as well as in mixed culture. Across the nutrient levels, the height increment was 213-332% in pure culture and 264-383% in combination with *Alnus* and 264-359% with *Coriaria*. Facilitative effect of *Alnus* on oak was comparatively greater than *Coriaria.* The facilitative effect of *Alnus* and *Coriaria* was maximum at the lowest nutrient level (20% and 30%, respectively), and then tended to decrease with increasing nutrient level up to N4 (11% and 9% by *Alnus* and *Coriaria,* respectively). Response to nutrient enrichment was lower in *Pinus* than other species.

Table 2.1: Percent increment in seedling height of target species (*P. roxburghii, C. torulosa* and *Q. floribunda*) and nurse species (*A. nepalensis* and *C. nepalensis)* along the nutrient gradient in pure and mixed cultures.

Species combination	N1	N2	N3	N4
P. roxburghii with *P. roxburghii*	173	257	308	313
P. roxburghii with *A. nepalensis*	304	411.1	439	412
P. roxburghii with *C. nepalensis*	286	384	433	421
C. torulosa with *C. torulosa*	866	1128	1145	1119
C. torulosa with *A. nepalensis*	986	1344	1353	1214
C. torulosa with *C. nepalensis*	1057	1316	1345	1251
C. torulosa with *Q. floribunda*	828	1107	1136	1098
Q. floribunda with *Q. floribunda*	213	287	318	332
Q. floribunda with *A. nepalensis*	265	327	342	383
Q. floribunda with *C. nepalensis*	247	306	340	359
Q. floribunda with *C. torulosa*	116	194	209	235
C. nepalensis with *C. nepalensis*	1105	1309	806	652
C. nepalensis with *P. roxburghii*	901	986	637	496
C. nepalensis with *C. torulosa*	821	873	514	454
C. nepalensis with *Q. floribunda*	1020	1183	680	607
A. nepalensis with *A. nepalensis*	1075	901	759	660
A. nepalensis with *P. roxburghii*	738	787	586	445
A. nepalensis with *C. torulosa*	692	762	556	388
A. nepalensis with *Q. floribunda*	840	884	628	498

Table 2.2: Percent facilitation by *A. nepalensis* and *C. nepalensis* on height growth of target species (*P. roxburghii, C. torulosa* and *Q. floribunda*) along the nutrient gradients.

Target species	% facilitation by *A. nepalensis*				% facilitation by *C. nepalensis*			
	N1	N2	N3	N4	N1	N2	N3	N4
P. roxburghii	40.0	37	30	25	30.2	24.6	20.3	16.2
C. torulosa	32.0	30.0	27.0	16.0	25.0	21.3	19.0	11.0
Q. floribunda	30.0	23.0	19.1	11.9	20.4	16.9	14.1	9.3

Total seedling dry mass

Seedling dry mass of all target and nurse species along the nutrient gradient at two years of age are presented in Fig. 3.1. In all target species, the dry mass of 2-years-old seedling increased with increasing nutrient supply. However, the difference between dry mass at N3 and N4 nutrient level was insignificant.

In *Pinus*, the seedling mass ranged from 3.23-11.62 g seedling^{-1} when grown alone and from 7.45-18.48 g seedling^{-1} when grown with *Alnus*. The facilitation of *Alnus* was maximum at N1 level (130%) and then continuously decreased up to N4 nutrient level (56%). The facilitation of *Coriaria* was less than the facilitation of *Alnus*, but the response to different nutrient levels was similar. In N1 nutrient level the facilitation was 115% and decreased with increasing nutrient level N4 (58%) (Table 3.1).

Across the nutrient levels in *Cupressus* the seedling biomass ranged from 6.01-15.92 g seedling^{-1} in pure culture, from 15.16-30.28 g seedling-1 with *Alnus*, and

14.50-25.00 g seedling^{-1} with *Coriaria*. The facilitation of *Alnus* was again highest at N1 level (150%) and minimum at N4 level (88%).

In *Quercus*, the mass ranged from 2.25-9.30 g seedling^{-1} in pure culture and 6.07-10.90 g seedling^{-1} with *Alnus* and 5.40-11.00 g seedling^{-1} with *Coriaria*. However, with *Cupressus* it decreased from N1-N4 nutrient level. Facilitation effect of *Alnus* was highest at N1 (129%) and sharply declined towards the higher nutrient level N4 (19%).

In the two nurse species (*Alnus* and *Coriaria*) when grown with the other species, the dry mass increased upto N2 level, and then decreased in the higher part of the nutrient gradient.

Responses of shoot and root biomass to different nutrient levels were similar to that of total seedling dry mass.

Analysis of variance for seedling dry mass at two years indicated that difference between species, level of competition, treatment as well as their interactions were highly significant ($P<0.01$; Table 3.2) .

Table 3.1: Percent facilitation by *A. nepalensis* and *C. nepalensis* of seedling dry mass of target species (*P. roxburghii, C. torulosa* and *Q. floribunda*) along the nutrient gradients.

Target species	% facilitation by *A. nepalensis*				% facilitation by *C. nepalensis*			
	N1	N2	N3	N4	N1	N2	N3	N4
P. roxburghii	130	90	60	56	115	75	50	58
C. torulosa	150	114	94	88	141	95	59	51
Q. floribunda	169	59	21	17	129	46	25	19

Table 3.2: 3-factor analysis of variance for seedling dry mass of study species along the nutrient gradients.

Tests of Between-Subjects Effects

Dependent Variable: SEEDLING

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	53894.386[a]	76	709.137	1452.774	.000
TREATMEN	1053.052	3	351.017	719.113	.000
SPECIES	11428.544	4	2857.136	5853.278	.000
COMBINAT	389.992	3	129.997	266.319	.000
TREATMEN * SPECIES	1419.019	12	118.252	242.256	.000
TREATMEN * COMBINA	16.660	9	1.851	3.792	.000
SPECIES * COMBINAT	1972.910	11	179.355	367.437	.000
TREATMEN * SPECIES COMBINAT	161.434	33	4.892	10.022	.000
Error	74.195	152	.488		
Total	53968.581	228			

a. R Squared = .999 (Adjusted R Squared = .998)

Discussion

In the experiments based on pot-culture of seedlings, we examined the nursing effect of two nitrogen-fixing actinorhizal plants: *Alnus nepalensis* and *Coriaria nepalensis* on some important forest species, *viz., Pinus roxburghii, Quercus floribunda* and *Cupressus torulosa* of central Himalaya. While *Alnus* (alder), *Coriaria* and *Pinus* (pine) are effective colonizers of the landslide and other disturbed sites, *Quercus* (oak) is important late successional species of central Himalayan forests (Singh and Singh 1992). *Cupressus* (cypress) has restricted distribution and seldom forms a large stretch of forest on steep and eroding slopes (Troup 1921). Both oak and pine are evergreen with single year leaf lifespan, alder and *Coriaria* are sub-evergreen, and cypress is evergreen with several years of leaf lifespan. On roots of alder and *Coriaria*, the actinomycetes (*Frankia*) form nodules that are capable of biological nitrogen fixation. Both have also mycorrhizal association. These features enable these plants to thrive in nutrient-poor soils and increase their potential in forestry as nurse plant (Gautheir *et al.,* 1983).

The occurrence of *P. roxburghii, C. torulosa*, *A. nepalensis* and *C. nepalensis* species is associated with nutrient-poor sites and that of the oak with nutrient-rich sites, indicating thereby that during the succession nutrients in soil increases and with the progress of succession community biomass increases (Chaturvedi, 1983; Rawat 1983), and availability of light to seedlings declines (Singh and Singh, 1992).

In all target species, seedling growth (height, diameter and seedling dry mass) increased with increasing nutrient level. In each experiment facilitative effect of nitrogen fixers (alder, *Coriaria*) was evident, so was the competitive effect of target species (pine, cypress and oak) on it (Figs. A-F). Since the positive effect of facilitation was more than the negative effects of competition, mixtures had higher values than the average of monocultures. The facilitative effect of alder and *Coriaria* was maximum at lowest nutrient level (N1) and then continuously decreased with increasing nutrient level. However, the effect of competition of target plant on nurse plant was maximum at the highest nutrient level (N4) and decreased with decreasing nutrient level. Bertness and Callaway (1994) hypothesized that competition increases in importance toward the more productive part of the environmental gradient, whereas facilitation is more important under harsh conditions. The facilitation of *Coriaria* was less than the facilitation of alder, possibly due to its (*Coriaria*) spreading habit. Clearly, combination of species, species number and abiotic environment interact to determine ecosystem function. Our experiments indicate that when individuals of different species grow together, more than one interaction may be involved, and their effects change with changing environment.

Since alder overtops the associated species, the negative effect on alder growth in mixture cannot be due to the competition with heterospecific individual. It seems that as soil nutrients increase, alder's ability to fix nitrogen declines, and this trend is sharpened by the presence of heterospecific individual. *Coriaria* individual is less adversely affected by the presence of heterospecific individual and whatever reduction in its growth occurs is more than compensated for by its facilitative effect resulting in more growth in mixture than in monoculture (Fig. M). Our study indicates that the growth of facilitator is reduced in the presence of species facilitated and the combined dry mass production in mixed culture is increased when facilitator species is smaller and slow growing than the species as whose growth is facilitated. Thus, the impact of species diversity depends on the interaction between individuals of species and that can vary from one combination to another.

The root:shoot ratios were relatively lower towards the higher nutrient gradients (Table 4.0). Several authors have reported a decrease in the root:shoot ratio of plants treated with N-fertilizers (Hocking, 1972; Reddy *et al.* 1976; Luxmoore, 1971; Chapin, 1980), and root and shoot allocation patterns may be adjusted to alleviate imbalances in resource availability (Chapin, 1980; Zangerl and Bazaaz, 1983). Plants from infertile

habitats maximize nutrient uptake through high root:shoot ratio (Nye and Timber, 1977). Rapidly growing species from high-nutrient habitats show considerable plasticity in root:shoot ratio generally having higher ratios at low availability and lower ratios at high availability than do species from low nutrient habitats (White, 1973; Grime and Curtis, 1976; Grime, 1979). Parrish and Bazzaz (1982a) have explained the decrease in proportion of biomass in roots of perennial species with increasing nutrient concentration in two ways. First, the plants could have some sort of feedback system enabling them to reduce root growth if a small root system could provide enough nutrient and water for shoot; and second, the higher concentration of nutrients could kill the root tips, limiting root growth.

In presence of nurse plant, root: shoot ratio of target species decreased in all nutrient levels. This may indicate that nutrient supply was greater in mixed culture due to biological nitrogen fixation by nurse plant. All the target species showed greater height and mass with increasing soil nutrient concentration.

Both *Coriaria* and alder come as an early succession species in freshly exposed soil of landslide-affected sites which are often rocky and severely eroded. A relatively heavy demand for nitrogen in nitrogen-poor soil is met through biological N-fixation in alder and *Coriaria*. The production of nitrogen-rich litter and mineralization increases soil fertility. In the course of succession, these species are replaced by seral species in the natural habitats (Sharma and Ambasht, 1988). Thus, the higher fertility suppresses the growth of alder and *Coriaria* by inhibiting the nitrogen supply to these nodules forming species associated with mycorrhizal fungi. The nodule biomass declines with increasing soil fertility. The replacement of alder and *Coriaria* by target species as found in the present experiment represents the facilitation model of succession. Parrish and Bazzaz (1982b) have explained that in monoculture, individuals, very likely have similar and widely overlapping fundamental niches, and each may be affected equally by the presence of the other, because of the great similarity in their genetic identity and consequent limitation in variation in capabilities of using a given resources. However, when the individuals with similar fundamental niche are of different species, as in mixed culture, it is likely that in competition one will be considerably better than the other one at obtaining resources. As a result, under competition growth of one species is reduced. The difference in individual's weight between species was lowest at the lowest nutrient level, indicating that there were not enough nutrients present for the individuals to manifest genetic differences strongly and develop competitive hierarchies (Parris and Bazzaz, 1982). Competition between species has been shown to be more intense at higher fertility level (Mahmood and Grime, 1976; Austin and Austin, 1980). The results presented here, however, should be considered only a starting step towards the understanding of role of resources in facilitation. This study shows that competitively superior species may not only suppress the growth of its competitor, it may also realize better growth in its presence. Thus, there is a scope for exploring the usefulness of forest species "as a nurse species" in forestry practices. However, there are several limitations in this study. First, experiments have focus on a short period of the life of long-lived plants. Second, different resources as adults than as seedlings may limit plants. Third, environmental conditions in poly-houses where seedlings were grown are different from those of outside. High humidity and nighttime condensation of vapours and warmer temperatures differentiate the poly-house environment for the natural one. However, results presented here clearly indicate that they may be important determinants of the sequence of replacement of species in this region.

In this experimental study based on pot cultures, we have considered tree seedlings of the Western Himalayan forest, grown in both monocultures and mixtures of two species along a nutrient gradient. We examined whether seedling mass was greater in mixture than in monoculture and how it was modified due to variation in species growth form and soil nutrient level. The species pairs only included the species that occur together in natural communities. We assume that a mixture of two species is twice as diverse a monoculture. We considered various functional types, such as

nitrogen fixing species known for their facilitating role, and broadleaved evergreen species and conifer species, considered highly competitive in behavior (Singh *et al.*, 1984). We assume that interacting individuals of species and their interaction with the abiotic conditions influence ecosystem properties. In the Western Himalayan forest generally, interactions occur among the individuals of 2-4 tree species.

Table 4.0: Root:shoot ratio of target species (*P. roxburghii, C. torulosa* and *Q. floribunda*) and nurse species (*A. nepalensis* and *C. nepalensis*) along the nutrient gradients in pure and mixed cultures.

Species combination	N1	N2	N3	N4
P. roxburghii with *P. roxburghii*	0.598	0.442	0.401	0.392
P. roxburghii with *A. nepalensis*	0.574	0.380	0.302	0.282
P. roxburghii with *C. nepalensis*	0.570	0.363	0.329	0.317
C. torulosa with *C. torulosa*	0.522	0.491	0.432	0.425
C. torulosa with *A. nepalensis*	0.495	0.412	0.381	0.371
C. torulosa with *C. nepalensis*	0.401	0.379	0.312	0.301
C. torulosa with *Q. floribunda*	0.516	0.412	0.417	0.398
Q. floribunda with *Q. floribunda*	1.172	1.041	0.980	0.947
Q. floribunda with *A. nepalensis*	1.061	0.981	0.971	0.792
Q. floribunda with *C. nepalensis*	1.195	0.988	0.853	0.723
Q. floribunda with *C. torulosa*	0.924	1.001	1.106	0.973
C. nepalensis with *C. nepalensis*	0.669	0.478	0.387	0.398
C. nepalensis with *P. roxburghii*	0.563	0.414	0.315	0.289
C. nepalensis with *C. torulosa*	0.555	0.495	0.320	0.337
C. nepalensis with *Q. floribunda*	0.554	0.483	0.331	0.369
A. nepalensis with *A. nepalensis*	0.746	0.706	0.612	0.614
A. nepalensis with *P. roxburghii*	0.828	0.785	0.737	0.649
A. nepalensis with *C. torulosa*	0.802	0.737	0.676	0.606
A. nepalensis with *Q. floribunda*	0.791	0.714	0.665	0.687

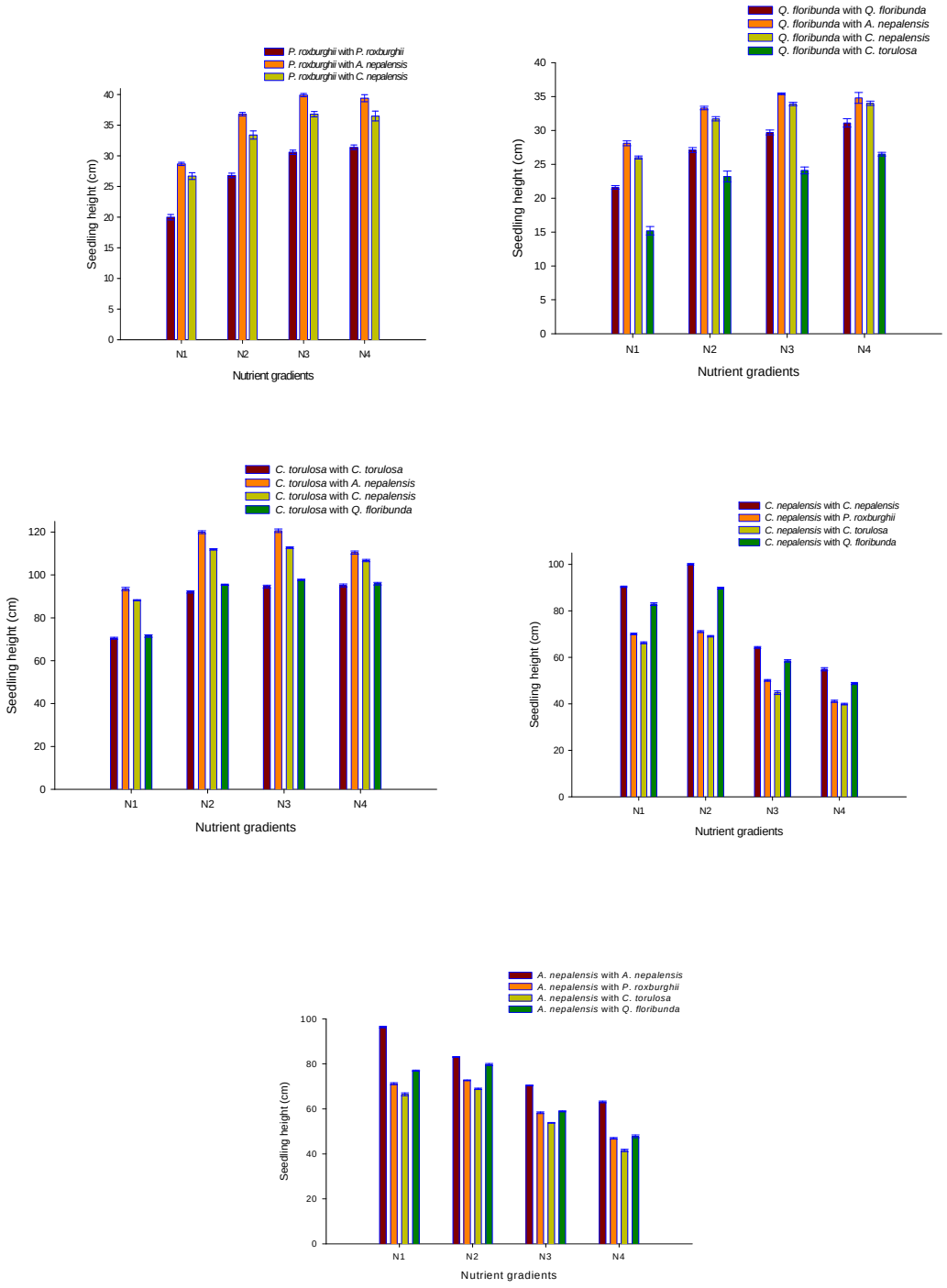
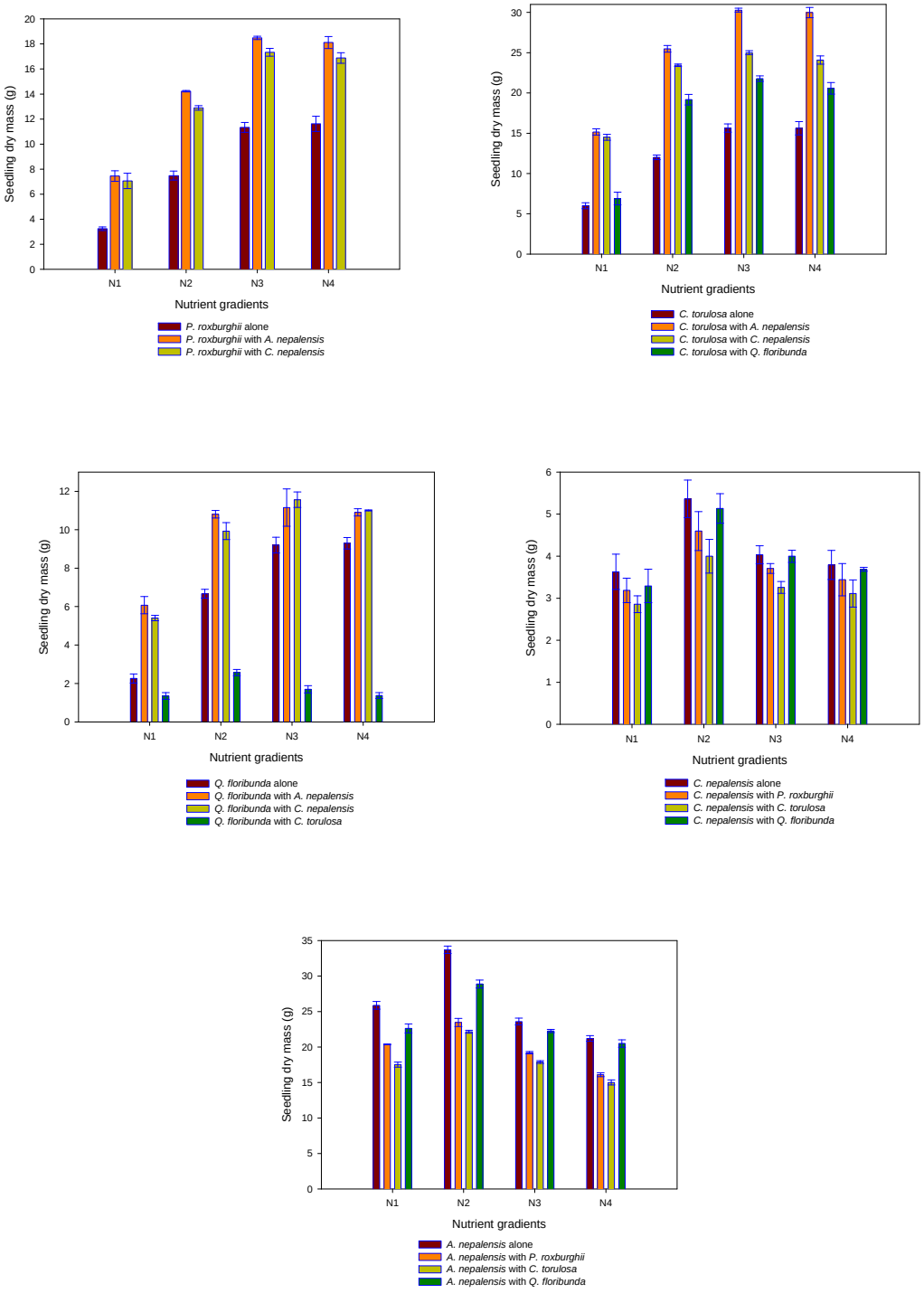


Fig 2.1: Average seedling height (cm ± SE) in different combinations of target [*P. roxburghii*, *C. torulosa*, *Q. floribunda*] and nurse species [*A. nepalensis*, *C. nepalensis*] along the nutrient gradients at yr-2.



Figs. 3.1: Average seedling biomass (g seedling^{-1} ± SE) in different combinations of target [*P. roxburghii, C. torulosa, Q. floribunda*] and nurse species [*A. nepalensis*, *C. nepalensis*] along the nutrient gradients at yr-2.

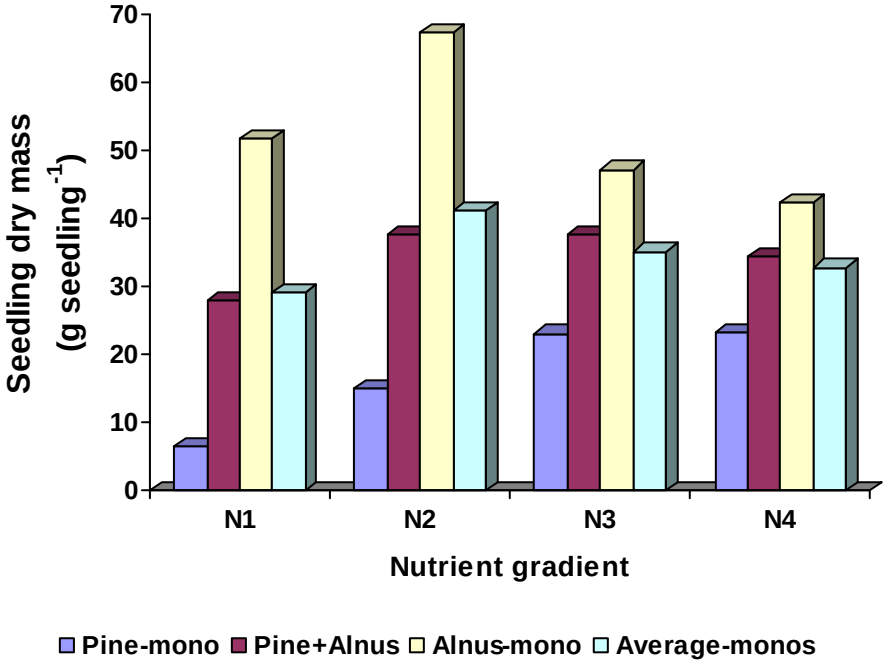


Fig. A: The sum of dry mass in mono and mixtures of pine and *Alnus* (g seedling^{-1}) along the nutrient gradients.

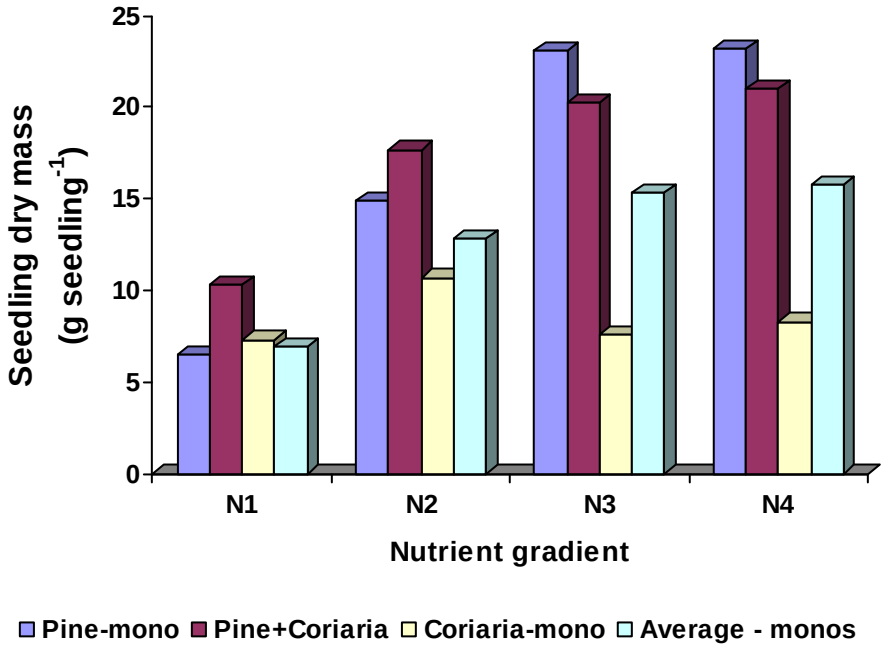


Fig. B: The sum of dry mass in mono and mixtures of pine and *Coriaria* (g seedling^{-1}) along the nutrient gradients.

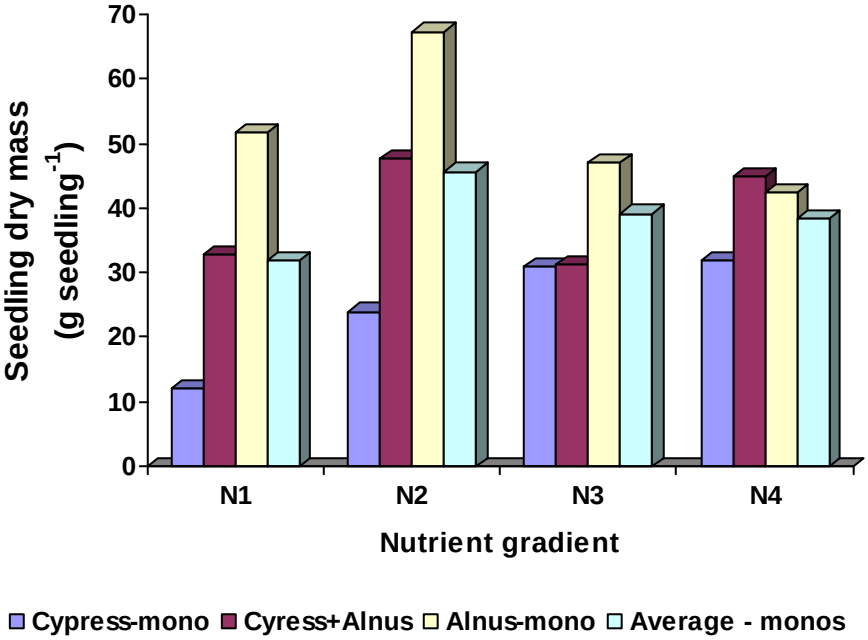


Fig. C: The sum of dry mass in mono and mixtures of cypress and *Alnus* (g seedling^{-1}) along the nutrient gradients.

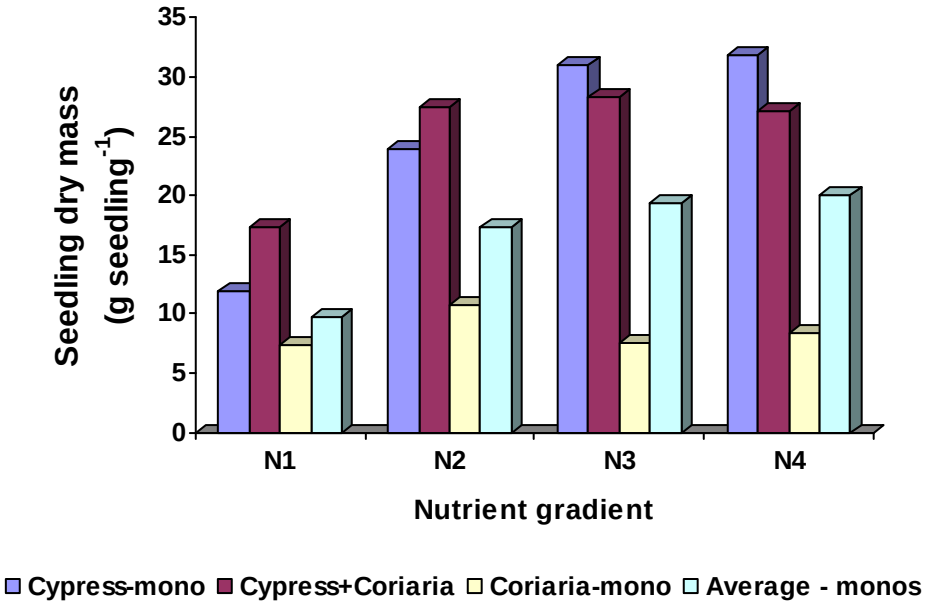


Fig. D: The sum of dry mass in mono and mixtures of cypress and *Coriaria* (g seedling^{-1}) along the nutrient gradients.

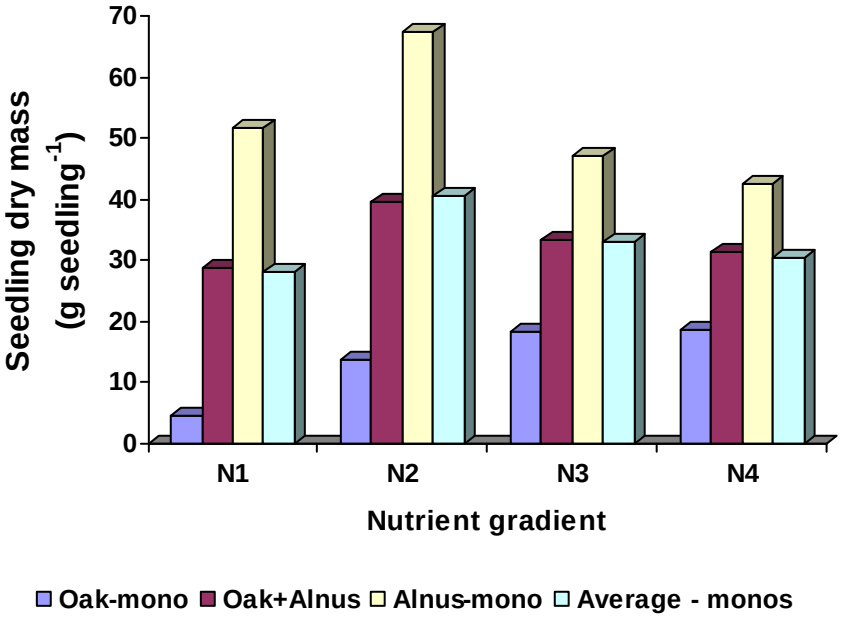


Fig. E: The sum of dry mass in mono and mixtures of oak and *Alnus* (g seedling^{-1}) along the nutrient gradients.

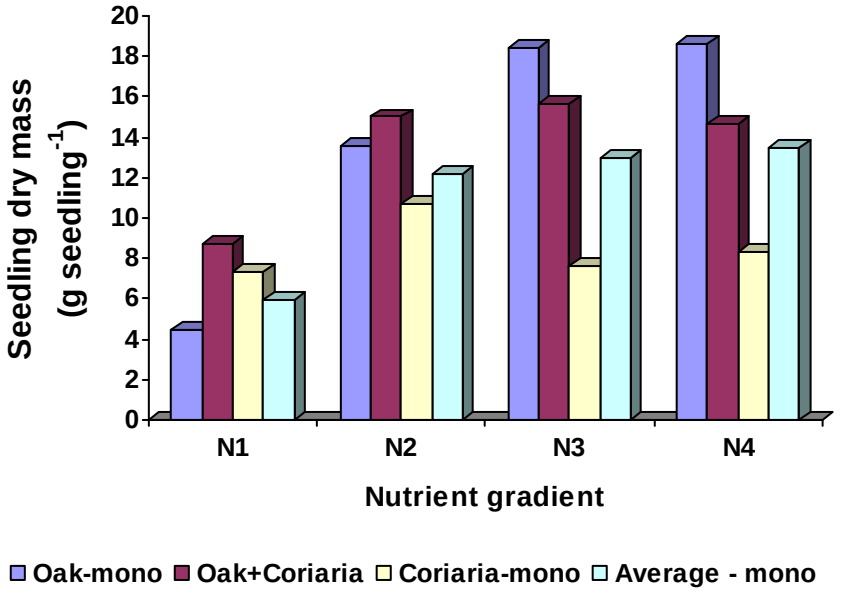


Fig. F: The sum of dry mass in mono and mixtures of oak and *Coriaria* (g seedling^{-1}) along the nutrient gradients.

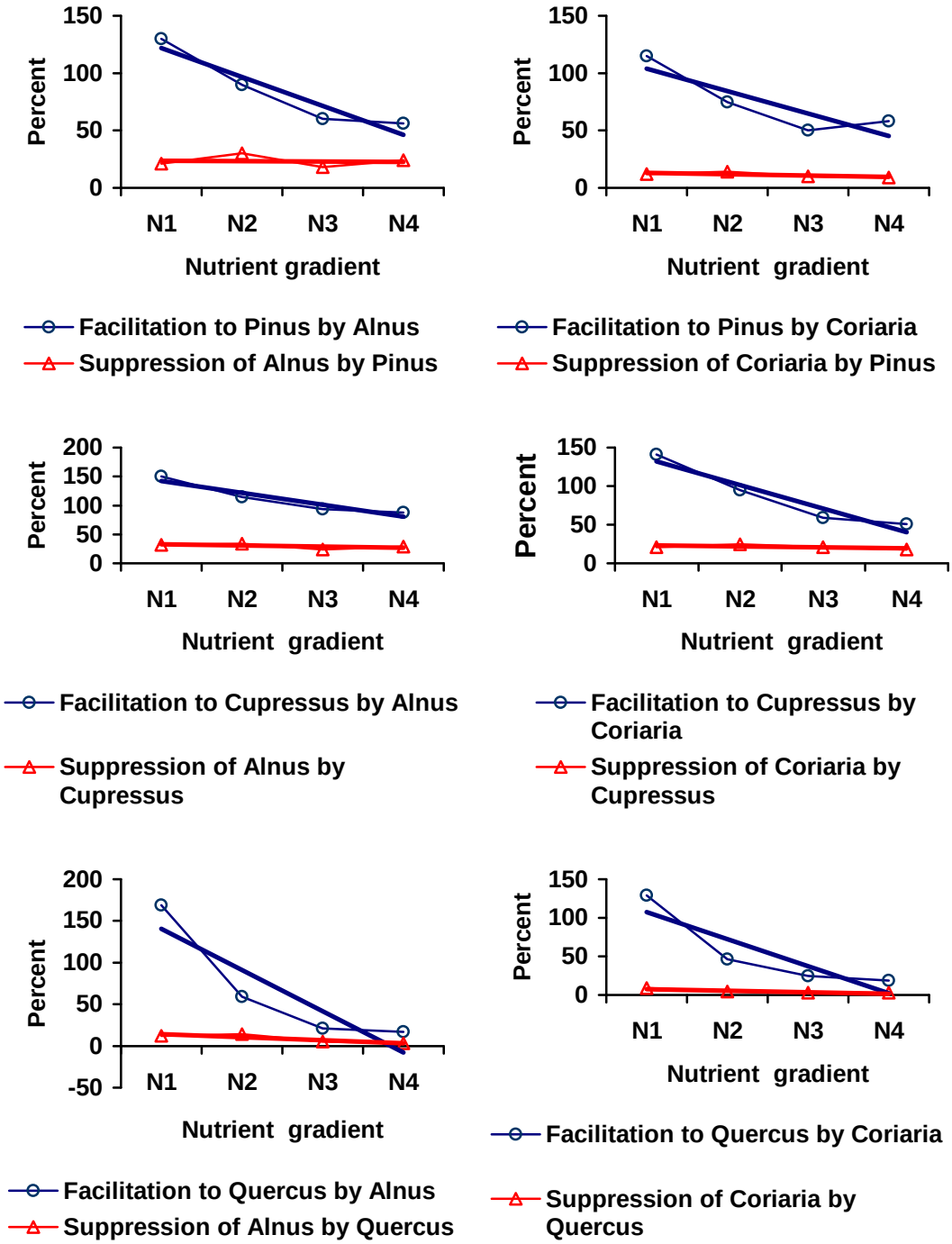


Fig. M: Values of facilitation and suppression of seedlings along a nutrient gradient (N1-N4). Facilitation was calculated as percent increase in growth of a seedling in a mixture over its growth in monoculture. Suppression is percent decrease in seedling growth in mixture over than in monoculture. In all combinations facilitation exceeded suppression, consequently the combined mass of seedlings of any two species was greater in mixture than the combined mass in monocultures.

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Polymerization in fluidics and stabilzable bio active molecular complexes of variable structures

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Abstract

Fluids are bio active molecules which exhibit special chemical and physical properties. Dielectric properties lead to multiple reactions, aggregation or polymerization. The life is a special polymerized molecular structure, environmental modification that completely depends upon concentration gradient. The transition alters the states of fluids - gels -soft matter- matter and visa versa. The nutritional elements in the life cycle of the plants and animals results several polymer structures. The P^H of plant sap and blood alters the body structural properties. Proper proportions leads to the stable structures can be standardized as good health and deviation defines hazards in health conditions. This paper sounds keen observation of natural and synthetic polymers and polymerization. Body health is directly proportional to intake of nutritional ingredients and environmental conditions. It is also depends upon molecular stress, strain, orientation, temperature with other physical properties inside & outside. Stabilization of the body & required elemental compositions specifies the environment, nature of food, medicinal buffering systems. [Nature and Science. 2009;7(4):51-57]. (ISSN: 1545-0740).

Key words: Buffer, Cell Structure, Elements, Enzyme, Fluids, Polymer

Introduction

The nature reflects its every content in terms of several states of the matter. Matter is made up of smaller units like atoms and molecules of fluids and solids. Fluids are having property of flow with bio active sensation. The proper proportion of molecular complex compounds with a chance factor is life. The life is complex molecules; exhibit the special property of growth and sensational moments with character of reproduction. Life is chance factor which is a proper combination of fundamental elements (Punchabhuta's) having history of 5000 yrs. [Vedas] civilization. Civilization is united by diversity of life and its distribution. Nature is blooming with several kinds of life unites like plants, herbs, shrubs, microorganisms, creatures, insects, birds, animals…..etc. Human beings are intellectual creature who is making best use of the nature & its products for his survival, since from the older days. Skill full way of using easily available herbs, Serbs, plants, food grains and natural resources to maintain the good condition of the body, Height to weight physic, leading spiritual satisfactory life by natural exercises like yoga, to sustain healthy peacefully longer life, is best art of living[Charka & Susrth].

This science of life is called Ayurveda. The natural food habits and spiritual thinking's, meditation, exercises like yoga are cultivated processes & recycling from older days [Veda's and Ayurveda]. All modern developments are the summary of the old ideas still requires the refreshments. Ancient concepts of the clear ideas lights up the new better intelligence and techniques. Every individual has unique in nature and their secrecy of the health lies secret only. The supreme guides of the Ayurveda are *Charka* and *Sushrut Samhitha* and both with more condensed form Asthang hridhaya. Some times Ayurveda is called the summary of the *Righ* and *Athara* Veda, because 10572 &5977 hymns explain use of herbs, serbs, anatomy. physiology, diseases & surgery etc. Sushruta was a famous surgeon. Classification of the various

diseases and critical operation at eye, nose, heart and brain etc. still making highway for the modern medicines and surgery. Detailed explanation of the intake, health hazards and remedy is a quite amazing one. After clear observation of the natural "Ayurveda" today's modern medicine and the developments in the surgery is a just few steps of progress only. Today's imbalanced chemical combination drugs, costlier surgery affecting the struggling body for the more uncertainty. Once again more persons are just looking back history of the natural treatment

Discussion

Polymerization and life

Life started with very first simple subunits as monomers, linking together forming many complex polymers. This difference in polymerization kinetics is sufficient to create patterned structures like RNA & DNA towards the protein synthesis and the cell like structure with membrane and micro-organisms by self replication. Several modification and reproduction is resulted changing in life nature. The choice of different factors like initiator, initiator concentration and light intensity directly affect the rate of photo initiation and, subsequently, the rate of polymerization.. During polymerization, the growing length of the polymer chains causes an increase in the viscosity of the reaction mixture. The increase in viscosity leads to a reduction in the mobility of the growing kinetic chains or macro radicals. The universal solvent water plays a major role in metabolic activities, supported by light, heat, wind, pressure and gravitational energies[1-2].The environmental conditions supports the life for the proper growth is called as healthy growth and ideal environment. But earth's motion and seasons enforces the physiological changes and variable metabolic activities in the life. Human beings are intellectual creatures making best use of raw nature and several adaptation skills.

pH and buffers

Patterned hydro gel structures have potential application in micro fluids[2-4] tissue engineering[5-7] and other soft matter technologies. The molecules of the fluid are acidic and basic state plays key role in every process. Hydro gels have been widely used for a variety of biomedical applications due to their biocompatibility and tissue-like physical properties[8]. Specifically, patterned hydro gels have been used to design micro fluidic devices with stimuli-responsive channels [9,10] cell-based micro arrays[11,12]. Water is nothing but H^+ and OH^- ions acts as universal solvent. The pH of aqueous solutions has main role in metabolic activities. Which is a temperature dependant directly affects the biomolecular process. The control of pH in biological systems is achieved by the action of efficient buffering systems, due to metabolic production of acids (lactic) and bases(ammonia).The pH of the blood in venous limits 7.36 to7.4 and of arterial 7.38 to7.42. The viscous fluid blood flows through the veins by capillary flow method optimizing the body pressure. For healthy body every part or the cell is having communication by special nerves and controlled by the brain. Healthy mind leads to the healthy body. The buffering systems are H_2CO_3 , HCO^-_3 HPO_4, H_2PO_4, HBO_2 etc. potentates and protein present in blood, lymph and spinal fluids & exudates. Biological fluids contains Ca^{+2}, Na^+, K^+, Cl^-, HPO_4^{2-}, HCO_3^- with several other trace elements and compounds [13,14].These ions activities determines rate of reaction and chemical equilibrium.

Influencing factors

The environmental conditions and their parameters influence the rate of reaction, mainly gravitational, electromagnetic radiations, heat, pressure, etc. The quantity and intake nature of food is directly proportional to the health .usually the fluids [15]. Colloidal & gel like mass structures accelerates the rate of reaction and liberation of the energy. The difference of concentration gradient and equilibrium position attains by diffusion, osmosis through the cellular membrane. The macro, micro and nano molecular clusters with polar molecules automatically balance the rate of reaction. The in & out flow, the intake food, quantity, form, time and environmental conditions influence the rate of reaction. Every body is having its own resistance towards the variation. Imbalanced adsorption of the polar molecules that results irregularities in health like fat formation, calcium crystals, tumors [17], excitements, high & low b.p, mental disorders and other health hazards. The extent to which an enzyme's activity is limited to a certain

substrate or a certain type of reaction is referred to as the specificity of the enzyme like superoxide dismutase, catalase – the fastest enzymes which act together to protect cells against oxidative stress.

Proteins and Enzymes

Proteins are polymers with amino acids as their monomers. Amino acids are carboxylic acids with amines attached to the carbon. There are 20 common amino acids but one amino acid (glycine) are chiral, with the alpha carbon. Those used in the body are always the L stereoisomer. Most amino acids differ only in the composition of the side chain[18]. Regardless of their many different biological functions, all proteins are chemically similar. Proteins are polymers of amino acids & have nonpolar side chains, neutral side chains. Two or more amino acids can link together by forming amide bonds, usually called *peptide bonds* when they are in proteins. This structure alone determines the overall shape of a protein molecule, whatever its size[16]. Structure of angiotensin II is a blood-pressure regulating hormone present in blood plasma. Enzymes are catalysts for biological reactions the reactant in an enzyme-catalyzed reaction is known as the enzyme's substrate to protect cells against oxidative stress. Many enzymes include nonprotein portions known as cofactors in such enzymes; the protein part is called an apoenzyme. Only the apoenzyme and cofactor together are active as a catalyst. The cofactors are either inorganic ions usually metal ions, or small organic molecules called coenzymes. Modern understanding makes it clear that most molecules are not rigid, but flexible. The E-S interaction is better described by Induced-Fit Model. Since enzymes are composed of only L-amino acids, they catalyze the reaction of only one pair of optical isomers. Lactate dehydrogenate catalyzes the removal of hydrogen from "left-handed" L-lactate but not from "right-handed" D-lactate. Enzymes must be able to respond appropriately to the constantly changing conditions as we eat, sleep, exercise, or fall ill. Any process that starts up or increase the action of an enzyme is called activation. Any process that slows down or stops the action of an enzyme is called inhibition. Inhibition of several types is important for natural enzyme control can also be utilized in medications that modify enzyme activity. Many drugs rely on enzyme inhibition for their therapeutic effect. Some inhibitors are poisons because they prevent an enzyme from carrying out a necessary function. Some molecules could bind to the enzyme's active site and thereby prevent the usual substrate from binding to the same site [7, 14, 18]. As a result, the enzyme would be tied up. The inhibitor competes with substrate for binding to the active site – competitive inhibition[20]. Competitive inhibition is reversible.

Enzyme + Inhibitor----------→ Enzyme-Inhibitor complex

If the bond between an inhibitor and an enzyme is not easily broken, the result is irreversible i.e, inhibition – enzyme cannot return to an active state. Most irreversible inhibitors are poisons e.g. heavy metals such as Hg or Pb bind covalently to the sulfur atoms in the –SH group of cysteine. Phosphate-containing insecticides such as malathion and phosphate- based nerve gases like sarin are irreversible inhibitors of the enzyme Acetyl cholinesterase (AChE).

Elemental compositions

Conservation laws of the nature always true forever. The nutrients of the plants depend upon the traces of the elements and compounds present at that place. Metabolic activities of the bio molecular cells remain same irrespective of life class. But the ingredients differ according to the polymerization. The birth of life - food cycle-development-saturation-biodegradation maintains the flow of trace elements almost constant. But the scarcity & aggregation defines the special occasion. The special aggregation of the atoms and molecules is the complex molecular polymer and dielectric properties increases in the polymerization[20]. The bio-active stabilized proper proportion biomolecular complexes are grouped broadly by common characters in plant and animal kingdoms sub groups with different class and categories for easier understanding. The following figure shows the essential trace elements present in a organisms and single cell.

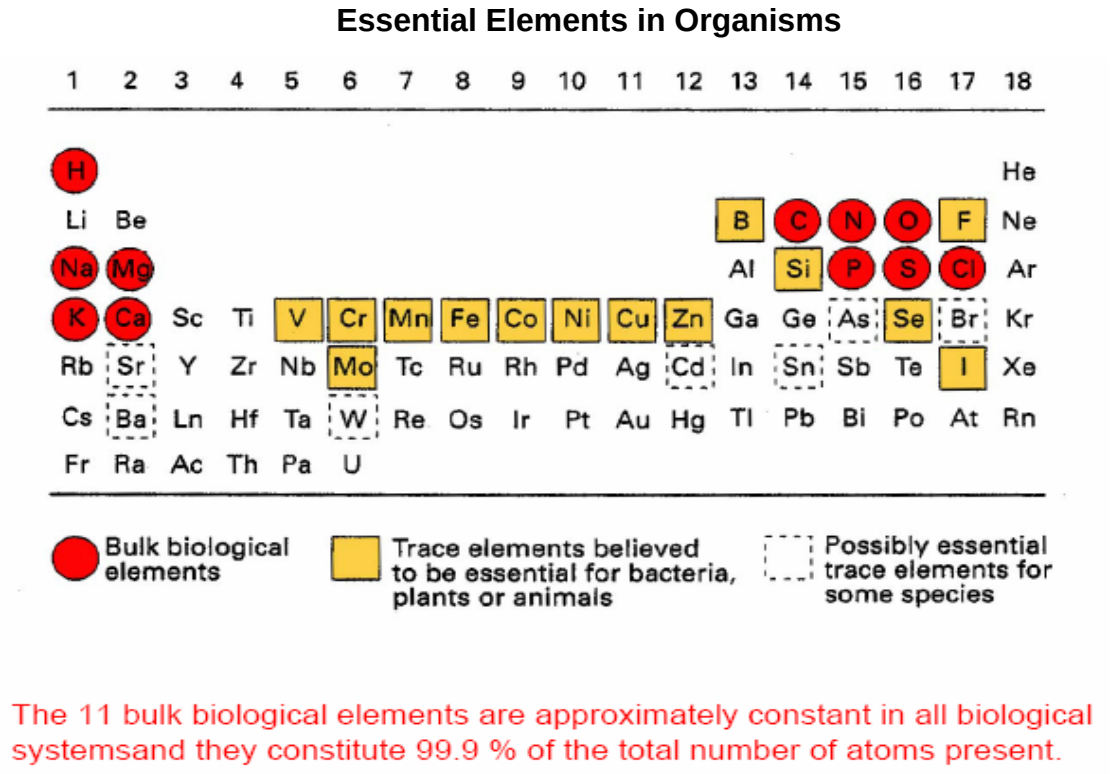


Fig.1: Essential Elements in Organisms shows different composition

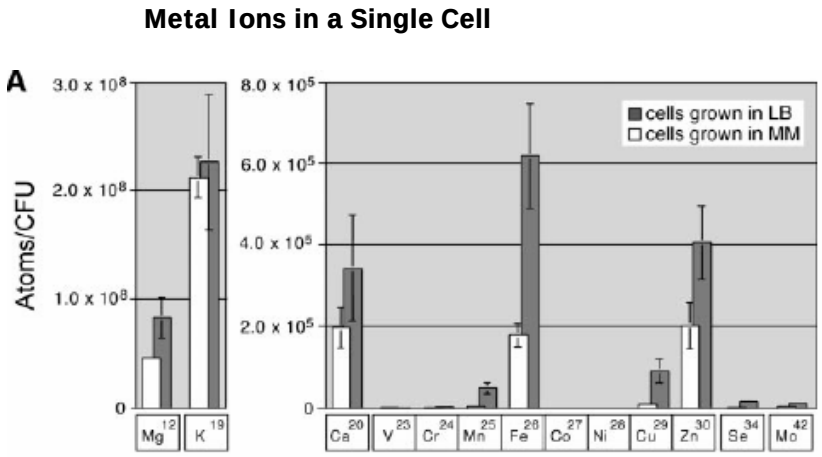
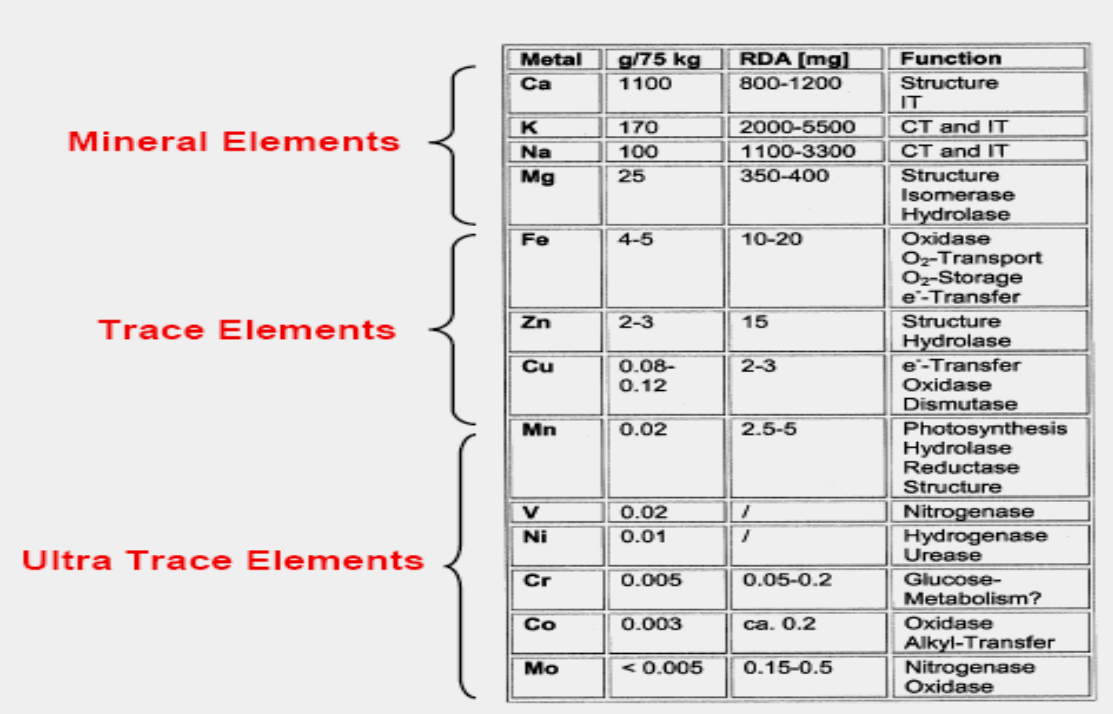


Fig.2 It is possible to detect the number of metal ions in a single cell by inductively coupled plasma mass spectrometry (ICP-MS). The results Shown above are for *E. coli*.[21].

The above two figures shows that the fundamental elemental composition of the any organisms. The red color circles specify the essential bulk biological elements in all biological systems reflect same number of atoms. The yellow square specifies the trace elements required for the bacteria, animals and plants. But

the dotted square possible essential trace elements towards limited species. The atomic configuration and developmental aspects in each tissue depends upon the dielectric properties of units and subunits of the cell structure. However the tissue engineering [7,14,18] is the very complex phenomenon cannot be predetermined. The metal ions in a single cell is detected by E.coli by ICP-MS is shown in the figure 2. The multicullular structures of proper proportional elemental composition in a cell almost remain same (E.coli). The rate of cellular aggregation with different tissues of the bodies of proper elemental composition reflects the variable structures. The constant rate of polymerization results the time dependant stable structure. But the abrupt variation rate of reaction leads to the instability and decay. However environmental influencing factors are many & some times it is a chance factor. That is why we observe the same species with different weight fractions. The figure 3 shows the elemental gram weight fractions in the 75 kg human body for specified functions.



Metal	g/75 kg	RDA [mg]	Function
Ca	1100	800-1200	Structure IT
K	170	2000-5500	CT and IT
Na	100	1100-3300	CT and IT
Mg	25	350-400	Structure Isomerase Hydrolase
Fe	4-5	10-20	Oxidase O_2-Transport O_2-Storage e^--Transfer
Zn	2-3	15	Structure Hydrolase
Cu	0.08-0.12	2-3	e^--Transfer Oxidase Dismutase
Mn	0.02	2.5-5	Photosynthesis Hydrolase Reductase Structure
V	0.02	/	Nitrogenase
Ni	0.01	/	Hydrogenase Urease
Cr	0.005	0.05-0.2	Glucose-Metabolism?
Co	0.003	ca. 0.2	Oxidase Alkyl-Transfer
Mo	< 0.005	0.15-0.5	Nitrogenase Oxidase

Conclusion

Fluids plays key role in every life process. Their dielectric properties and concentration gradient results are aggregation of the molecules. The molecular aggregation defines several structures. The polymerization is influenced by several factors. The rate of the reaction depends upon elemental composition & the environmental condition like light, wind, humidity and water that alter the concentration gradient of the fluid molecules. The fluid molecular stress and strain generates the temperature. The proper knowledge of elemental composition of nutrients, food and medicinal systems stabilizes the polymer structures. Polymers are part and partial unites of the life.

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3/2/2009

Impediments To Educational Development Of Primary School Pupils In Ogbomoso Oyo State Nigeria

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ABSTRACT: Announcement across the World today perfectly reflects the scope of the sound knowledge receives from the primary schools. Additionally, the competence of Specialists, Engineers, Scientists, Bankers, technologist, Technicians must acquire their skills formally or informally are firmly connected and rated by other foundation in the primary schools. Among the major responsibilities of primary education are to train the child ability to reason logically and develop concepts which are formulated upon basic knowledge. In this paper, we study the impediments to educational development of primary school pupil in Ogbomoso, Oyo state - Nigeria. The study revealed that there. [Nature and Science. 2009;7(4):58-62]. (ISSN: 1545-0740).

KEYWORDS : Impediments, Primary School Pupils, Educational Development

INTRODUCTION

Comments and Statements about the state of our schools indicate that the standards of education have fallen (Fagbamigbe, 1986 and Lawal, 1996). Many references have been made to English Language and Mathematics as prerequisites to success in overall examination. Those who point to the controversial issue possibly implies that the performance of Public Schools in Nigeria is declining when compared with past standards. In fact, an individual observation of primary school pupils in Oyostate unveils the traces of dividing educational quality. Questions are now being asked about what could be the cause of falling standards of education. (Joseph S. Owoeye, 2000).

Evidence has shown that Foundation is very important in a building. A building with a weak Foundation may likely be a bad building which cannot with stand tension. The building that is good is the one whose foundation is solid. This analogy can be applied to educational development of a child. Primary education is the foundation of any educational attainment in life. The quality of education obtained by a pupil at primary school would determine the performance of such a student at secondary and tertiary levels. Additionally, the competence of Specialists, Engineers, Scientists, Bankers, Technologist, and Technicians who acquire their skills formally or informally are firmly connected and rated by their Primary education foundation. Among the major responsibilities of Primary education are to train the child the ability to reason logically and develop concepts which are formulated upon basic knowledge.

It is important to have it in mind that Primary education is the foundation for subsequent education and training, academic and vocational and for some people, preparation for modern economic effort. Thus Primary education must lay the foundations for an industrial and agricultural labor force in which the most common skill is that of functional literacy and also provide a sufficiently rigorous preparation for more advance level of education.

According to Akinboye (1980) in his book titled “Psychology of Discipline in Contemporary Nigerian Education System” stated that the aims of primary education in Nigeria today are to train the children physically, intellectually, morally and spiritually.

Ukeje (1979) in his own book titled "Foundation of Education" pointed out that the aims of primary education is to make it possible for one to live as full and as happy a life as possible.

The most important reason for that is to help one to learn and to appreciate things in life- good books, art, health, law, government rending source etc. rather than pursuit of money for its sake and for the power that it brings.

STATEMENT OF PROBLEM

It is an undisputed fact that Nigeria needs Improvement in the educational development of primary school in Oyo state. Why does the country need that, one may ask? To the question, a good answer is to improve or solve the impediments to educational development of primary school pupils in Oyo state- Nigeria.

The present state of primary education system as compared with the past has assumed a declining trend, which is to the dissatisfaction of the general public; therefore the need arises as to look into the impediments to educational development of primary school pupils in Oyo state- Nigeria. Attempts were therefore made to test the following hypothesis.

Ho_1 ÷ There is no significant difference between family socio-economic status and educational development of primary school pupils.

Ho_2 ÷ There is no significant difference insufficient funding of primary school and educational development of primary school pupils.

Ho_3 ÷ There is no significant difference between lack of professional guidance and counseling service and educational development of primary school pupils.

PURPOSE OF THE STUDY

The purpose of this study is to look at the impediments to educational development of primary school pupils in Oyo state- Nigeria, and make suggestion(s) on how to improve and promoting educational development of primary school pupils. Also advocate convincing the policy makers.

METHODOLOGY

The investigation of the study was carried out ex-post facto. A multi-stage probability proportion to size (MPPS) sampling technique was used to determine the number of schools to be chosen as sample. These schools spread across Ogbomoso North and South local government area in Ogbomoso, Oyo state-Nigeria. Five primary schools were selected in each local government to carry out the study. Secondary data were collected from 10 schools five each from the two local governments: [Ogbomoso North and South local government] The Schools were randomly selected on the basis of the following formula:

$$S = \frac{L}{O} * \frac{M}{I}$$

Where:

S = Number of sample from the selected Local Government Area.
L = Number of primary schools in the each Local Government.
O = Total number of primary schools in Ogbomoso Township.
M = Maximum number of schools to be sampled.

We critically examine and have the following outcomes from the questionnaires that were given to the respondents. Out of 150 questionnaires 100 were returned.

Table 1: Distribution of Respondents by their Personal Characteristics

Variable	No of Respondents	% Scored		
Age:				
Below 20	-			
21 - 30	18	18		
31 - 40	52	52		
41 - Above	30	30		
Total	**100**	**100**		
sex:				
Male	39	39		
Female	61	61		
Total	**100**	**100**		
Marital Status:				
Single	11	11		
Married	89	89		
Divorced	-	-		
Widow/Widower	-	-		
Total	**100**	**100**		
Religion:				
Christianity	64	64		
Islam	36	36		
Traditional	-	-		
Free Thinker **Total**	-	-		
	100	**100**		
EDUCATION:				
Sec. Schl. Cert.	1	1		
Grade II Cert.	3	3		
N.C.E./ OND	90	90		
B.Sc /B.A / B.Ed	3	3		
HND	3	3		
M.Ed / PhD	-	-		
Total	**100**	**100**		
Occupation:				
Trading	-			
Teaching	100	100		
Civil Servant	-			
Clergy	-			
Others	-			
Total	**100**	**100**		

What constitutes to impediments of educational development of primary school pupils in Oyo state, Nigeria, can be discussed on there main points

- **ON THE PART OF THE GOVERNMENT**
-

1. **Increase in the number of primary school pupils / poor renumeration / irregular promotion:** According to Aderounmu and Eliametatlor (1983) in their book titled "An Introduction to the Administration of School in Nigeria" asserted that increase in the number of primary school pupils without corresponding increase in the basic infrastructure vis-à-vis teachers, poor renumeration vis-à-vis irregular promotion, salaries and allowances not promptly paid at months end, and all contributing factors impending education development of primary school pupils.
2. **Lack of educational teaching equipment / materials:** On the 10th Nov, 1992, Nigeria Daily Newspaper, page 4 enumerated some causes of falling standard of pupils' educational development in primary school nowadays. It claimed that the schools lacked necessary equipments including school furniture .The paper agreed that there are instances when parents were forced to contribute money to buy Chalks, desks, benches and other minor teaching materials for use in school, describing the situation as dangerous to the future of education and pupils educational development in general.
3. **Government educational policies:** The introduction of educational policy like, continuous assessement . According to W.O Aderounmu et al; in the book titled " Nigerian Certificate in Education series published for the Ondo state College of Education Ikere – Ekiti, observed that the continuous assessement programme introduced by the Government requires much time and devotion on the part of the teachers.
4. **Lack of personnel:** The process of assessement is characterized by metriulous keeping of records.when there is shortage of special personnel to handle records keeping,
5. **Re - evaluation of School Curriculum:** The Curriculum in our school need to be re – evaluated and the government the Government need to look into the improvement of the Curriculum.

- **ON THE PART OF THE PARENT**

1. **Socio – economic Status of Parents:** According to Chapman he said that "the socio – economic status can be judged by income, occupation, education, culture and the standard of living of an individual in a society"

In 1973, Ogunlade conducted a reseach on the family socio – economic status and educational development of some pupils in Western State of Nigeria. His findings showed that Children from literate homes had educational achievement than those from illiterate homes.

2 **Non provision of educational material by Parents**: Many Parents fail to provide necessary school materials like School Uniform, Text books, Exercise books, Pens and Rulers. Failure to have these, the pupil may be Sent out or going to the classroom lately, he will surely miss some subjects taught while he was not in the class. Take home assignments are generally given to pupils from their textbooks and pupils without such textbooks will either not do them or will do well at the end of the examination, this factor impedes educational development of such pupils.

- **ON THE PART OF THE PUPIL(S):**

1 **Absence from school:** Pupil constant and proper attendance at School Constitute on improvement factor which influence his educational development both nature and academic. Such Pupil would have enough time to prepare himself for Classes more so text and assessment. Rate of School attendance serve as a dominant factor impending educational development of primary school pupils.

2 **Failure to do assignment:** Failure of the pupil(s) to be doing the assignment given to them serve as impediment to educational development of primary school pupil(s)

3/2/2009

Flavonol glycosides from antioxidative activity guided fractionation of aqueous-ethanolic extract of *Bauhania retusa* Roxb.

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ABSRACT: *Bauhania retusa* Roxb., a traditional medicinal and leguminous fodder plant of Kumaun hills, is widely distributed in sub-tropical and terai regions of Himalaya ranging altitude from 3000ft. to 4,500ft. Leaves of the plant have widely been used for curing cough, bronchitis, diuretic, diabetes, dysentery, sores, liver-dysfunction, inflammation, ulcers, piles and skin diseases by various tribal folks of Kumaun Himalaya. Leaf flavonoids derived from various food and fodder plants have widely been recommended for curing diseases related to oxidative stress. The n-BuOH fraction from aqueous-ethanolic extract (1:1) of the leaves of *Bauhania retusa* was fractionated on cellulose cc using 30 to 50% HOAc. All the dark purple fluorescing bands observed on cc were eluted and collected separately by monitoring with UV light. The combined fraction derived from the elute of dark purple fluorescing bands gave antioxidative activity against the methanolic solution of DPPH free radical in UV-VIS spectrophotometer at 515 nm. The flavonol glycoside, Quercetin-3-O-rutinoside, Kaempferol-3-O-α-δ-rhamno-pyranosyl-(1→2)-β-D-glucopyranoside-7-O-α-δ-rhamnopyranoside, Quercetin-3-O-α-δ-rhamnopyranosyl (1→2)-β-D-glucopyranoside-7-O-α-δ-rhamnopyranoside and Quercetin-3-O-β-D-glucosyl(1→2)glucoside-7-O-β-D-glucoside were identified from antioxidative active fraction of aqueous-ethanolic of aqueous-ethanolic extract of *Bauhania retusa*. [Nature and Science. 2009;7(4):63-65]. (ISSN: 1545-0740).

Key Words: *Bauhania retusa* Roxb., antioxidative activity, flavonol.

INTRODUCTION

Bauhania retusa Roxb., a member of Caesalpionioideae group of leguminous phanerogamic plant, is a tree bearing broad entire leaves, white-pinkish flowers and brown-yellow pods. Genus *Bauhania* comprises 300 species with cosmopolitan in distribution. Most of the *Bauhania* species of Kumaun Himalaya have been reported as a traditional medicines for curing number of ailments like diabetes, inflammation, snake bite, dysentery, sores, liver disorders, ulcers, piles and skin diseases by various ethnic groups (Chopra *et al*., 2002; Pande and Jain, 2002; Singh, 2002). Flavonoidal compounds, a diversified group of polyphenolic heterocyclic compounds and integral constituents of food and fodder plants, have widely been used for diseases related to oxidative stress like diabetes, strokes, arthritis, cancer, inflammation and antibiotic. *Bauhania* extracts have widely been identified as antioxidants and are rich source of flavonoidal compounds (Rahman and Begum, 1966; Gupta *et al*., 1980; Kumar *et al*., 1990). Many flavonol glycosides have been obtained from some fodder species of *Bauhania* (Salatino *et al*., 1998; Yahara *et al*., 1994).

MATERIAL AND METHODS

1. **Authentification of plant species**: *Bauhania retusa* was identified by Prof. P.C. Pande, Department of Botany, Kumaun University S.S.J. Campus Almora Uttarakhand, India. The leaves, pods and twigs bearing flowers have been deposited (Vouchar speciman No. 40) in the Chemistry Department of Kumaun University SSJ Campus, Almora Uttrakhand.
2. **Extraction and Separation of Flavonoids**: The leaves of *Bauhania retusa* was collected from Lohaghat (District Champawat of Uttrakhand) sites of Kumaun. About 3 kg air dried powered leaves of *B. retusa* was extracted sequentially with 70% EtOH and 50% EtOH by cold percolation for many days. The two extracts were filtered and combined and evaporated to dryness under reduced pressure at 70^0 in Rota evaporator until H_2O layer remained. It was partitioned with CH_2Cl_2 and BuOH successively. The BuOH soluble was fractionated on cellulose cc (Merck) using H_2O-AcOH as an eluent. On eluting cc with 30% HOAc and 40% HOAc, a number of dark

purple fluorescing bands were observed and were eluted and collected separately by monitoring cc with UV light. All elutes derived from dark purple fluorescing bands were combined and concentrated under reduced pressure. The residue was chromatographed on Whatman No.3 PC using BAW (n-BuOH-AcOH-H2O, 4:1:5, V/V, upper layer) as a developing solvent. Three dark purple fluorescing broad bands observed on PC under UV light at Rf 47, 40 and 36, representing FRAC-01, FRAC-02 and FRAC-03 respectively.

3. **Antioxidative active screening**: Each fraction was examined for antioxidative activity by the standard thin layer autobiography methods (Cuendet *et al*., 1997 and 2000).
   TLC thin layer plate of FRAC-01, 02 and 03 were spotted and developed with CHCl3: MeOH (3:1). The dried and developed plates were sprayed with methanolic solution of DPPH free radical. Three antioxidative active yellow spots were observed on TLC of FRAC-01 while no antioxidative active spots were observed with FRAC-02 and FRAC-01.
4. **Isolation of flavonoids from antioxidative active FRAC-01**: The eluate of FRAC-01, an antioxidative active fraction, was chromatographic on Whatman No.1 PC using repeated development with BAW (4:1:5, upper layer). Two major dark purple fluoresent bands were observed on PC at Rf 56 and 48 and were cut and eluted separately with 70% MeOH. The elute of faster moving band gave compound (1) and three compound (2-4) were isolated from slower moving band by sephadex LH-20 cc using 40% MeOH as an eluent.

RESULT AND DISCUSSION

Compound **(1),** gave a molecular ion at m/z 609 $[M - H]^-$ in FAB-MS (-ve) and other prominent ions identified at 463 $(m/z\ 609\text{- rha})^-$ and 301 (m/z 463-gluco), indicating successive elimination of terminal rhamnose and glucose from Quercetin. It was further supported by complete acid hydrolysis of (1) with 2NHCl and gave Quercetin (CoPC), rhemnose (CoPC) and glucose (CoPC). The compound (1) was identified as Quercetin-3-O-rutinoside by comparison of spectroscopic and physical data with authentic sample or the reported values in literature.

Compound **(2)** gave a molecular ion at m/z 739 (FAB-MS -ve) calculated for $C_{33}H_{40}O_{19}$. It gave positive tests with Mg + HCl, $FeCl_3$ and α-naphthol. Acid hydrolysis of 2 with 2NHCl gave Kaempferol (CoPC), galactose (CoPC) and rhamnose (CoPC). H_2O_2 oxidation of 2 affored a flavonol glycoside 2(a) and robinose sugar (CoPC), indicating the robinose sugar is attached at C-3 of Kaempferol. The compound 2(a) was identified as Kaempferol-7-O-α-δ-rhamnoside (CoPC). Hydrolysis of 2 with α-rhamnosidase gave Kaempferol 3-O-β-D-galatoside (CoPC) and rhamnose (CoPC). Partial acid hydrolrsis of 2 with 0.1N HCl gave Kaempferol-3-O-galactoside-7-O-α-δ-rhamnoside and Kaempferol-7-O-α-δ-rhamnoside (CoPC). On the basis of FABMS and hydrolytic methods the compound (2) was identified as kaempferol-3-O-α-δ-rhamnosyl (1→6)-galactoside-7-O-α-δ-rhamnoside. The structure of 2 was further supported by `HNMR (in DMSO-d_6, 400m Hz).

`HNMR showed two meta coupled and two ortho coupled symmetrical doublets in aromatic region at (δ) 6.48 (1 H,d,J = 1.0Hz), 6.81 (1Hk,d,J = 1.0Hz), 8.14 (2H,d,J = 8.5Hz) and 6.93 (2H,d,J = 8.5Hz) represent H-6, H-8, H-2`/6` and H-3`/5`, respectively of Kaempferol. Three anomeric proton signals at δ5.53 (1H, d, J = 7.5Hz), 5.10 (1H, d, J = 1.0Hz) and 5.60 (1H, d, J = 7.5Hz) for C-3-glucosyl, rhamnosyl attached to C-2`` of C-3-glucosyl and rhamnosyl, C-7 respectively. Two methyl signals appeared at 0.90 (1H, d, J = 6.0Hz) and 1.22 (1H,d,J = 6.0Hz) for H-6``` and H-6```.

Compound **(3)** gave a molecular ion, at m/z 755 (FAB-MS -ve) and other prominent ions identified at m/z 609 (m/z 755-rham.), 447 $(m/z\ 609\text{-glaco.})^-$ and 301 (m/z 447-rham.), supporting the release of two moieties of rhamnose and one molecule of galactose from Quercetin. It has further been supported by complete acid hydrolysis of (3) with 2NHCl and gave Quercetin (CoPC), galactose (CoPC) and rhamnose (CoPC). The `HNMR of sugar region of (2) was found similar with the sugar region of compound (3). Thus, the compound (3) was identified as Quercetin-3-O-α-δ-rhamnopyranosyl (1→6)-galactoside-7-O-α-δ-rhamnopyranoside.

Compound (4): FABM (-ve) of (4) gave a molecular ion at m/z, 747 $(M-H)^-$ and other prominent ions at 463 $(m/z\ 747\text{-sopnrose sugar})^-$ and 301 $(462\text{-gluco})^-$ represent release of three moiety of glucose from Quercetin. Complete acid hydrolysis of (4) gave Quercetin (CoPC) and glucose (CoPC). H_2O_2 oxidation of compound (4) afforded Quercetin-7-O-β-D-glucoside (CoPC) and sophorose sugar (CoPC). Thus, sophorose sugar was released from C-3 of Quercetin. The compound (4) was identified as Quercetin-3-O-sophoroside-7-O-β-D-glucoside. `HNMR of (4) in DMSO-d6 (400 MHz) gave five doublets in aromatic region at 7.69 (1H,d,J = 2.0, 8.5, Hz), 7.51 (1H,d,J = 2.0 Hz) and 6.80 (1H,d,J =

8.5 Hz), 6.74 (1H,d,J = 2.0 Hz) and 6.41 (1H,d,J = 2.0Hz) represent H-6`, H-2`, H-5`, H-8 and H-6 respectively of Quercetin. Three anomeric proton signals appeared at 5.80 (1H, d, J = 7.5Hz), 5.16 (1H, d, J = 7.5Hz) and 4.67 (1H, d, J = 7.5Hz) represent 3-glucose-H-1``, 7-glucose-H``` and 2``-terminal glucose H-1``` respectively. Thus compound (4) was identified as Quercetin-3-O-β-D-glucosyl (1→2) glucoside-7-O-β-glucoside.

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Capacity Building And Training Requirement For Effective Fisheries And Aquaculture Extension In Nigeria - A Review

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ABSTRACT: This paper examines the importance of capacity building and training requirement for the benefit of fisheries and aquaculture extension in Nigeria. It involves the analysis of secondary data bothering on the management of agricultural extension in Nigeria over time vis-avis various agricultural development programmes in the past in which fisheries and aquaculture extension was apparently de-emphasized or neglected at the implementation phase. It is therefore recommended as a way forward towards actualizing the Unified Agricultural Extension System objective that a favorable reform in the Nigerian agricultural extension system will be a necessary avenue to put fisheries and aquaculture extension in proper perspective for effective service delivery which will be a good entry point to achieving the millennium development goals of combating hunger, extreme poverty and attainment of food security in Nigeria. [Nature and Science. 2009;7(4):66-71]. (ISSN: 1545-0740).

Keywords: Aquaculture, capacity building, training, extension

Introduction

The educational and knowledge status of most extension staff fell short of much desired goal of bringing about rural and agricultural development through qualitative and committed extensions service. Yet this grade of extension workers constitutes the contact point between the extension service and farmers in the rural areas. Knowledge is a product of field experience and consistent training over time. These have not been forth-coming as the inability of the extension outfit to actualize such important incentives has been hinged on various reasons which hovers on inadequate funds, lack of willpower on the part of the past governments, misplaced priority, official mismanagement and general lack of commitment and sacrifice on the part of some extension agents. The predominance of poorly trained extension staff upon whom many categories of farmers depend has not been able to achieve a great deal of success in rural development where agriculture is the major occupation. Agricultural development constitutes a complex network of interrelationship amongst all the agricultural sub sectors including fisheries, livestock and crop agriculture. It is therefore important that concerted efforts be put in place by all concerned stakeholders in agriculture for improved capacity building necessary for effective service delivery. This paper specifically aimed at discussing capacity building in the fisheries subsector of agriculture vis-avis the extension - training requirement for the fisheries extension officers. Analysis of secondary data was adopted and attempt was made at assessing of the status and potential fisheries and aquaculture in Nigeria, make an overview of past efforts aimed at promoting agricultural development in relation to fisheries and aquaculture in Nigeria and finally discuss the extension training requirement of the extension staff while effort was made to suggest suitable modalities in line with the policy of Unified Agricultural Extension System as a way forward to achieving result oriented fisheries and aquaculture extension in Nigeria.

Status and Potential of Fisheries and Aquaculture in Nigeria

The land, water and fisheries resources of Nigeria are quite encouraging, a coastline of about 800km and vast sea area of about 256000 square kilometers. The first 300km western coast is blessed with inter-connecting lagoons while the renaming 5000km coastline consists of deltaic channels of River Niger and its tributaries. The country has extensive ecosystem estimated to cover over 0.5 million hectares (Tobor, 1993). IFAD report (1998) further observed a favourable hydrology of the country dominated by two major river systems, the Niger and Benue into which over 370 smaller rivers covering about 10.8 million hectares. Similarly Nigeria is blessed with Lake Chad along with 12 other man-made lakes with considerable surface area and a diversity of fish resources which is capable of yielding more than half a million tones of fish annually if fully developed (Tobor, 1993). The greatest yet untapped potential however lies in aquaculture, which is capable of supplying up to 1.3 million tones of fish from the vast areas of perennial fresh and brackish water swamps (IFAD, 1998).

Although the contribution of fisheries to the G.D.P is small (3-4%), it occupies a very significant position in the primary sector providing employment for over five hundred thousand people and contributing to over 40% of the animal protein intake of the people particularly the resources poor. Tobor (1993) observed that national demand for fish is well over a million metric tons of which about 360,000 tones is produced locally while about 40,000 tones is imported annually leaving a short fall in excess of a quarter of million tones. This is in contrast to an estimated potential production of 1.83 million tones. It is self evident that the full realization of the agricultural potential of any country be it developed or developing economies depends on the country's information management capabilities. In Nigeria, the management of agricultural (fisheries, crop, livestock or forestry) information is saddled on the extension department of the Agricultural Development Programme (ADP) under the aegis or the Federal Ministry of Agricultural and Rural Development. The concept of the Agricultural Development Project is premised on the fact that interrelated factors comprising the right technology, effective access to physical inputs and effective market strategy are essential to modernizing any aspect of Nigeria's agriculture. The commitment of the government to this concept led to the establishment of ADP in every state of Nigeria including Abuja, the Federal Capital Territory (FCT) (Akpoko, 1993)

It must be noted however, that allied to the establishment of the ADPs, the Federal Government of Nigeria experimented on many related programmes aimed at boosting food production in the country. Foremost among these are:

- River Basins Development Authority (RBDA**)** This programme was started in the mid 1970 specially designed for identifying and developing water resources of the river basins, which are important for supporting and promoting agriculture. It had an extension component, which gives advisory service to the farmers in the project areas. The programme was criticized for over emphasizing adhoc extension operations with inadequate research-extension-farmer linkages, which led to weak, and vague technical recommendations.
- The National Accelerated food production Programme (NAFPP) launched in 1973 with specific emphasis on the production of six basic food crop-cassava, maize, millet, rice, sorghum and wheat. In its operationalization, it involves extension, research and agro-service components.
- Operations Feed the Nation (OFN). The Federal Military Government launched this in 1976. In principle, the OFN emphasized self-sufficiency in food production especially in crops and livestock agriculture. It was criticized however for achieving little in practice as it was shrouded in excessive propaganda (Akpoko, 1993).
- Green Revolutions Programme (GRP). The Shagari Administration launched this programme in 1979 and it was aimed at achieving self-sufficiency in food production within five years. The extension component of the programme was faulted for spending much time in input distributions at the expense of the educational function.

Given the brief expositions of the operationalization of the various program above, a critical analysis of each of the past government efforts shows that the entire extension component lacked commitment to fisheries development. Fisheries and aquaculture have always been lumped up with other sub sectors of agriculture on paper in the formative period while little is done on fisheries extension in practice. The development has led to mismanagement of our fishery resources in most of our water bodies due to lack of effective co-ordinations and inadequate information for proper resources utilization by the fisherfolks. Similar trend applies to culture fisheries, which are known to be the greatest untapped potential of about 1.3 million tones of fish annually for the country. It must be emphasized that this is only realizable with proper planning in favour of effective extension activities beyond the present epileptic level where everything is left at the mercy of government

Present Status of Fisheries and Aquaculture Extension in Nigeria

History has shown that the first major attempt at promoting fisheries extension in Nigeria dated back to the colonial days when fisheries development division in the agricultural department was created in 1945 (Mijidadi and Arokoyo, 1988).

Within this period extension activities concentrated on improving the efficiency of fishing gear, craft and post harvest handling. At this time, the use of nylon fishing net, hooks, cement made sinkers, ropes, buoys and flags, improved processing and cement anchors were actually in vogue, and indeed

marked the beginning of improved fishing technologies introduced in the 1960s (Bolorunduro and Bukar, 1989).

The post colonial period witnessed the creation of the National Accelerated Food Production Programme in 1973 which paid lip service to fishing development, in favour of crop agriculture and the Special Fish Development Project of the Directorate of Food, Roads and Rural Infrastructure (DFRRI) introduced in 1986 by Babangida Administration. The formation had impact on capture fisheries through the provision of input at subsidized rates, training in the use of mechanized canoe, advocated the change from the use of dug-out canoes to medium-sized on-shore fishing vessels and within the first four years of the later, the project was reported to have published four extension guides to arouse public interest in fish farming (Akpoko, 1993).

At present, the sole agency saddled with the responsibility of transferring fisheries and aquaculture technologies from research to farmers in Nigeria is the Agricultural Development Programme, which began as an integrated agriculture development project in enclave areas in the mid 70s. The ADP utilizes the on-farm adaptive research approach as a strategy for effective transfer of agricultural technologies in the country through its extension officers (Okoye, 2000). The adoption of Unified Agricultural Extension System (UAES) through a resolution passed by National Council on Agriculture (NCA) in 1990 created a new concept, which supposedly prepare the extension agents/officers to have scheduled, and regular contact with farmers and solely deliver messages on crops, livestock, agro forestry, land management and fisheries. The extension services delivery in the country before this NCA resolution on adoptions of UAES, had been on paralled arrangement characterized with bringing assorted message to the farmers by different groups, a situation which was criticized from confusing the farmers due to lack of co-ordination.

The question therefore is, to what extent has the UAES been effective in the transfer of fisheries and aquaculture technologies? It is practically evident that it requires high degree of commitment to personnel training and intensified capacity building for any extension agent to be a catalogue of knowledge covering the wide and diverse disciplines as mentioned earlier. The ADPS have the difficult task of ensuring that the added responsibility needed by every extension agent in order to meet the expectation of Nigerians and specifically fish farmers and fisherfolks on this concept of unified Agriculture extension services is realized. The implication is that more money has to be expended on recruiting, training and retraining of officers couple with adequate and periodic monitoring and evaluation for desirable result to be achieved in fisheries /aquaculture extension.

Training Requirement for Fisheries and Aquaculture extension officers.

The concept of Unified Agricultural Extension System (UAES) which put additional intellectual and technical responsibility on the change agents has created the urgent need for building more capacity with regard to the extension personnel through recruitment of relevant people of varying qualifications and subjecting them to training and retraining. This implies that more funds would have to be sourced by the extension outfit in order to liaise with training institutions such as the universities for specialized training needs of extension officers in fisheries/aquaculture. In a similar vein, specific institutions and other local centers could be established to handle the training of village extension officer who often make scheduled contact with artisanal fishermen. For the benefit of efficiency and cost effective extension operations specialized training institutions such as National Agriculture Extension Research Liaison Services (NAERLS) and the Agriculture and Rural Management Training Institute (ARMTI) could be equipped to give regular training on fisheries extension/aquaculture management for higher officers. This is important for promoting knowledge, skills and positive attitudinal change among extension agents. Specific areas requiring improvement though training in the fisheries/aquaculture extension include:

1. Technical Know - how
2. Clientele's problems Diagnostic ability
3. Techniques of communications
4. Management and administration of extension.

Categorization of Training Required by Fisheries/Aquaculture Extension Officers

For effective training of fisheries and aquaculture extensionist the following categorization is recommended.

1. Advanced training for senior extension officers.

Officers in this category include Subject Matter Specialist (SMS) and Agricultural Extension Officer (AEOs). These officers are first degree or HND certificate holders with formal training in agriculture but have nevertheless not been given the right training for their job. This training category has a systematic approach such as follows:

a. **Special Supplementary courses**:

This is necessary to augment the residual knowledge of the trainee. The training could be planed and implemented in close cooperation's with the fisheries administration and sponsors of fisheries and aquaculture development programmes, similar to this training is the Monthly Technology Review Meeting (MTRM) of Agriculture Development Programme (ADPs). It is a type of training that places special emphasis on practical work rather than theoretical orientation.

b. **Induction course**:

This course is required for the fisheries/aquaculture extensionist in the following ways;

i. It introduces the new officer to the social-economic circumstances in the development region e.g fisherfolks communities
ii. Informs him about the aims of past and present development activities and difficulties encountered by the development institutions, which could be socio-economic, cultural or religious.
iii. It arouses the officer awareness of the relevant institutions, superior officers and the subordinates.
iv. Familiarizes the officer with his range of work, his responsibilities, and the rules and procedures that he must follow.
v. Motivate the officer to approach his/her work with positive attitude.

c. **On- the Job training**

After the induction course the fishery extensionist can take full responsibility of his job with regular supervision from experienced colleague who visit and counsel him on regular basis. He could also attend short duration course monthly for he can exchange ideas with other officers on problem encountered.

d. **Continuous Further Training**:

This is an additional training the extension officer receive while doing his/her job in order to improve his qualifications for his present or future work. Area of focus under continuous further training includes;

i. Refresher course:-These are aimed at refreshing the memory and to bring the participant extension officer up to date in order to cope with the content and method of the extension programmes. These courses are good opportunities to remedy deficiencies are in training that has become apparent during the season but also to introduce new, positive experience in his work.
ii. Special Courses:- These are carried out at irregular intervals when further training on a specific topic becomes necessary for a successful fisheries/aquaculture extension e.g. fish disease control or water quality management. These courses usually deal in detail with fish production techniques, pond management or out break of parasites and diseases in fishpond that need urgent solution.
iii. Seminars: seminars involve the fisheries extension trainee participating and tackling problem himself or herself and then working out solutions together. Expert may make little contribution while seminar leader uses his skills to guide the discussion, structure the contribution of individuals and direct them toward solutions. Expert may intervene where necessary.

Seminars are valuable in boosting the technical know-how of the extensionist because they involve active learning that result is productive solutions to problems of strategy, planning and formulation of idea. It also encourages team work cooperation and reduction of bureaucratic behaviour in extension organization.

iv Extension conference: All fisheries extensionist should be allowed to attend annual conferences of extension officers where they discuss past and future extension programme and also exchange ideas.

e. Further training aboard: where adequate level of training is not available in the country, training of our fishery and aquaculture extensionist should be allowed and sponsored for overseas training if they are professionally and personally suitable and are prepared to continue to work in their field and the organization on return. It is also important that the substance and aims of further training can clearly be defined in consultation with the training centre abroad

In addition to the above training needs all fishery/aquaculture extensionist must undergo basic training in extension methodology in order to enable the officers to:

- Analyse the socio-economic conditions of fisherflolks and fishfarmers.
- Identify people of influence and communication structures
- Spot obstacles to fisheries and aquaculture extension and eliminate them
- Apply the principle of free discussion
- Form groups to be counseled.
- Use a variety of extension methods s are teaching aids

Conclusion

Agricultural extension in Nigeria is an old development strategy for the agricultural sector. It has long been used to manage agricultural information in Nigeria with specific reference to the transfer of technological innovations to farmers in crop, livestock, forestry and ideally fisheries and aquaculture. Fisheries and aquaculture extension have not had a fair share of this development policy for Nigerian agriculture over time probably due to inadequacy in prioritization, which seemingly created the observed long and secondly, the relative newness of aquaculture in Nigeria compared to crop and livestock farming. This situation is further worsened by the inadequate funding systems of the Agriculture Development Programme (ADPs), which creates epileptic periods of activity and dormancy in the organization. This implies that if urgent actions are not evolved and more productive and result oriented reform operationalized in the Nigerian extension systems which will cover all relevant aspect of agriculture, fisheries and aquaculture would still be the neglected subsector suffering misplaced priority in the scheme of things.

Unified Agriculture Extension Systems of the ADPs seemed to have facilitated unification of message transfer to farmers but much still need to be done in the area of capacity building and added training requirement for the extensionist especially in fisheries and aquaculture. This will require more funds, more planning and more coordination on the part of the government, relevant institutions, individual stakeholders and donor agencies. This will go a log way in making fisheries and aquaculture research and information management a rallying point in the development of Nigerian agriculture

Recommendations

In order to use fisheries/aquaculture management as an entry point to achieving the millennium development goals of combating hunger and reducing poverty in Nigeria, proper management of the extension component of fisheries is imperative. This task is achievable through effective training and retraining of fishery/aquaculture extensionist for good performance.

Cost effective and efficient fisheries/aquaculture extension is only achievable through adequate funding and sufficient fund could be sourced for this great challenge thought the following ways;

i. Funding of extension management and administration should not be left only at the mercy of the federal government. Both state and local governments should be clearly involved constitutionally so that ambiguities in the level of involvement do not arise when finding is to be provided.

ii. Stakeholders should be properly coordinated and all institutions requiring the service of fishery extensionist should facilitate linkage with appropriate institutions for extension training in the needed areas.

iii. Private organizations, donor agencies and interested individuals should be encouraged to assist government in promoting fishery and aquaculture extension through the provision of fund in sufficient magnitude.

iv. The federal, state and local government should facilitate the establishment of Agricultural Extension Trust Fund for which fisheries and aquaculture should have a fixed percentage. This action point should be supported by law from the National Assembly with further encouragement from the state houses of assembly of all the 36 states.

v. Aquaculture and fisheries training centres should strengthen the aspect of extension training which involves major courses that is capable of enhancing practical competence of extension workers through the use of latest proven technologies in fisheries

vi. Extension officers should be encouraged with necessary incentives and *morale* boosters in addition to salaries in order to create a better sense of belonging along side other civil servants.

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Effect of Seed Size on Quality within Seed Lot of Pea and Correlation of Standard Germination, Vigour with Field Emergence Test

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Abstract: Seeds originating from the same seed lot contain different seed sizes and can affect seedling establishment, growth and yield. The objective of the present investigation was to observe the effects of seed size on seed quality within seed lot of pea crop. One commercial seed lot of pea cultivar (*Pisum sativum*) was size graded into three categories: large (seeds retained on a 2.36 mm screen), medium (seeds retained on a 2.0 mm screen) and small (ungraded). For statistical analysis, a completely randomized split plot design with twelve replicates (four replicates for each size grade) was used. Mean 1000 seed weight for each category was 250 g, 164 g and 126 g respectively. The graded seeds were tested for standard germination, vigour and field emergence. Large and medium size seeds have high seedling survival, growth and establishment under unfavourable condition, i.e. under field condition than small seeds. From the result of the AA test it can be suggested that to use as a carry-over seed it would be suitable to use the medium (average) size seeds. These findings support the hypothesis of larger seeds those have superior performance to small seeds as the relation was found significantly positive. It also concluded that a seed lot cannot be expressed as vigour by testing a sample of a lot as the lot consists of different sizes of seeds. [Nature and Science. 2009;7(4):72-78]. (ISSN: 1545-0740).

Key words: Correlation, field emergence, large seed, seed vigour, unfavourable condition.

Introduction

Quality seed increases the productivity of agricultural crops and always pay dividend to the growers. Among the quality attributes seed size and seed weight invariably expressed as seed density which is the most important. The seed size indicated the amount of reserve food supply for seedlings. Small and shrivelled seeds do not contain as much food to give the plant a vigorous start as the bold and plumby seed. In pea the pod produced earlier has larger in seed size than those produce later.

Seed size is the main factor that affects seeding rates. Environmental variation affects the seed size and results in production of smaller seeds under unfavourable conditions. Seeds thus produced affect the germination seed vigour, field stand and processing recovery (Dighe and Patil, 1981). The possible effect of seed size on germination is associated with the length of the structures that form the seedling, but not necessarily with the subsequent biochemical conversion of storage reserves into germinating tissues (Soltani *et al*. 2002). Wood *et al.* (1977) reported that the seed size does not affect the yield "per-se", but better field stand with vigorous seedlings helps to perform ideally under the existing environment. Bigger seeds are associated with greater seed vigour and germination (Maranville and Klegg, 1977; Kalingrayar and Dharmalingam, 1980, Dighe and Patil, 1981) in sorghum. Often farmer retained seeds, lacked seed vigour reflected in the seedling emergence, seedling vigour and seedling biomass (Marcroft *et al*, 1999).

Large seed size significantly increased seedling survival compared to small seed size by 25%. Results indicated that the post-sowing compaction and increased seed size may benefit seedling survival (Rohitha *et al*, 2004). The use of differing seed size physical parameters as discriminating criteria for seed among varieties and different species has been previously reported (Dehghan-Shoar *et al*. 1998; Illipronti *et al*. 1999; Keefe, 1999). Nerson (2002) showed that small muskmelon seeds had the lowest percentage germination, emergence, and the lowest seedling growth demonstrating that there is an association between seed physical parameters and seed quality.

The lack of relationship between seed size and germination could be explained by the more important influence of embryo size or weight; in general, larger seeds have larger embryos, which is associated with

increased germination (Lopez-Castaneda *et al*. 1996). Further, Mian and Nafziger (1994) showed that different seed sizes influence the water potential and, therefore, the speed of germination. That difference can also affect the uniformity of germination and subsequent seedling development (Seiwa and Kenji, 2000) that causes a higher germination percentage in seeds with greater size, even though this may not necessarily occur with smaller seeds (Liu *et al*. 1993; Adkins *et al*. 1996; Duval and NeSmith, 2001). As seed size is a widely accepted measure of seed quality by different workers, study was conducted to determine whether seed size really affects seedling establishment and to its' vigour. Therefore, present investigation aims to observe the effects of seed size on seed quality within seed lot of pea crop.

Materials and Methods

The experiment was conducted on a commercial seed lot of pea cultivar (*Pisum sativum*), collected from local farming area of Uttarakhand (India). The seeds were graded into three categories: large (seeds retained on a 2.36 mm screen), medium (seeds retained on a 2.0 mm screen) and small (ungraded). Mean 1000 seed weight taken for each category was 250 g, 164 g and 126 g respectively. The graded seeds were tested for germination, vigour and field emergence. For statistical analysis, a completely randomized split plot design with twelve replicates (4 replicates for each size grade) was used.

Test Weight: The seed sample with highest seed weight (1000 seed weight) was considered as vigorous. The size of the seed was graded from a sample taken randomly of a seed lot.

Sand Method (SM): Four replications (40 seeds to each replicate) for each seed size category were planted on Petri plates. Plates were placed in a germination chamber at 25ºC for 7 days. Normal and abnormal seedlings were assessed for percentage germination (ISTA, 2008) and dead seeds recorded.

Between Paper (BP): The seeds were germinated for 7 days in between two layers of papers by loosely covering the seeds with an additional layer of germination paper and placed on germination trays. Same temperature and condition were given as on the sand method. Assessments were also conducted according to the ISTA norms.

Accelerated Aging (AA): The dry seeds are subjected to accelerated aging test; stress of high temperature 42^0 C and near 100% relative humidity for 72 hours. For this four replicates for each size grade, 40 seeds to each replicate, were distributed over an aluminium screen in germination plastic boxes. The boxes were filled up with 40 ml of distilled water and kept in a germination chamber and then seeds were placed for germination test (ISTA, 1996).

Seedling Length (SL): Length of 5 normal seedlings grown in moist towel paper kept at optimum temperature (25^0C) at 45^0 was measured in cm on the day of final count. Separate measurement was taken for shoot and root. The sample showing maximum mean seedling length was considered as vigorous.

Seedling Dry Weight (SDW): The weight of seedling for each replicate was taken in gm, excluding the cotyledon on 8^{th} day after oven drying at 100^0C for 24 hr and noted. The sample exhibiting the maximum seedling dry weight was recorded and considered as vigorous.

Seedling Growth Rate (SGR): Twenty seeds were placed in straight line on a paper towel moistened with distilled water and kept at an angle of 45 in a germinator at a temperature of 25^0C. Only eight competitive normal seedlings were selected for observation and remaining seedlings were removed. For the next 5 days the length of each seedling was measured daily in cm. Seedling growth rate was determined by dividing the mean increase in length from each previous measure by the number of days the seedling had been in the germinator. Sum of each count at the end of the test period is expressed as seedling growth rate (Copeland, 1976).

SL1/F1 + (SL1 – SL2)/F2 + ... + [SLn – SL(n-1)] / Fn

Where, SL1 mean seedling length at first count
SL2 mean seedling length at second count
SL1-SL2 mean increase in length in second count

F1 days to first count
Fn days to final count

Field Emergence (FE): Field experiments were carried out at the field Research Centre of Seed Science & Technology: 40 seeds to each replicate were manually sown in 1.5 m long and 0.25 m apart rows at 3 cm depth. Four plots were prepared for each seed size grade. Emerged seedling counting and evaluation was carried out when the seedlings showed well characterized apparent plumule over the soil surface.

Statistical analysis (Correlation coefficient): The data obtained from different test parameters were subjected to the Pearson correlation coefficient analysis. It is the mutual association between variables without implying any cause and effect relationship. In this analysis, simple correlation coefficients were computed between pair of characters using the SPSS program.

RESULTS AND DISCUSSION

Test weight v/s Field emergence: Test weight exhibited significant positive correlation with mean field emergence and negative correlation with standard germination tests (Table 1). Seed size was strongly correlated with days to germination; smaller seeds germinated faster than larger seeds. The time from sowing to emergence was significantly related to different seed sizes. Small seeds may be expected to imbibe water faster than large seeds, so small seeds may germinate faster. However, large seeds have more nutrients stored within the seeds so their plants may be expected to grow taller than plants from smaller seeds.

Between papers, Sand method v/s Field emergence: Mean maximum germination percent in between paper and sand test was recorded in small seed grade which was in contrast to field emergence percent (Table 1). But small seed grade reveals significant positive relationship between replications of germination and seedling emergence [Table 2(A) & 2(B)] in both the methods which contradicts the observations of Nerson (2002), Lopez-Castaneda *et al*. (1996). The findings on standard germination tests support the report of Calton and Edgar (1971). Moreover, mean experimental finding values (Table 1) apparently confirm that increase seed size affect decrease germination index, seedling length with enhance mean germination time which support the results of Kaya *et al.* (2008).

Accelerated aging v/s Field emergence: In the entire replications of different seed size grade, accelerated aging exhibited significant positive correlation with field emergence [Table 2(C)]. The mean maximum germination (90%) after accelerated aging was recorded in medium grade (Table 1) which showed that vigour test values can be directly used to predict field emergence. It also indicated that it has the property to retain its viability for prolong time period which support the findings of Aguiar (1979) and Rohitha *et al*, (2004), that average seed size may increase seedling survival than large and small seed size. The findings also support the hypothesis that large seeds have superior performance to small seeds or that small seeds have lower vigour under unfavourable conditions.

Seedling length v/s Field emergence: The Seedling length recorded from medium seed grade shows significant negative association with field emergence. But positive association was found in small and large size grade [Table 2(D)]. The mean maximum seedling length was recorded in small and medium grade with same result (Table 1). It explains that initial seedling size and length is positively related to seed size; the result was consistent to the reports of Moebenburg (1996), Kaya *et al.* (2008), Soltani *et al*. (2002). Improve seedling vigour by large-sized seed in field emergence and accelerated aging has been discussed by Murray *et al*, (1984).

Seedling dry weight v/s Field emergence: A significant negative correlation was found in medium size grade and significant positive relation in large and small seed grade [Table 3(E)]. But maximum mean seedling dry weight was recorded in medium grade followed by small and large (Table 1) which showed relationship with accelerated aging test value. The analysis showed that dry matter content is a component of seedling vigour. Faster dry matter component accumulation allows plants to better tolerate many stresses. But when the seedling vigour was concerned average or medium size seeds showed the high seed vigour and longevity which was in accordance of the report of Woodstock (1976) and Khare & Bhale (2000).

Seedling growth rate v/s Field emergence: A significant negative correlation was found with respect to seed size and mean field emergence (Table 1). This result can equate the report of Komba *et al*, (2007) that large seeds do

not have superior performance to small seeds. The relation between seedling growth rate and field emergence test also supports the findings of Adkins *et al*. (1996). The lowest mean field emergence percentage was recorded in small seed and findings were consistent with those of Karivarhadaraaju *et al*. (2001) and Liu *et al*. (1993). Murray *et al*, (1984) found improved seedling vigour by large-sized seed and consistent result was found in field emergence and accelerated aging.

Table 1. Mean values of four replicates of different tests parameters with respect to different seed size grade. The values are expressed on the basis of number of normal seedlings after analysis. Test weight is expressed on mean 1000 seed weight.

Seed size grade (mm*)	TW (g)	BP	SM	AA	SL (cm)	SGR	SDW (g)	FE
Large (>4.5)	250	32.00	33.50	26.00	5.0	0.65	0.225	36.50
Medium (>3.35 & <4.5)	164	36.00	34.00	36.00	6.4	1.76	0.276	33.50
Small (<3.35)	126	38.00	36.00	28.50	6.4	2.03	0.231	25.50

Acronym: TW = Test weight, BP = Between paper, SM = Sand method, AA = Accelerated aging, SL = Seedling length, SGR = Seedling growth rate, SDW = Seedling dry weight, FE =Field emergence,
*size of aperture of the screen

Table 2. Correlation coefficient analysis by Spearman's 1-tailed method using SPSS program. The analysis is based on the values of replications of respective seed sizes. Relationships were analysed between Field emergence with Between paper (A), Sand method (B) Accelerated aging (C) Seedling length (D) Seedling dry weight (E). [Note: (**.**) Cannot be computed because recorded variables (values) are independent, but they are considered as no relation.]

Seed Size Grade		**Between Paper**	**Field Emergence**
Large	Correlation Coefficient	1.000	.738
	Sig. (1-tailed)	.	.262
	N	4	4
Medium	Correlation Coefficient	1.000	-.500
	Sig. (1-tailed)	.	.500
	N	4	4
Small	Correlation Coefficient	.	.
	Sig. (1-tailed)	.	.
	N	4	4

(A)

Seed Size Grade		**Sand Method**	**Field Emergence**
Large	Correlation Coefficient	1.000	.389
	Sig. (1-tailed)	.	.306
	N	4	4
Medium	Correlation Coefficient	1.000	.000
	Sig. (1-tailed)	.	.500
	N	4	4
Small	Correlation Coefficient	1.000	-.056
	Sig. (1-tailed)	.	.472
	N	4	4

(B)

Seed Size Grade		Accelerated Aging	Field Emergence
Large	Correlation Coefficient	1.000	.778
	Sig. (1-tailed)	.	.111
	N	4	4
Medium	Correlation Coefficient	.	.
	Sig. (1-tailed)	.	.
	N	4	4
Small	Correlation Coefficient	1.000	.056
	Sig. (1-tailed)	.	.472
	N	4	4

(C)

Seed Size Grade		Seedling Length	Field Emergence
Large	Correlation Coefficient	1.000	.211
	Sig. (1-tailed)	.	.395
	N	4	4
Medium	Correlation Coefficient	1.000	-.738
	Sig. (1-tailed)	.	.131
	N	4	4
Small	Correlation Coefficient	1.000	.105
	Sig. (1-tailed)	.	.447
	N	4	4

(D)

Seed Size Grade		Seedling Dry Weight	Field Emergence
Large	Correlation Coefficient	1.000	.632
	Sig. (1-tailed)	.	.184
	N	4	4
Medium	Correlation Coefficient	1.000	-.211
	Sig. (1-tailed)	.	.395
	N	4	4
Small	Correlation Coefficient	1.000	.316
	Sig. (1-tailed)	.	.342
	N	4	4

(E)

Conclusion

It may be concluded from this experiment that positive relation was found between large seed grade and field emergence in all the test parameters. However, standard germination values can't be directly used to predict field emergence. The research has revealed that large and medium size seeds have high seedling survival, growth and establishment under unfavourable condition, i.e. under field conditions. It also concluded that seed vigour differs among seed sizes in a seed lot. These data support the hypothesis that large seeds have superior performance to small seeds. The result also suggested that to use as a carry-over seed it would be suitable to use the medium size seeds.

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Model for Predictive Analysis of the Quantity of Water Evaporated during the Primary-Stage Processing of Bioceramic Material Sourced from Kaolin

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Abstract: Model for predictive analysis of the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin has been derived.The model; $\alpha = e^{(\ln t/2.1992)}$ shows that the quantity of evaporated water during the drying process is dependent on the drying time, the evaporating surface being constant. It was found that the validity of the model is rooted on the expression $(\ln t/\ln\alpha)^N = \text{Log}\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value was found to be less than 22% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. [Nature and Science. 2009;7(4):79-84]. (ISSN: 1545-0740).

Keywords: Model, Prediction, Water Evaporation, Bioceramic Material.

1. Introduction

Ceramics have been found [1] to comprise various kinds of non-metallic and inorganic materials. These include naturally occurring clays (kaolin) which is mainly alumino-silicates ($Al_2O_3.2SiO_2.2H_2O$) and vary concentrations of carbides, nitrides, and oxides. Ceramic material can exist in the crystalline, amorphous, or glassy states.

Bioceramics and associated biomedical devices are used in so many parts of the human body. Because human life and well being often depend on these devices there are stringent controls and constraints placed upon the application of devices and materials that can be used. It has been found [2] that when a prosthetic device is placed into the body, two aspects must be taken into account:

Biofunctionality:

This concerns the effect of the physiological environment on the material (bioceramics)/device. The material must satisfy its design requirements in service. The varied functions of bioceramics include: load transmission and stress distribution; eg bone replacement, articulation to allow movement; eg artificial knee joint, and space filling; eg cosmetic surgery[2].

Biocompatibility

This is associated with the effect of the prosthetic device/material (and any degradation product) in the body. The material is not expected to degrade in its properties within the environment of the body and must not cause any adverse reactions within the host body.

Nature of the physiological environment:

Studies [2] carried out on the effect of the physiological environment on biomaterials/device show that NaCl aqueous solution (0.9M) containing organic acids, proteins, enzymes, biological macromolecules, electrolytes and dissolved oxygen, nitrogen compounds, and soluble carbonates are environments where biomaterials and devices can operate favourable. It was found [2] that pH ≈ 7.4 is normal for physiological extracellular fluid. It has been discovered that cells (eg. inflammatory cells and fibrotic cells) secrete several complex compounds that may significantly affect an implanted biomaterial. Applications of these biomaterials/devices have been found [2] to be also dependent on mechanical environment: static, dynamic, stress, strain and friction.

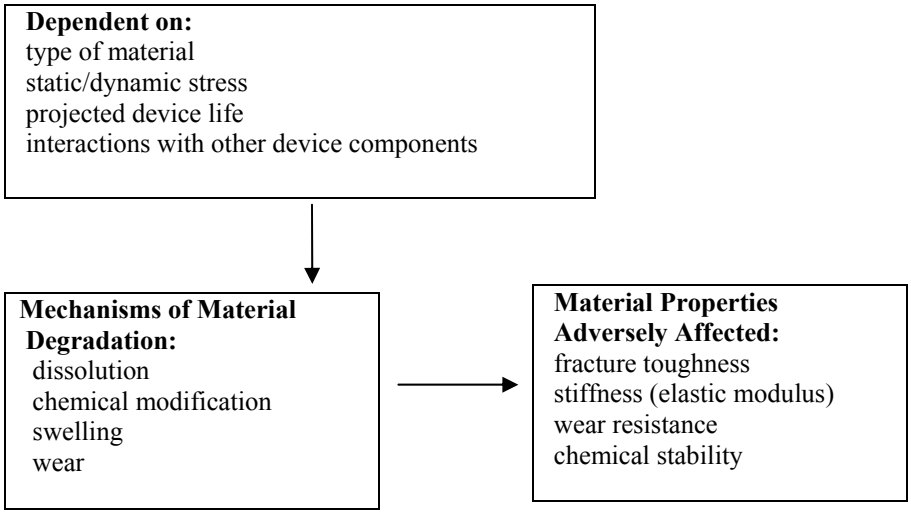

Fig. 1: The effect of the physiological environment on materials/devices [3]

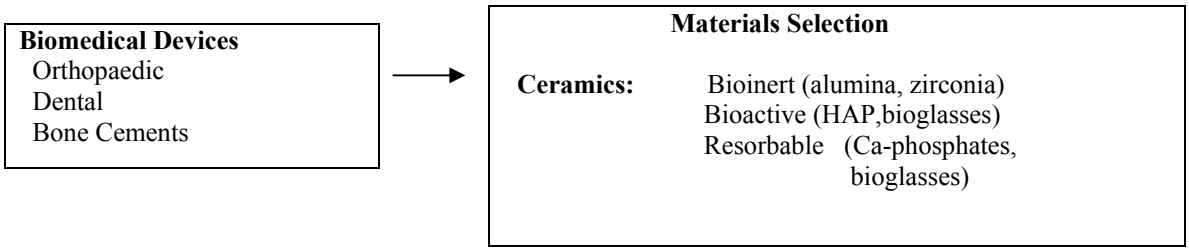

Fig.2: Material selection for functional performance [3]

Bioceramic Materials (Functional Properties)

Ceramics are stiff, hard and chemically stable and are often used in situations where wear resistance is vital. Of the large number of ceramics known, only a few are suitably biocompatible. These ceramics can be grouped according to their relative reactivity in physiological environment. The main problem with ceramic component is that they are brittle and relatively difficult to process.

Studies [4] reveal three types of bioceramics; bioinert, bioactive and resorbable ceramics. Bioinert ceramics includes; alumina (Al_2O_3), partially stabilized zirconia (ZrO_2) and silicone nitride (Si_3N_4). For these materials, foreign body response equals encapsulation. They were found [4] to be extremely stable and elicit minimal response to host tissues. The functional properties of bioinert ceramics were found [3] to include; high compressive strength, excellent wear resistance and excellent bioinertness. Alumina has been found [3] to be a traditional bioinert material being chemically inert and highly stable oxide. It has been discovered that alumina has low fracture toughness and tensile strength implying that it can be used in compression only. Applications of alumina were found [3] to include; femoral head of total hip replacement (polycrystalline) and single crystal (sapphire) in dental implants. It has been found that zirconia combines with a metal oxide dopant (stabilizing oxide-MgO or Y_2O_3) to form a ceramic known as partially stabilized zirconia (PSZ). This ceramic exhibits excellent toughness compared to other ceramics. This was found to be as result of a process known as transformation toughening [5]. This involved an

energy absorbing phase change at the front of propagating crack tip which slows down the advancement of cracks. PSZ has been found to be useful in hip joint prosthesis.

It has been found [5] that bioactive ceramics direct chemical bond with tissue and in particular, bone. They are surface-reactive but has low solubility, allows fixation of implants in the skeletal system. Investigations [4] carried out on the properties of bioactive ceramics reveal that they are hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and bioglasses, having low mechanical strength and fracture toughness. It was reported [3] that bioactive ceramics can find application in: coatings used on stainless steel, Ti and CoCr for tissue on-growth, bone filler for dental and maxillofacial reconstruction.

It has been found [4] that resorbable ceramics are chemically broken down by the body and resorbed. This type of ceramic was also found [3] to control dissolution rate by composition and surface area (density). Investigations [3] indicate that chemicals produced as the ceramic is resorbed, are processed through the normal metabolic pathways of the body without evoking any deleterious effects. Chemically resorbable ceramics is made up of calcium phosphate e.g., tri-calcium phosphate, $Ca_3(PO_4)_2$.This ceramic has found applications in bone repairs such as maxillofacial and periodontal defects, as well as in temporary scaffold or space-filler material [3].

It has been discovered that on drying clays through heating, water is given off. With time, a hard but porous piece forms. A swollen appearance might occur during the release of some gases, but overall shrinkage must occur when verifications set in leading to a strong dense piece [6].

The present work is to derive a model for predicting the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin (mined at Ukpor (Nigeria)). Evaporation of water from the kaolinitic clay ($Al_2O_3.2SiO_2.2H_2O$) occurred in the course of drying the clay (in the oven) during the extraction of alumina (Al_2O_3). The need for this extraction resulted from the indispensable role played by alumina (in biomedical engineering) as bioinert ceramics for total hip replacement.

2. Model formulation

Experimental data obtained from research work [7] carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data [7] shown in Table 1, gave rise to Table 2 which indicate that;

$$(\ln t/\ln\alpha)^N = \text{Log}\beta \quad \text{(approximately)} \tag{1}$$

Introducing the values of N into equation (1)

$$(\ln t/\ln\alpha)^{1.36} = \text{Log}\beta \tag{2}$$

Multiplying the indices of both sides by 1/1.36

$$\ln t/\ln\alpha = (\text{Log}\beta)^{1/1.36} \tag{3}$$

$$\ln t/\ln\alpha = (\text{Log}\beta)^{0.7353} \tag{4}$$

Introducing the value of β into equation (4)

$$\ln t/\ln\alpha = (2.9206)^{0.7353} \tag{5}$$

$$\ln t/\ln\alpha = 2.1992 \tag{6}$$

$$\ln\alpha = \ln t/2.1992 \tag{7}$$

$$\alpha = e^{(\ln t/2.1992)} \tag{8}$$

Where

(α) = Weight of evaporated water during the drying process (g)

(β) = Area of evaporating surface (mm^2)

N = 1.36; (Collapsibility coefficient of binder-clay particle boundary at the drying temperature of 110^0C) determined in the experiment [7].

t = Drying time (mins.).

Table 1: Variation of quantity of evaporated water with drying time [7]

t (mins.)	(β)	(α)
30	833	6.00
50	833	5.90
70	833	6.90
90	833	7.80
110	833	7.40
130	833	7.60

Table 2: Variation of $(\ln t/\ln\alpha)^N$ with Logβ

lnt	Logβ	lnα	$(\ln t/\ln\alpha)^N$
3.4012	2.9206	1.7918	2.3908
3.9120	2.9206	1.7750	2.9062
4.2485	2.9206	1.9315	2.9214
4.4998	2.9206	2.0541	2.9052
4.7005	2.9206	2.0014	3.1938
4.8675	2.9206	2.0281	3.2892

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 49mm, width 17mm, and breadth 9mm exposed to drying in the furnace while it was in wet condition. Initially, atmospheric levels of oxygen are assumed. Atmospheric pressure was assumed to be acting on the clay samples during the drying process (since the furnace is not air-tight). The grain size of clay particles used is 425μm, weight of clay and binder (bentonite) used (for each rectangular product); 100g and 10g respectively, quantity of water used for mixing; 2% (of total weight), drying temperature used; 110^0C, area of evaporating surface;833mm^2 and range of drying time used;(30-130)mins. These and other process conditions are detailed in the experimental technique [7].

The boundary conditions are: Atmospheric levels of oxygen at the top and bottom of the clay samples since they are dried under the atmospheric condition. No external force due to compression or tension was applied to the drying clays. The sides of the particles and the rectangular shaped clay products are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of the model-predicted α values and those from the experiment for equality or near equality.

Analysis and comparison between these α values reveal deviations of model-predicted α from those of the experimental values. This is believed to be due to the fact that the surface properties of the clay and the physiochemical interactions between the clay and binder, which were found to have played vital role during the evaporation process [7] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted α value to that of the corresponding experimental value (Table 3).

Deviation (Dv) (%) of model-predicted α values from the experimental α values is given by

$$Dv = \frac{DP - DE}{DE} \times 100 \quad (9)$$

Where DP = α values predicted by model

DE = α values obtained from experiment

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \quad (10)$$

Therefore

$$Cf = -100\left(\frac{DP - DE}{DE}\right) \quad (11)$$

Introduction of the value of Cf from equation (11) into the model gives exactly the corresponding experimental value of α [7].

5. Results and Discussion

The derived model is equation (8). A comparison of the values of α obtained from the experiment and those from the model shows low deviations hence depicting the reliability and validity of the model, (Table 3). The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 22% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. It was found that the validity of the model is rooted in the expression $(\ln t/\ln\alpha)^N = \log\beta$ where both sides of the expression are correspondingly approximately equal to 3.Table 2 also agrees with equation (1) following the values of $(\ln t/\ln\alpha)^N$ and $\log\beta$ evaluated from Table 1 as a result of corresponding computational analysis.

Table 3: Comparison between quantities of evaporated water as predicted by model and as obtained from experiment [7]

α_{exp}	α_M	Dv (%)	Cf (%)
6.00	4.6953	-21.75	+21.75
5.90	5.9230	+0.39	-0.39
6.90	6.9022	+0.03	-0.03
7.80	7.7377	-0.80	+0.80
7.40	8.4771	+14.56	-14.56
7.60	9.1459	+20.35	-20.35

Where α_{exp} = α values obtained from experiment [7]
α_M = α values predicted by model.

6. Conclusion

The model predicts the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin. It was found that the validity of the model is rooted in the expression $(\ln t/\ln\alpha)^N = \log\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 22% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model-predicted α values from those of the experimental.

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Fuelwood, Fodder Consumption and Deficit Pattern in Central Himalayan Village

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ABSTRACT

A study of traditional agroecosystem conducted to understand status of fuelwood and fodder in central Himalayan village. The fuel consumption is 418.86 MT and the annual fuel availability is 211.03MT, there is a deficit of 207.83MT. Total available fodder is 281.76 MT but the total consumption is 402.72 MT so there is a deficit of 207.83 MT. [Nature and Science, 2009; 7(4):85-88] (ISSN 1545-0740).

KEY WORDS: Fodder and Fuel deficit, Central Himalaya, Consumption and Sources

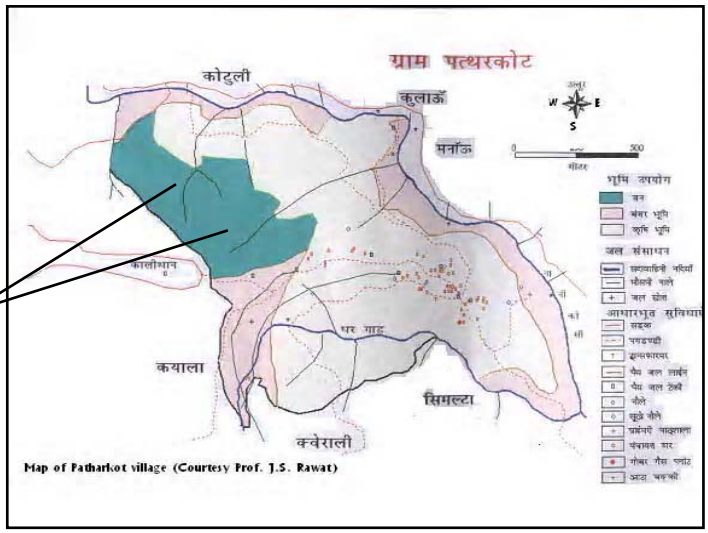
INTRODUCTION:

In many of the developing countries, forests are the main source of fuel wood, timber for house construction and fodder for livestock. Consequently, any depletion of this resource base can erode living standards as well as ecosystem stability (Shah, 1982; Pant and Singh, 1987). In the central Himalaya traditional agriculture is the main source of the fodder and forests are the source of fuel energy. The livelihoods of the hill people are mainly dependent on marginal agriculture on the one hand and rearing livestock on the other. Fodder and fuelwood is collected by lopping/ the vegetative biomass. Moreover in Uttarakhand hills, it is well known that women are mainly responsible for the collection of fuelwood and fodder. Since vegetation is already in a degraded stage in most of the areas further exploitation of natural resources is a degrading factor. Villagers rear these animals for the milk that adds to their income. (Chandra et al.). The major constraint in the central and north – western Himalaya in improving livestock and increasing milk production is that the livestock feeds are inadequate and unbalanced. The most serious problem is the unavailability of green forage, particularly in winter, causing deficiency of protein and vitamins, resulting in low milk production, shortened breeding span and decreased working capacity of bullocks. (Palni et al). Aims of the study were to (i) To assess the status of the fuel and fodder in the study village (ii) list out the sources and species of fuel and fodder species in central Himalayan village.

STUDY AREA:

The study was conducted in the village Patharkot, block- Hawalbagh district – Almora which is situated at Uttarakhand. The total population of the village is 733 out of them 433 male and 300 female members. The

literacy rate of the village is very good and is approximate 95%. The total land area of the village is 163.62 ha out of them Van panchyat is 33.40 ha, agriculture area is 65.97 ha and other is 64.24 ha. The other area includes wastelands and settlements. The no. of agriculture fields in the study site are 7395. The farming system prevalent in the hills is subsistence-farming framers cultivate the crops in their land for the living. The land holdings are smaller in size with majority farmers coming in the marginal category. Agriculture is heavily dependent on energy flows from uncultivated lands such as forests and grasslands recycled in to manure through livestock. (Mahadev, Ashish 1979).



Community Forest- Major source of fuel and fodder

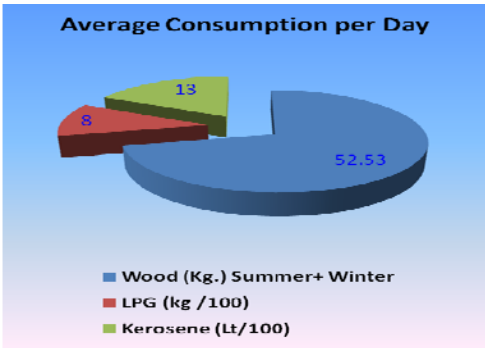
Resource Map of Study Site:

METHODOLOGY:

The study was based on the primary survey and data collected through PRA method by approaching the villagers representing different age groups and gender also. The collected information was recorded and tabulated also. This data used for the status of fuel and fodder status in the central Himalayan village. Lists of families were procured from Gram Panchyat / Gram Shabha or family register for the secondary information about the village. Estimation of the actual amount of fodder and fuelwood consumed was carried out based on regular observation in the village. The source and supply of fodder for animals was quantified as contribution of agricultural by- products, forest and tree fodder from agroforestry by estimation in the villages and by measuring the daily ration of food concentrates given to the animals by each household. Use of firewood by human population was also quantified under different categories viz. firewood for cooking, water heating, house warming, animal food preparation and festivals. Villagers were asked to burn a known weight of firewood and observations were taken for time and quantity of firewood. (Sundriyal, 1994).

FUELWOOD CONSUMPTION:

Among the natural resources of Uttarakhand, forests are the most important, both economically and environmentally (Sati, 2005). The forest is the main sources of the fuel in the state. The use of fuel wood is the only form of energy for cooking. Men, women and children from nearby forested areas collect firewood. At higher elevations people collected firewood during winter months only and store it in heaps for the whole year, whereas, at lower elevations collection is made throughout the year. Due to collection of huge amount of firewood, forests near to the villages are subjected to rapid degradation and over exploitation. A very small fraction of firewood comes from the agriculture fields. (Sundriyal, 1994). The total forest area of the fuel collection for the village is 33.40 ha and the per household forest area in the village is 0.63 ha. According to primary survey of the village the fuel consumption is 418.86 MT and the annual fuel availability is 211.03 MT, there is a deficit of 207.83 MT. Per family fuel consumption is 52.53 kg. (Summer – 17.28 kg. Winter – 35.25 kg.). Consumption of Kerosene is 0.13 lt. and LPG is 0.08 kg. Per day per family. The major fuel wood species are *Quercus leucotrichophora, chir Pinus roxburghii, Myrica esculanta and Pyrus pashia*.



Van Panchyat Area	33.40 ha
Total household	53 (NoS.)
Per household forest area	0.63 ha
Fuel requirement per year	418.86 MT
Availability per year	211003 MT
Deficit	207.83 MT
Major fuel wood species	Banj Oak, Chir Pine, Kafal, Mehal etc.

Status of Fuelwood in village Patharkot

FODDER CONSUMPTION:

In the Himalayan region, the domestic animals provide main drought power for agriculture system. They also process crop residues, provide essential organic manure and generate farm income when they are sold (Thapa et al., 1991). The domestic animals are depending on forest as well as agricultural residues also. The main type of fodder, sources of fodder area under fodder production and total production is quantified by regular observation and survey method in the village. The maximum fodder comes from agricultural and forest areas in the months of May to July, which is 197.58 MT (based on dry matter). Villagers collect the dry grasses from grass fields which are locally known as ***Mange.*** Fodder collected from these grasslands is 62.70 MT., the other sources of fodder are agricultural residues and tree species. The main tree species of fodder are *Grewia optiva, Celtis australis, Quercus leucotrichophora* etc. The total available fodder is 281.76 MT but the total consumption is 402.72 MT so there is a deficit of 207.83 MT.

Fodder	Sources	Area (ha)	Quantity (dry matter)
Green Grasses	Agricultural Fields and Forest	15 ha	197.58 MT
Dry Grasses	Grasslands (**Mange**)	9.04 ha	62.70 MT
Tree Fodder	Agricultural Fields	NA	3.91 MT
Agricultural residues	Agricultural Crops	15 ha	6.99 MT
Oak leaves	Forests **(Bajani)**	33.04 ha	10.78 MT

Fodder Status in village Patharkot

CONCLUSION AND FUTURE PROSPECTS:

Based on the statistics of fuel and fodder it has been noticed that in the central Himalayan village have a problem of fuel and fodder. Due to lack of fodder the livestock is very poor in this village so, there is a wide concept gap between villagers for the marketing of milk and other products. The fuel consumption is 418.86 MT and the annual fuel availability is 211.03 MT, there is a deficit of 207.83 MT. The major fuel wood species are *Quercus leucotrichophora, chir Pinus roxburghii, Myrica esculanta and Pyrus pashia.* The main tree species of fodder are *Grewia optiva*, *Celtis australis*, *Quercus leucotrichophora* etc. Total available fodder is 281.76 MT but the total consumption is 402.72 MT so there is a deficit of 207.83 MT. Afforestation with ecologically as well as socio - economically viable species will not only fulfill the demand of local villagers but also provide other basic needs of central Himalayan people.

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