An International Journal

Nature and Science

ISSN 1545-0740

Volume 7 - Number 6 (Cumulated No. 27), July 15, 2009

Marsland Press

P.O. Box 21126, Lansing, Michigan 48909, the United States

525 Rockaway PKWY, #B44, Brooklyn, New York 11212, the United States

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P.O. Box 21126
Lansing, Michigan 48909
The United States
Telephone: (517) 349-2362
Email: sciencepub@gmail.com;
naturesciencej@gmail.com;
Websites: http://www.sciencepub.net;
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Nature and Science

ISSN: 1545-0740

Volume 7 - Number 6 (Cumulated No. 27), July 15, 2009

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Coag-flocculation kinetics and functional parameters response of Periwinkle shell coagulant (PSC) to pH variation in organic rich coal effluent medium

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ABSTRACT

The coag-flocculation performance of PSC as affected by pH variation in coal washery effluent has been investigated at room temperature using various dosages of unblended PSC. In addition, coag-flocculation parameters such as α, K, β_{BR}, K_R, ε_p and $\tau_{1/2}$ are determined. Turbidity measurement was employed using the nephelometric (turbidimetric) standard method while PSC preparation was adapted from the works of Fernandez-Kim. The maximum PSC performance is recorded at α of 2, K of $1.6667x10^{-3}m^3$/ kg.s, dosage of 0.4 kg/m^3, pH of 6 and $\tau_{1/2}$ of 8.0s while the minimum performance is recorded at α of 2, K of $8.833x10^{-5}m^3$/ kg.s, dosage of 0.5kg/m^3, pH of 10 and $\tau_{1/2}$ of 218s.The minimum value of coagulation efficiency, E (%) recorded is > 95% .In general, the computed parameters lie within range of previous works .The results obtained confirmed that the theory of fast coag-flocculation holds for coag-flocculation of the coal washery effluent using the coagulant investigated and at the conditions of the experiment. [Nature and Science. 2009;7(6):1-18]. (ISSN: 1545-0740).

Keywords: Coag-flocculation,Kinetics,CoalEffluent,Periwinkle shell,coagulation

INTRODUCTION

Traditionally ,coag-floculation is aimed at the removal of suspended colloidal particles when the stabilized colloids are aided to overcome their repulsive forces leading to the aggregation of the particles into flocs.(Ma et al ,2001;Di Terrlizzi, 1994;Edzwald,1987;O'meila, 1978)
Among the factors that affect the process are raw effluent quality, temperature, pH, chemical and bacteriological parameters etc(jin,2005).

Coag-flocculation may be achieved by any of the common coagulants such as alum, lime etc. The coagulation behaviors of these common coagulants have been well investigated with little or no attention given to the coag-flocculation potential of many animal and plant derivatives. To this end, a focus is hereby given to the study on periwinkle shell as potential source of coagulant derivative. Periwinkle shell is a natural carbohydrate biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean. (Fernadez-Kin, 2004).

PSC is a non-toxic, biodegradable and biocompatible polymer. Previous results obtained from the study on crustacean derived coagulants highlight promise of renewable polymeric materials with extensive application in removal of colloidal particles in wide range of effluent media (Fernandez-Kin, 2004)

However, in spite of the abundance of periwinkle in our local communities in Nigeria, little or no comprehensive work has been reported. Against this backdrop, this work endeavors to explore and generate interest in the utilization of periwinkle shell as coagulants. As a step in this direction, this work focuses on coagulation performance and kinetics of PSC under varying pH of coal washery effluent, a typical medium for this kind of study. Thus, if well developed, PSC can serve as a suitable replacement either in total or in part for the inorganic coagulants.

THEORETICAL PRINCIPLES AND MODEL DEVELOPMENT

The rate of successful collisions between particles of sizes i and j to form particle of size *k* is (Thomas *et al*, 1999; Jin, 2005):

$$N_{ij} = \varepsilon_p \beta (i,j) n_i n_j \quad \ldots 1$$

where N_{ij} = the rate of collisions between particles of size i and j(mass concentration / time)
ε_p = collision efficiency
$\beta(i,j)$ = collision factor between particles of size i and j
$n_i n_j$ = particle concentration for particles of size i and j, respectively.
Assuming monodisperse, no break up and bi particle collision , the general model for perikinetic coag-flocculation is given as (Swift and Friedlander 1964 ; Jin, 2005)

$$\frac{dn_k}{dt} = \frac{1}{2}\sum_{i+j=k}\beta(i,j)n_i n_j - \sum_{i=1}^{\infty}\beta(i,k)n_i n_k \quad \ldots 2$$

where $\frac{dn_k}{dt}$ is the rate of change of concentration of particle of size k (concentration / time). β is a function of the coag-flocculation transport mechanism .The appropriate value of β for Brownian transport is given by Von Smoluchowski (1917):

$$\beta_{BR} = \frac{8}{3}\varepsilon_p \frac{k_B T}{\eta} \quad \ldots 3$$

where k_B is Boltzmann's constant (J/K)

T is Absolute temperature (K)

The generic aggregation rate of particles (during coagulation / flocculation) can be derived by the combination of equations 2 and 3 to yield:

$$-\frac{dN_t}{dt} = KN_t^{\alpha} \quad \ldots 4$$

where N_t is total particle concentration at time t, $N_t = \sum n_k$ (mass / volume)

K is the α^{th} order coag-floculation constant

α is the order of coag-floculation process

And $K = \frac{1}{2}\beta_{BR}$ …5

where β_{BR} is collision factor for Brownian transport

Also, $\beta_{BR} = \varepsilon_p K_R$ …6

combining equations 4,5 and 6 produce:

$$-\frac{dN_t}{dt} = \frac{1}{2}\beta_{BR}N_t^{\alpha} \quad \ldots 7$$

$$= \frac{1}{2}\varepsilon_p . K_R N_t^{\alpha} \quad \ldots 8$$

where K_R is the Von smoluchowski rate constant for rapid coagulation (Van Zanten, *et al* 1992)

However $K_R = 8\pi R_p D'$ ….9

$$R_p = 2a \quad \ldots 10$$

where D' is particle diffusion coefficient

a is particle radius.

From Einstein's equation: $D' = K_B T / B$ …11

From Stoke's equation : $B = 6\pi\eta a$ ….12

where B is the friction factor

η is the viscosity of the fluid

combining equations 9 and 11 give:

$$K_R = \frac{8\pi R_p K_B T}{B} \quad \ldots 13$$

combining equation 12 and 13 give:

$$K_R = \frac{8\pi R_p K_B T}{6\pi\eta a} \quad \ldots 14$$

Putting equation 10 in 14 yields:

$$K_R = \frac{8}{3}\frac{K_B T}{\eta} \quad \ldots 15$$

combining equations 8 and 15 give:

$$-\frac{dN_t}{dt} = \frac{1}{2}\varepsilon_p\left(\frac{8}{3}\frac{K_B T}{\eta}\right)N_t^{\alpha}$$

$$= \frac{4}{3}\varepsilon_p \frac{K_B T}{\eta} N_t^{\alpha} \quad \ldots 16$$

Comparing equations 4 and 16 show:

$$K = \frac{4}{3}\varepsilon_p \frac{K_B T}{\eta} \quad \ldots 17$$

For perikinetic aggregation, α theoretically equals 2 as would shown below (Fridrikhsberg, 1984; Hunter, 1993):

From Fick's law,

$$J_f = D' 4\pi R_p{}^2 \frac{dN_t}{dR_p} \quad \ldots 18$$

Integrating equation 18 at initial conditions $N_t = 0$, $R = 2a$:

$$\frac{J_f}{D' 4\pi}\int_0^{R_p} \frac{dR_p}{R_p{}^2} = \int_{N_0}^{N_t} dN_t \quad \ldots 19$$

Therefore $N_t = N_0 - \frac{J_f}{4\pi D' R_p} \quad \ldots 20$

It implies,

$$0 = N_0 - \frac{J}{4\pi D'(2a)} \quad \ldots 21$$

Therefore $J_f = 8\pi D' a N_0 \quad \ldots 22$

$$= \frac{1}{2}\cdot K_R \cdot N_0 \quad \ldots 23$$

For central particle of same size undergoing Brownian motion, the initial rate of rapid coag-flocculation is:

$$-\frac{dN_t}{dt} = J_f \cdot \varepsilon_p \cdot N_0 \quad \ldots 24$$

$$= \frac{1}{2}\cdot K_R \cdot \varepsilon_p \cdot N_0 N_0$$

$$= \frac{1}{2}\cdot K_R \cdot \varepsilon_p \cdot N_0^2$$

$$= \frac{1}{2}\cdot \varepsilon_p \cdot \frac{8}{3}\frac{K_B T}{\eta}\cdot N_0^2$$

$$= \frac{4}{3}\varepsilon_p \frac{K_B T}{\eta} \cdot N_0^2 \quad \text{...25}$$

$$\equiv \frac{4}{3}\varepsilon_p \frac{K_B T}{\eta} N_t^2 \text{ at } t > 0$$

Hence, from equation 25, $\alpha = 2$

However in real practice, empirical evidence shows that in general $1 \le \alpha \le 2$ (WST, 2003; Menkiti, 2007).Based on this , what is required to evaluate *K* is to determine the line of better fit between $\alpha = 1$ and 2 while the experimental data are fitted into linearised form of equation 4.

Hence, for $\alpha = 1$,equivalence of equation 4 yields:

$$\frac{dN}{dt} = - KN \quad \text{...26}$$

Integrating within the limits, produces

$$\int_{N_0}^{N} \frac{dN}{N} = -\int_0^t K dt \quad \text{...27}$$

Equation 27 yields:

$$\ln N = \ln N_0 - Kt \quad \text{...28}$$

$$\ln\left(\frac{1}{\left[\frac{1}{N}\right]}\right) = \ln N_0 - Kt$$

$$\ln\left(\frac{1}{N}\right) = Kt - \ln N_0 \quad \text{...29}$$

Plot of $\ln\left(\frac{1}{N}\right)$ vs. t gives a slope of *K* and intercept of $(-\ln N_0)$.

For $\alpha = 2$; equivalence of equation 4 yields:

$$\frac{dN}{dt} = -KN^2 \quad \text{…30}$$

Hence:

$$\int_{N_0}^{N} \frac{dN}{N^2} = -K\int_0^t dt \quad \text{…31}$$

$$\frac{1}{N} = Kt + \frac{1}{N_0} \quad \text{...32}$$

Plot of $\ln\left(\frac{1}{N}\right)$ Vs t produces a slope of *K* and intercept of $\frac{1}{N_0}$.

For the evaluation of coagulation period ($\tau_{1/2}$), from Equation 32:

$$N = \frac{N_0}{1 + N_0 Kt} \quad \text{....33}$$

$$= \frac{N_0}{\left[1 + \frac{t}{\left(\frac{1}{N_0 K}\right)}\right]} \quad \text{...34}$$

let $\tau = \left[{}^{1}\!/_{N_0 K} \right]$ ...35

Hence:

$$N = \frac{N_0}{1 + (t/\tau)} \quad \ldots 36$$

When $t = \tau$, equation 36 becomes

$$N = {}^{N_0}\!/_{2} \quad \ldots 37$$

Therefore as $N_0 \rightarrow 0.5N_0; \tau \rightarrow \tau_{1/2}$

Hence $\tau_{1/2} = {}^{1}\!/_{(0.5N_0 K)}$ ...38

For Brownian aggregation at early stages ($t \leq 30$ minutes), equation 2 can be solved exactly, resulting in the expression (Holthof *et al*, 1996):

$$\frac{N_{m(t)}}{N_0} = \frac{\left[{}^{t}\!/_{2}\left(\frac{1}{KN_0} \right) \right]^{m-1}}{\left[1 + \frac{t}{2\left(\frac{1}{KN_0} \right)} \right]^{m+1}} \quad \ldots . 39$$

Where $\tau = \left(\frac{1}{N_0 K} \right)$

Therefore $\frac{N_{m(t)}}{N_0} = \frac{\left[{}^{t}\!/_{2\tau} \right]^{m-1}}{\left[1 + {}^{t}\!/_{2\tau} \right]^{m+1}}$ 40

Let $2\tau = \tau'$

Therefore $\frac{N_{m(t)}}{N_0} = \frac{\left[{}^{t}\!/_{\tau'} \right]^{m-1}}{\left[1 + {}^{t}\!/_{\tau'} \right]^{m+1}}$ ...41

Equation 41 gives a generic expression for particle of *m-th* order .Hence,

For singlets *(m=1)*

$$N_1 = N_0 \left[\frac{1}{\left(1 + {}^{t}\!/_{\tau'} \right)^2} \right] \quad \ldots 42$$

For doublets *(m=2)*

$$N_2 = N_0 \left[\frac{\left({}^{t}\!/_{\tau'} \right)}{\left(1 + {}^{t}\!/_{\tau'} \right)^3} \right] \quad \ldots . 43$$

For triplets *(m=3)*

$$N_3 = N_0\left[\frac{\left(t/\tau'\right)^2}{\left(1+t/\tau'\right)^4}\right] \quad \ldots.44$$

Conversion of Turbidity (NTU) to TSS *(mg/l)* yields (Metcalf and Eddy,2003):

TSS *(mg/l)* = $(TSS_f).T$ ...45

Where T = turbidity (NTU)

(TSS_f) =Conversion factor to TSS

EXPERIMENTAL METHODS

The procedure for processing of the periwinkle shell to coagulant is adapted from the work of Ferdinandez-Kin (2004).The jar test were conducted based on standard Bench Scale Nephlometric Method for the Examination of water and waste water.(WST,2003;AWWA, 1985)

RESULTS AND DISCUSSION

The values of coag-flocculation reaction parameters are presented in tables 1 to 5.For the case of $\alpha = 1$, it is a shift from theoretical expectation but in line with empirical evidence (WST,2003).Generally, the value of α affects that of K inversely .Since K is rate per concentration and K is associated with energy barrier (KT) , it is understandable that for higher α to be obtained , a lower K is a necessary condition for such phenomenon (Fridkisberg,1984) .The K and α values are to a large extent consistent with previous works on Brownian coagulation (Van Zanten and Elimelechi,1992). $K(=0.5\beta_{BR})$ values appreciably is less sensitive to a given pH as the dosage of PSC changes from 0.1kg/m^3 to 0.5kg/m^3.Variation in K_R is generally minimal as presented in the tables 1 to 5 .This is because K_R is a function of viscosity and room temperature both of which did not vary considerably during the study.

The discrepancies in α, K and K_R are explained by the unattainable assumption that mixing of particles and coagulant throughout the dispersion is 100 % efficient before any aggregation occurs. The effect of this limitation will be local increases in particle ratios during the mixing phase given uneven distribution of particles / coagulant complexes. The complexity of this system makes it hard to have all the values of $\alpha = 2$.(Yates *et al* , 2001).Another account is the interplay between the van der waal forces and the hydrodynamic interactions which typically reduces the theoretically predicted K by a factor of 2.However, since K also depends on the type of counter ion, additional short range forces represent the most likely explanation for any other remaining discrepancies (Holthof *et al* ,1997;Holthof *et al* ,1996).

At nearly invariant values of K_R ,ε_p directly relates to $2K = \beta_{BR}$. The consequence is that high ε_p results in high kinetic energy to overcome the zeta potential.The implication is that the double layer is either reduced or the colloids destabilized to actualize low $\tau_{1/2}$ in favour of high rate of coagulation. The results show that high values $\tau_{1/2}$ corresponds to low ε_p and K, and indication of repulsion in the system.$\tau_{1/2}$ values lie within the range of previous works where milliseconds had been obtained. (Hunter, 1993).

TSS (Kg/m^3) VS TIME PLOTS.

The TSS Vs time plots are presented in Figs 1 to 5.As single particles flocculate into large aggregates ,the turbidity of the dispersion decreases and the transmission intensity increases. This behavior simply reflects the complex dependence of turbidity on particle number (dropping) and particle size (increasing) over time (Yates *et a*l, 2001).The consequence of this is the highest rate of coagulation usually recorded at the early time of the coag-flocculation process. This is supported by $\tau_{1/2}$ that lies within seconds' .Fig 1 to 5 indicate that the best coagulation was obtained at pH of 6.

EFFICIENCY, E (%) VS TIME

Plots of E (%) Vs time are presented in Figs 6 to 10 .The E illustrates the effectiveness of the PSC to remove turbidity from the effluent .The plots show that the least E > 95% ;supports the low values of $\tau_{1/2}$ .This justifies the theory of fast coagulation (Von Smoluchowski , 1917)which validates the real life application of coagulation in which 90% of particle removal is usually achieved within the first five minutes of coag-flocculation .With the exception of Fig 8 ,the highest and lowest E were recorded for pH of 6 and pH of 10 respectively.

PLOT OF E (%) VS pH

This is presented in Fig 11.It indicates the performance of various doses of the PSC at varying pH. Interestingly, all the doses have a similar trend having their maxima at pH = 6 and minima at pH = 10.All the doses have the same E at pH of 2, indicating that the doses do not affect the efficiency at pH = 2.However, the optimum doses at pH = 6 is 0.4kg/m^3 PSC.

PLOT OF E (%) VS DOSAGE (kg/m^3)

This is presented in Fig 12.It confirms the observation made in Fig 11.The optimum dose is 0.4kg/m^3 at pH = 6.However,it should be noted that between 0.1kg/m^3 to 0.3kg/m^3 PSC, pH = 2 gives a better performance .As already observed, pH = 10 gives a poor performance for all doses.

PARTICLE DISTRIBUTION PLOTS:

These are presented in Fig 13 to 17; one for each of the tables 1 to 5 for the optimum *K* .The trend is similar for all the curves. The particle distributions expected in a typical coag-flocculation process are shown in these figures. It is notable that the curves N_i *Vs t*, beginning with twins (doublets), passes through a maximum because they are absent at the initial instant *(t = 0,* N_2 *= 0)* and at the end of coag-flocculation process *(t =* ∞*,* N_2*=0)*. The number of primary particles (singlets) can be seen to decrease more rapidly than the total number of particles. For all consolidated particles, the curves pass through maxima whose height lowers with an increase consolidation.

The curves are expected in coag-flocculation where there is absence of excessive colloidal entrapment and high shear resistance. Mainly, the dominant mechanism in these graphs are charge neutralization combined with low bridging to ensure moderate speed of coag-flocculation as represented in Figs 13 to 17.The discrete nature of formation of N_1,N_2 and N_3 is associated with moderate energy barrier..

Table 1:Coag-flocculation Functional Parameters for varying pH and constant dosage of 0.1kg/m^3 PSC

Parameter	pH =2	pH = 4	pH = 6	pH = 8	pH = 10
A	1	2	2	1	2
R^2	0.9607	0.987	0.9931	0.9856	0.7777
K	$1.4483\times10^{-3}s^{-1}$	$3.3333\times10^{-4}m^3/kg.s$	$6.6667\times10^{-4}m^2/kg.s$	$3.9167\times10^{-4}s^{-1}$	$1.3333\times10^{-4}m^3/kg.s$
β_{BR}	$2.8967\times10^{-3}s^{-1}$	$6.6666\times10^{-4}m^3/kg.s$	$1.3333\times10^{-3}m^3/kg.s$	$7.8334\times10^{-4}s^{-1}$	$2.6667\times10^{-4}m^3/kg.s$
K_R	$1.4869\times10^{-17}m^3/s$	$1.4182\times10^{-17}m^3/s$	$1.5640\times10^{-17}m^3/s$	$1.4250\times10^{-17}m^3/s$	$1.3726\times10^{-17}m^3/s$
ε_p	$1.9480\times10^{14}m^3$	$4.7007\times10^{13}kg^{-1}$	$8.5249\times10^{13}kg^{-1}$	$5.4971\times10^{13}m^{-3}$	$1.9426\times10^{13}kg^{-1}$
$T_{1/2}$ (sec)	4.0 sec	39.2 sec	20.1 sec	49.60 sec	136.1 sec
-r	$1.4483\times10^{-3}C$	$3.3333\times10^{-4}C^2$	$6.6667\times10^{-4}C^2$	$3.9167\times10^{-4}C$	$1.3333\times10^{-4}C$

Table 2:Coag-flocculation Functional Parameters for varying pH and constant dosage of 0.2kg/m^3 PSC

Parameter	pH =2	pH = 4	pH = 6	pH = 8	pH = 10
A	1	1	1	2	2
R^2	0.9888	0.9896	0.967	0.9790	0.6623
K	$1.4933\times10^{-3}s^{-1}$	$1.0217\times10^{-3}s^{-1}$	$3.100\times10^{-4}s^{-1}$	$1.6667\times10^{-4}m^3/kg.s$	$1.3333\times10^{-4}m^3/kg.s$
β_{BR}	$2.9866\times10^{-3}s^{-1}$	$2.0434\times10^{-3}s^{-1}$	$6.2\times10^{-4}s^{-1}$	$3.3334\times10^{-4}m^3/kg.s$	$2.6666\times10^{-4}m^3/kg.s$
K_R	$1.431\times10^{-17}m^3/s$	$1.4150\times10^{-17}m^3/s$	$1.5463\times10^{-17}m^3/s$	$1.3880\times10^{-1}7m^3/s$	$1.3763\times10^{-17}m^3/s$
ε_p	$2.086\times10^{14}m^{-3}$	$1.4440\times10^{14}m^{-3}$	$4.0094\times10^{13}m^{-3}$	$2.4001\times10^{13}kg^{-1}$	$1.9374\times10^{13}kg^{-1}$
$T_{1/2}$ (sec)	4.0	13	43	117	136.0
-r	1.4933×10^{-3} C	1.0217×10^{-3} C	3.100×10^{-4} C	1.6667×10^{-4} C^2	1.3333×10^{-4} C^2

Table 3:Coag-flocculation Functional Parameters for varying pH and constant dosage of 0.3kg/m^3 PSC

Parameter	pH =2	pH = 4	pH = 6	pH = 8	pH = 10
A	1	2	1	2	2
R^2	0.8977	0.7815	0.9927	0.9627	0.7198
K	$1.4667x10^{-3}s^{-1}$	$3.3333x10^{-4}m^3/kg.s$	$5.6333x10^{-4}s^{-1}$	$1.6667x10^{-4}m^3/kg.s$	$1.3333x10^{-4}m^3/kg.s$
β_{BR}	$2.9334x10^{-3}s^{-1}$	$6.6666x10^{-4}m^3/kg.s$	$1.1267x10^{-3}s^{-1}$	3.3334x10-4m^3/kg.s	$2.6667x10^{-4}m^3/kg.s$
K_R	$1.7659x10^{-17}m^3/s$	$1.7173x10^{-17}m^3/s$	$1.7357x10^{-17}m^3/s$	$1.4786x10^{-17}m^3/s$	$1.4148x10^{-17}m^3/s$
ε_p	$1.6611x10^{14}m^{-3}$	$3.8819x10^{13}kg^{-1}$	$6.4911x10^{13}m^{-3}$	$2.2543x10^{13}kg^{-1}$	$1.8848x10^{13}kg^{-1}$
$T_{1/2}$ (sec)	4.0	39	24.0	117	136
-r	$1.46667x10^{-3}$ C	$3.3333x10^{-4}$ C^2	$5.6334x10^{-4}$ C	$1.6667x10^{-4}$ C^2	$1.3333x10^{-4}$ C^2

Table 4 :Coag-flocculation Functional Parameters for varying pH and constant dosage of 0.4kg/m^3 PSC

Parameter	pH =2	pH = 4	pH = 6	pH = 8	pH = 10
α	1	2	2	2	2
R^2	0.9662	0.947	0.9714	0.983	0.6616
K	$8.5517x10^{-4}s^{-1}$	$3.3333x10^{-4}m_3/kg.s$	$1.6667x10^{-3}m^3/kg.s$	$1.6667x10^{-4}m^3/kg.s$	$1.3333x10^{-4}m^3/kg.s$
β_{BR}	$1.7103x10^{-3}s^{-1}$	$6.6666x10^{-4}m^3/kg.s$	$3.3334x10^{-3}m^3/kg.s$	$3.3334x10^{-4}m^3/kg.s$	$2.6666x10^{-4}m^3/kg.s$
K_R	$1.424x10^{-17}m_3/s$	$1.3844x10^{-17}m^3/s$	$1.3861x10^{-17}m^3/s$	$1.2905x10^{-17}m^3/s$	$1.1373x10^{-17}m^3/s$
ε_p	$1.2058x10^{14}m^{-3}$	$4.8154x10^{13}kg^{-1}$	$2.4047x10^{-14}kg^{-1}$	$2.5829x10^{13}kg^{-1}$	$2.3445x10^{13}kg^{-1}$
$T_{1/2}$ (sec)	6.7	39.0	8.0	117.0	136.0
-r	8.5517x10-4 C	$3.3333x10^{-4}$ C^2	$1.6667x10^{-3}$ C^2	$1.6667x10^{-4}$ C^2	1.3333x10+ C^2

Table 5:Coag-flocculation Functional Parameters for varying pH and constant dosage of 0.5kg/m^3 PSC

Parameter	pH =2	pH = 4	pH = 6	pH = 8	pH = 10
A	2	2	2	2	2
R^2	0.928	0.9464	0.9744	0.9697	0.444
K	$5x10^{-4}m^3kg^{-1}s^{-1}$	$3.3333x10^{-4}$m3/kg.s	$6.6667x10^{-4}m^3/kg.s$	$1.6667x10^{-4}m^3/kg.s$	$8.3333x10^{-5}m^3/kg.s$
β_{BR}	$1x10^{-3}m^3/kg.s$	$6.6667x10^{-4}m^3/kg.s$	$1.3333x10^{-3}m^3/kg.s$	$3.3333x10^{-4}m^3/kg.s$	$1.6667x10^{-4}m^3/kg.s$
K_R	$1.4200x10^{-17}m^3/s$	$1.6622x10^{-17}m^3/s$	$1.7182x10^{-17}m^3/s$	$1.4924x10^{-17}m^3/s$	$1.4280x10^{-17}m^3/s$
ε_p	$7.0418x10^{13}kg^{-1}$	$4.0107x10^{13}kg^{-1}$	$7.759x10^{13}kg^{-1}$	$2.2334x10^{13}kg^{-1}$	$1.1671x10_{13}kg^{-1}$
$T_{1/2}$ (sec)	12	39.2	20	117	218
-r	$5x10^{-4}$ C^2	$3.3333x10^{-4}$ C^2	$6.6667x10^{-4}$ C^2	$1.6667x10^{-4}$ C^2	$8.3333x10^{-5}$ C^2

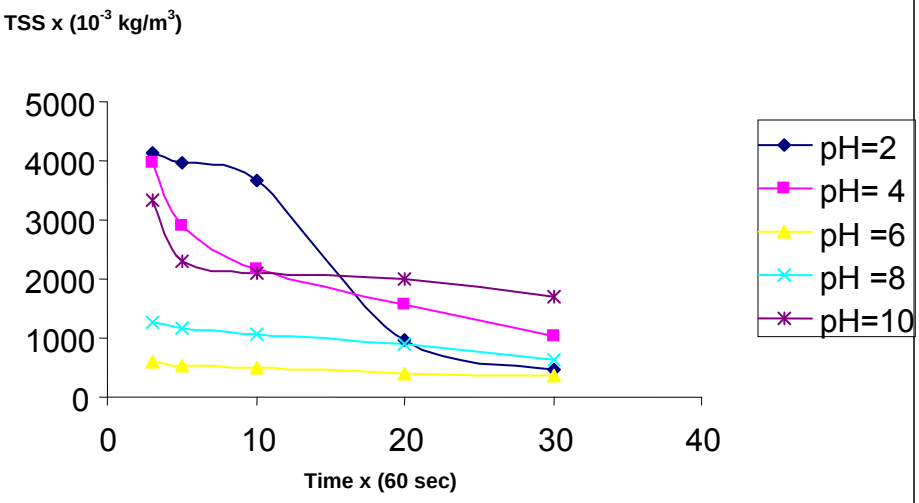

Fig 1:Plot of TSS Vs Coag-floccualtion Time for 0.1kg/m^3 PSC at varying pH

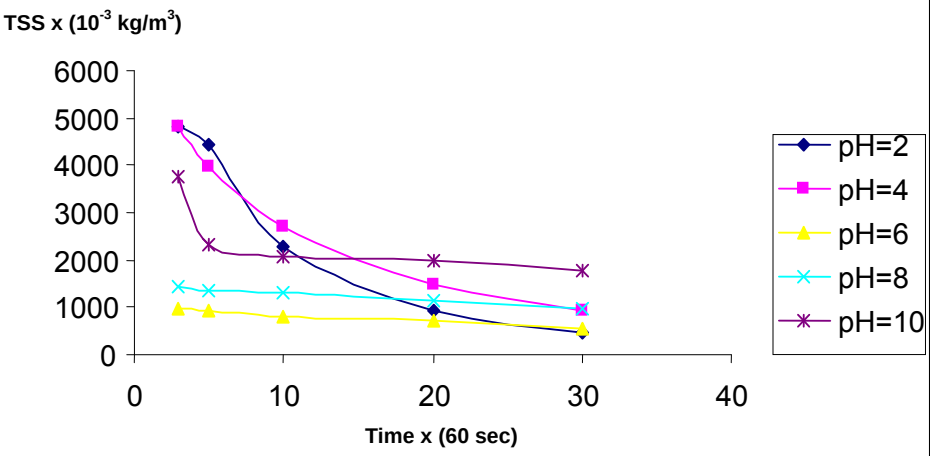

Fig 2:Plot of TSS vs Coag-flocculation Time for 0.2 kg /m^3 PSC at varying pH

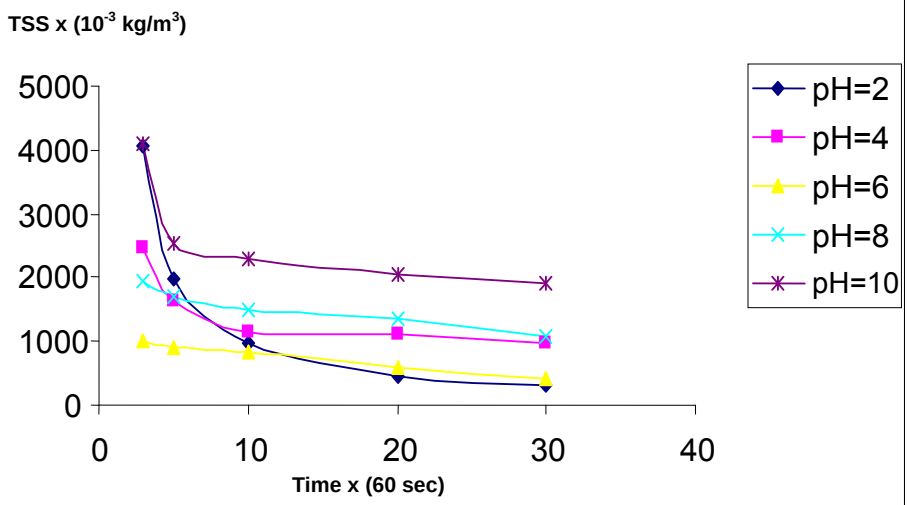

Fig 3:plot of TSS vs Coag-flocculation Time for 0.3kg/m^3 PSC at varying pH

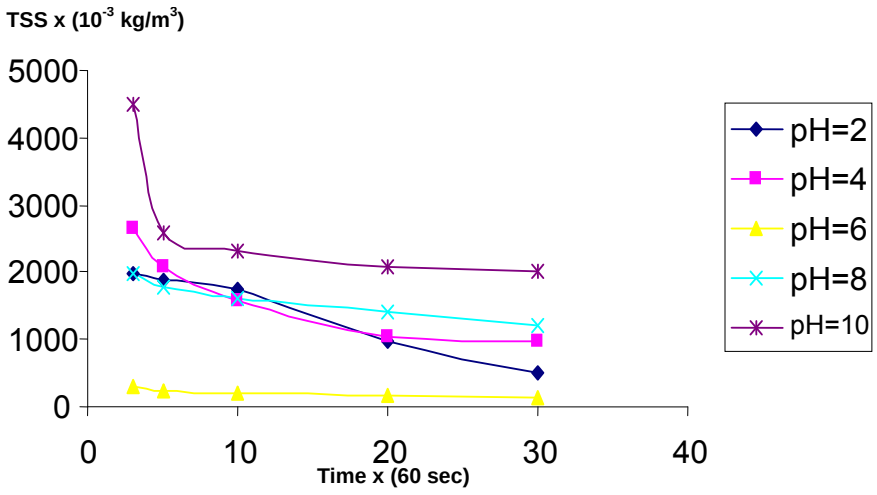

fig4: Plot of TSS vs Coag-flocculation Time for 0.4kg/m^3 PSC at varying pH

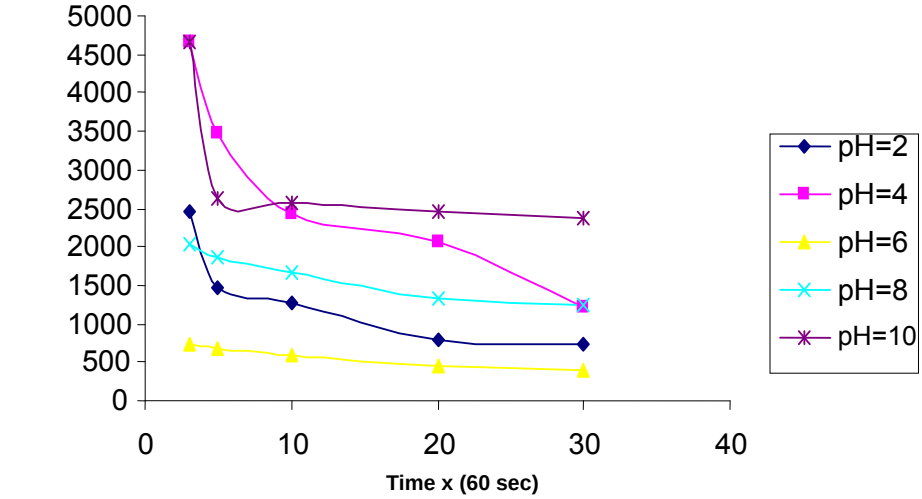

Fig5:plot of TSS vs Coag- flocculation Time for 0.5kg/m^3 PSc at varying pH

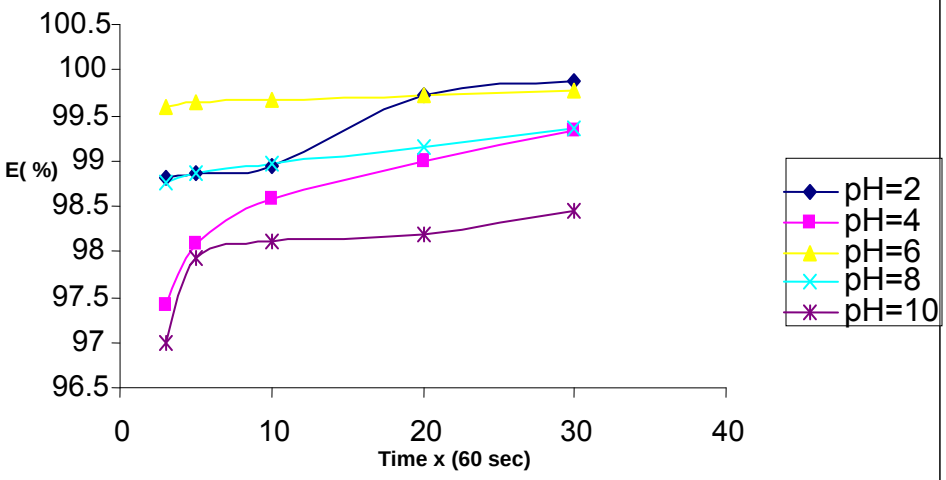

fig 6:Plot of E vs time at varying pH and constant dosage of 0.1kg/m^3 PSC

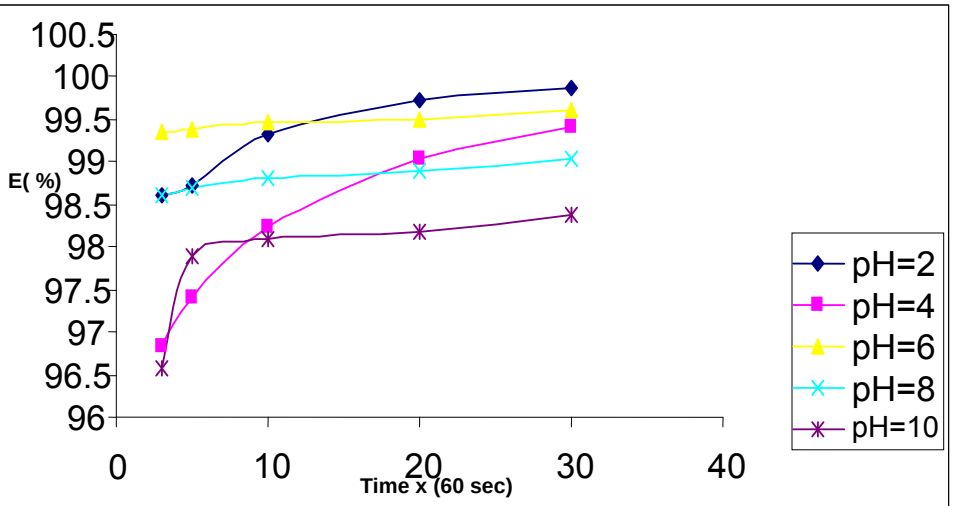

fig7:plot of E vs time at varying pH & constant dosage of 0.2kg/m^3 PSC

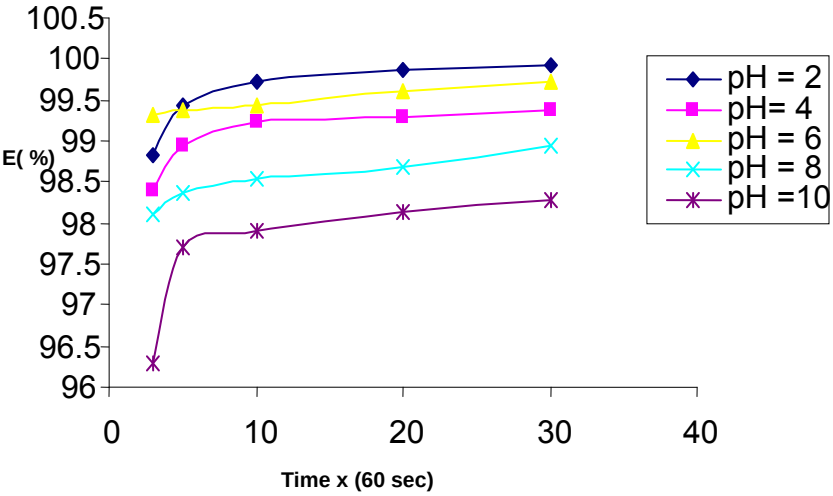

fig8:plot of E vs time at varying pH & constant dosage of 0.3kg/m^3 PSC

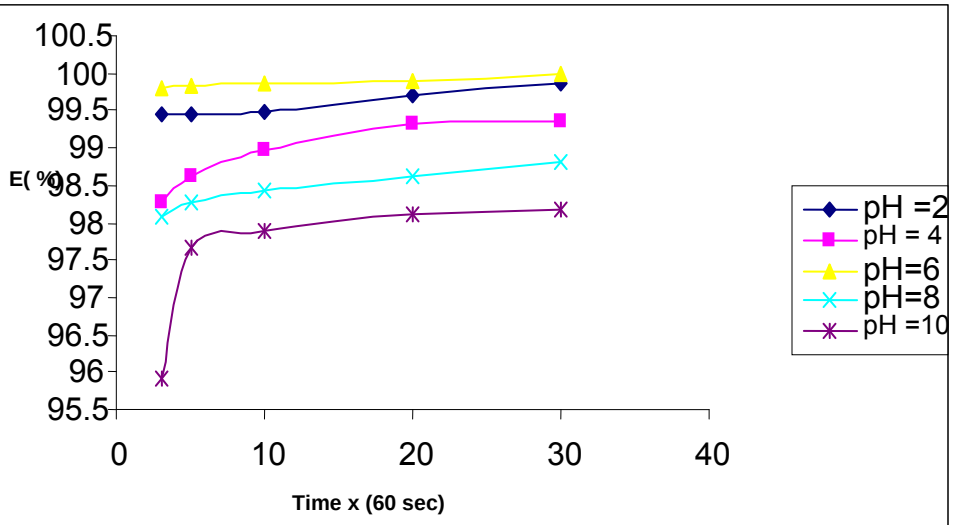

fig9:plot of E vs time at varying pH at constant dosage of 0.4kg/m^3 PSC

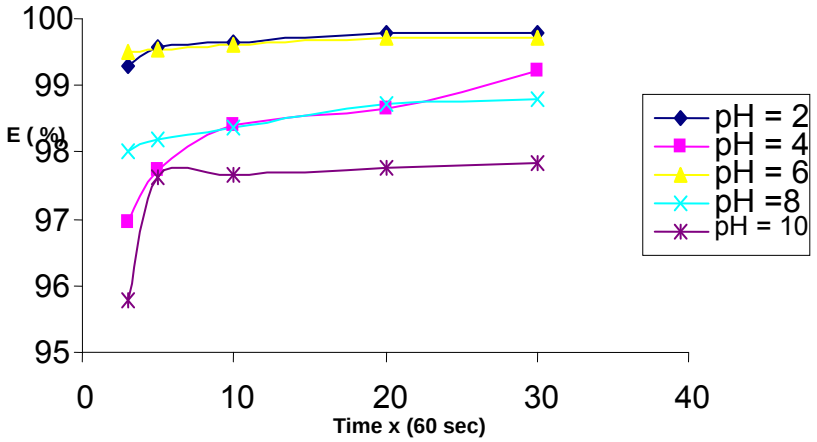

fig10:Plot of E vs time at varying pH & constant dosage of 0.5kg/m^3 PSC

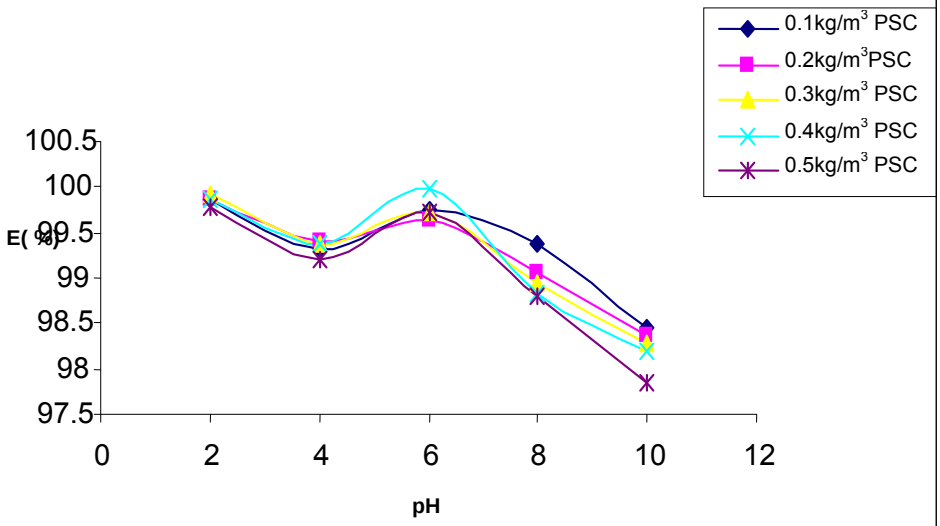

fig11:plot of Coag-flocculation efficiency Vs pH at 30 mins for varying dosages

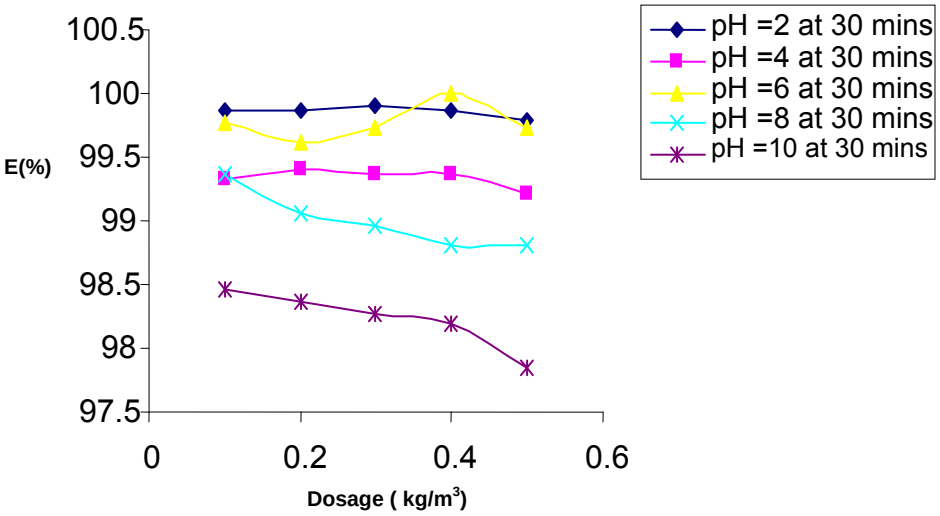

Fig 12:plot of Efficiency vs Dosage at varying pH & constant time

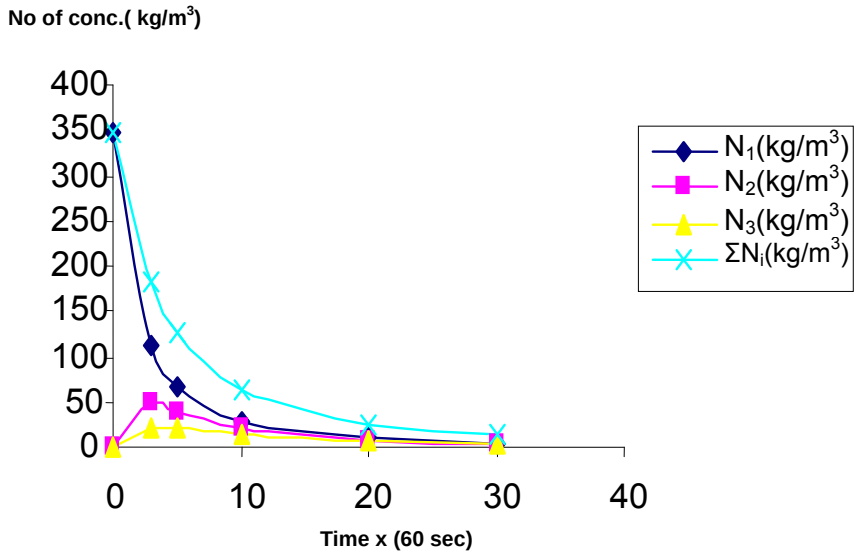

Fig 13:Particle distribution plot for 0.1kg/m^3 PSC at pH of 2

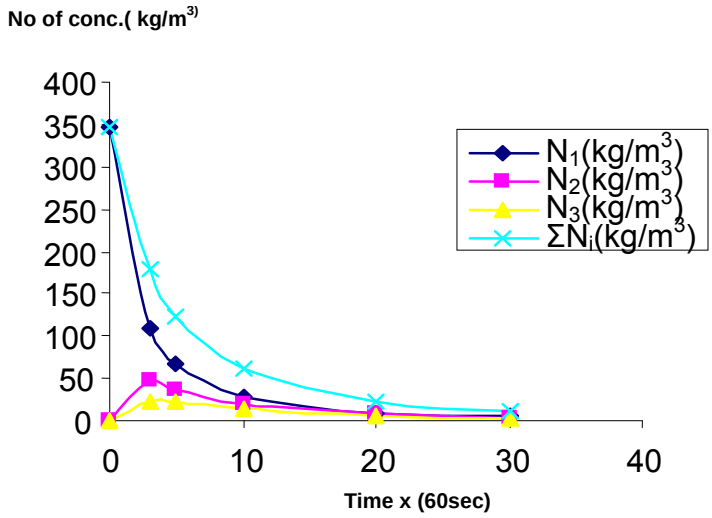

Fig 14:Particle distribution plot for 0.2kg/m^3 PSC at pH of 2

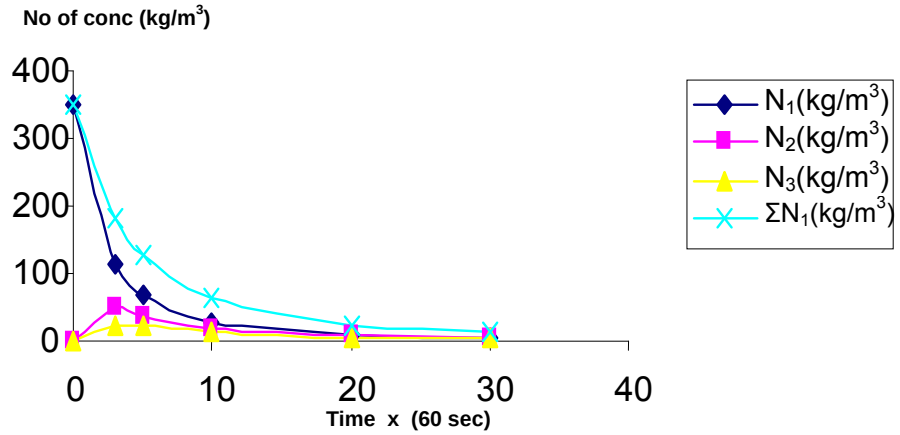

Fig 15: particle distribution plot for 0.3kg/m^3 PSC at pH of 2

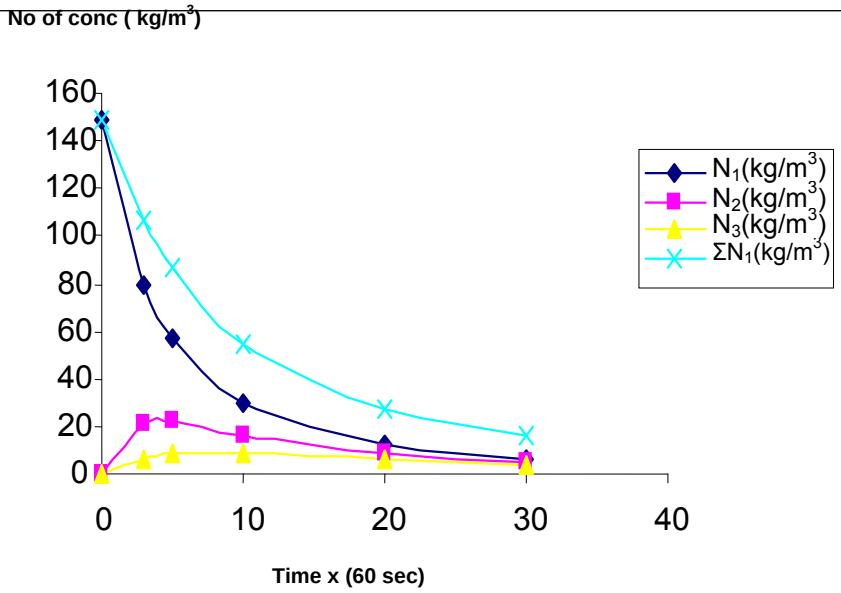

Fig 16: Particle plot distribution plot for 0.4kg/m^3 PSC at pH of 6

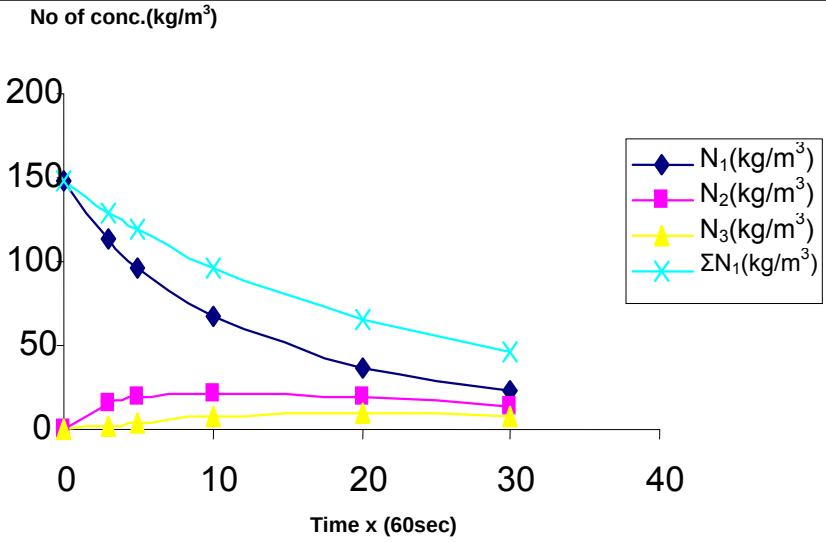

Fig 17:Particle plot distribution plot for 0.5 kg/m^3 PSC at pH of 6

CONCLUSION

The reduction of TSS recorded within the first 5 minutes and high level of E (%) presents the potential of PSC as Chitin derived coagulant that can be utilized in large scale water treatment.

The experimental results with respect to functional parameters highly agree with similar previous works (Jin;2005,Van Zanten and Elimelechi;1992,Holthof *et al* 1996)

NOMENCLATURE:

K :α^{th} order coag-flocculation constant
β_{BR}:Collision factor for Brownian Transport
ε_p:Collision Efficiency
$\tau_{1/2}$:Coagulation Period / Half life
E: Coag-floculation Efficiency
R^2: Coefficient of Determination
α: Coag-flocculation reaction order
$-r$: Coag-flocculation reaction rate

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4/1/2009

Effect of phosphorus nutrition on growth and mycorrhizal dependency of *Coriaria nepalensis* seedlings

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Abstract: *Coriaria nepalensis* Wall. is a common native shrub species of the Central Himalayan region between 1200 to 2500 m elevation. In present experiment growth and mycorrhizal dependency of *C.nepalensis* seedlings was observed under varying levels of phosphorus availability. For this, seedlings of *C. nepalensis* were raised from seeds of current year crop and kept under two inoculation treatments i.e. (i) inoculation of seedlings with VAM fungi and (ii) uninoculated control. For each treatment, there were four sub- treatments i.e. 0, 30, 60 and 90-ppm P_2O_5 levels. Plants were uprooted after 6 and 12 months after germination. Mycorrhizal development was determined by estimating the percentage of roots that were mycorrhizal. Height, dry mass and phosphorus content in different component of seedling were determined for each treatment.The results showed that VAM fungal inoculation resulted in 50-70% mycorrhizal colonization in *C. nepalensis* roots. The growth of inoculated as well uninoculated seedlings increased with increasing phosphorus level. The effect of VAM inoculation was maximum at 30-ppm phosphorus and towards higher P level the effect became insignificant. This indicates that the extent to which the *C. nepalensis* depended on VAM fungi for dry matter production decreased as the levels of soil P increased. [Nature and Science. 2009;7(6):19-24]. (ISSN: 1545-0740).

Key words: Phosphorus, inoculated, uninoiculated, dry mass, colonization

Introduction

The genus *Coriaria nepalensis* Wall. is an important plant having ability to grow and form monospecific stands on severely eroded hill slopes. It is an actinorhizal shrub having dual symbiotic association with nitrogen –fixing actinomycete *Frankia* and arbuscular mycorrhizal fungi (Tewari et al 2003). Being a nitrogen fixing species it depend on an adequate supply of phosphorus to achieve the potential for relatively rapid growth. Presence of mycorrhizal fungi can indirectly affect the carbon balance of the host through their effect on the phosphorus nutrition of the seedlings. They are well known to enhance the P-uptake of seedlings growing under P-poor conditions (Bowen 1973), this altered p nutrition alone have a substantial impact on the host carbon balance.

It is likely that the formation of VAM will be an important factor in the successful establishment of *C. nepalensis* and other mycorrhiza dependent plants (Jasper et al 1989) in the nutrient deficient soil of the degraded lands. Mycorrhizas are commonly associated with increased plant uptake of P from soil, though the response is determined, in part, by the P status of the soil relative to the physiological requirements of the plant. In present study efforts were made to evaluate the growth responses as well as its dependence on VAM fungi to attain maximum growth under varying levels of soil P.

Materials and Methods

Seedlings of *C. nepalensis* were raised from the seeds of the current year crop collected from a stand of *C. nepalensis* located at 1840 m (south-east aspect) near Nainital town (29°22' N lat. and 79°25' E long.) in the Kumaun region of the Central Himalaya. Seeds were rinsed and surface sterilized (30% H_2O_2 w/v for 20 min). These seeds were sown in polyethylene bags containing 1 kg commercial sand (autoclaved twice at 120^{o}C for 1 h, 2 days). After germination seedlings were transplanted in polybags containing 2 kg of sterilized soil and sand mixture. After establishment of seedlings there were two inoculation treatments i.e. (i) inoculation of seedlings with *Frankia* only and (ii) inoculation of seedlings with VAM fungi and *Frankia*. For each treatment, there were four sub- treatments i.e. 0, 30, 60 and 90-ppm P_2O_5 levels.

Mycorrhizal development was determined by estimating the percentage of short roots that were mycorrhizal as described by Reid et al (1983). Five seedlings were harvested just prior to the initiation of the treatment phase. Additionally five seedlings were harvested from each treatment at six and twelve month's interval. Immediately following harvesting, each seedling was separated into foliage, stem and roots. The foliage and stem were oven-dried for 48 h at 80 ^{o} C and weighed. The roots were thoroughly rinsed with tap water to remove adhering soil particles and then nodules and roots were separately oven

dried for 48 h at 80oC and weighed. Plant material was grounded and phosphorus concentration in different component was determined with a spectrophotometer following Jackson (1958).The growth rate (RGR) was determined using the classical growth- analysis equation:

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

where, t is time and W is plant dry weight at time 1 and 2 (Causton and Venus 1981).

Results

Mycorrhizal colonization

The results showed that the VAM fungi inoculum caused mycorrhizal colonization in *C. nepalensis* seedlings in all treatments (Fig.1). The colonization by VAM (MCP) tended to decrease with increasing levels of soil phosphorus (Table 1).

Plant growth

The increase in height and drymass in VAM-inoculated plants compared to uninoculated plants was maximum at P_1 level (16.7% in terms of height and 52% in terms of drymass) followed by plants at P_0 levels (14.8% in terms of height and 33.3% in terms of drymass).Irrespective of inoculation, the height and dry mass of seedlings increased with increasing P level (Table 1). However, the positive effect of inoculation decreased with increasing P level.

Morphological and physiological traits

Leaf weight ratio (LWR) increased with increasing P level in both inoculated and uninoculated plants while root: shoot ratio decreased with increasing P level ((Table 2). At each P level LWR increased in inoculated plants while root: shoot ratio decreased. Leaf number and leaf area per seedling increased with increasing P level and at P level both increased in inoculated plants. While Specific leaf area (SLA; $cm^2\ g^{-1}$) and Leaf area ratio (LAR $cm^2\ g^{-1}$) decreased with increasing P level and at each P level SLA and LAR decreased in inoculated plants (Table 2).

Mycorrhizal dependency

The mycorrhizal dependency(the extent to which a plant benefits from the presence of VAM fungi) of *C. nepalensis* on VAM inoculation increased with the soil P concentration upto P1 level and exhibited the lowest dependence at the highest soil P level (Table 1). The maximum dependence on the VAM inoculation was 34.2 % at P1 level and decreased more than 50% at highest P level.

P content and P utilization efficiency

Significant improvement in P uptake with increasing soil P levels was observed for roots and shoots of both inoculated and uninoculated plants (Fig. 2). The significant increase in p content in inoculated than uninoculated plants was observed at P_0 and P_1 levels and towards higher P levels (P_2 and P_3 levels) the increase was insignificant (Fig. 3). However, the P utilization efficiency was higher in uninoculated plants than their inoculated counter parts, with maximum for uninoculated (1159.51) and inoculated (1053.49) *C. nepalensis* seedlings at P_0 levels (Fig. 4).

Discussion

The response of actinorrhizal *C. nepalensis* inoculated with VAM indicated that this species is able to associate and from a tri-partite symbiotic association. Similar results have been reported for several other leguminous and non-leguminous nitrogen fixing plants (Xie et al 1995). Colonisation by VAM developed in all inoculated seedlings in all P treatments. The taproots as well as laterals adjacent to the taproot were colonised. Only hyphae and vesicles were present and arbuscules were absent. The growth of *C. nepalensis* was substantially increased by addition of phosphorus and by inoculation with VAM fungi. These results suggested that this species depend on VAM for uptake of phosphorus when grown in a nutrient-deficient soil. An increase in the total dry mass yield with increasing soil P level indicates the considerable demands for P by *C. nepalensis*. At each P level mycorrhizal inoculation resulted in an appreciable increase in the growth of seedlings. An increase in growth response due to VAM inoculation has been earlier reported by several worker using different test plants (see Becard and Piche 1989)

The mean concentration of P in the seedlings of *C. nepalensis* at each P treatment in the presence of VAM appeared to be higher than in the seedlings grown in the absence of the VAM. However, differences were not statistically significant towards higher P levels. It is known that phosphorus is a highly immobile element in soil and its demand is much higher than its mobility. Mycorrhizal plants can explore great volume of soil resulting in the increased flow of phosphorus from soil resulting in the increased flow of phosphorus from soil to plants (Webber 1992). Non- mycorrhizal plants growing in low to moderately fertile soils frequently require a supply of soluble phosphate in order to improve their P nutrition and growth. Such improvement in phosphorus uptake by roots occurs through improving physical exploration of the soil.

Root: shoot ratio decrease with increasing P level and at each P level root: shoot ratios were lower in inoculated plants as compared to uninoculated plants. At low P level seedlings plants enhance light interception by means of a high biomass allocation to leaves and the formation of thin leaves with high SLA leading to a high LAR. With increasing P levels leaf thickness increased this increasing photosynthetic capacity of leaf as indicated by decreased SLA and LAR. At each P level VAM inoculation resulted in decreased SLA and LAR.

In order to examine how VAM fungi can benefit *C. nepalensis* and what condition are required for their development, it is essential to understand that plant inoculated with VAM fungi differ markedly in their growth responses (Daft and Nicolson 1969). This growth stimulating effect, referred to as mycorrhizal dependency (Gerdemann 1975) is highly variable (Sanders et al 1977) and influenced by several factors such as plant species (Gerdemann 1968), and soil fertilization (Hayman and Mosse 1971). Mycorrhizal dependency of *C. nepalensis* seedling was maximum at P_1 level and decreased towards higher phosphorus level.

Table 1. Effect of VAM inoculation on growth of *C.nepalensis* seedlings at different P levels. Values in parenthesis indicate MIE (% mycorrhizal inoculation effect)

Treatments	Root colonization (%)	Height (cm seedling^{-1})	Root weight (g seedling^{-1})	Stem weight (g seedling^{-1})	Leaf weight (g seedling^{-1})	Nodule weight (g seedling^{-1})	Total weight (g seedling^{-1})	Relative growth rate (g g^{-1}d^{-1})
P_o	70	27	0.55	0.43	0.86	0.05	1.89	0.0055
P_o + VAM	67	31 (12.9%)	0.65 (15.4%)	0.58 (25.8%)	1.20 (28.3%)	0.13 (61.5%)	2.56 (26.2%)	0.0063
P_1	70	30	0.70	0.50	1.25	0.08	2.53	0.0063
P_1 + VAM	63	35 (14.3)	1.05 (33.3%)	0.85 (41.2%)	1.94 (35.6%)	0.15 (46.7%)	3.89 (34.9 %)	0.0075
P_2	61	32	0.75	0.75	1.67	0.12	3.29	0.0070
P_2 + VAM	58	36 (11.1%)	0.86 (12.8%)	0.88 (14.7%)	2.18 (23.39%)	0.18 (33.3%)	4.10 (19.75)	0.0076
P_3	55	35	0.98	0.76	2.04	0.15	3.39	0.0075
P_3 + VAM	50	39 (10.2%)	1.07 (8.4%)	0.85 (10.5%)	2.37 (13.9%)	0.20 (25%)	4.49 (12.47%)	0.0079

Table 2. Effect of VAM inoculation on various morphological and physiological traits of *C.nepalensis* seedlings at different P levels.

Treatments	LWR	R:S	Leaf (no seedling^{-1})	Leaf area (cm^2 seedling^{-1})	LAR (cm^{2}g^{-1})	SLA (cm^{2}g^{-1})
P_o	0.455	0.426	78	246	130.16	286.04
P_o + VAM	0.469	0.365	84	254	99.22	211.67
P_1	0.494	0.401	80	252	99.60	201.6
P_1 + VAM	0.498	0.390	86	260	66.83	134.02
P_2	0.507	0.309	84	266	80.85	159.28
P_2 + VAM	0.536	0.281	90	272	66.34	119.29
P_3	0.519	0.350	85	268	68.19	131.37
P_3 + VAM	0.528	0.332	90	280	62.36	118.14

LWR= Leaf weight ratio; R:S = Root: shoot ratio; LAR = Leaf area ratio; SLA= Specific leaf area

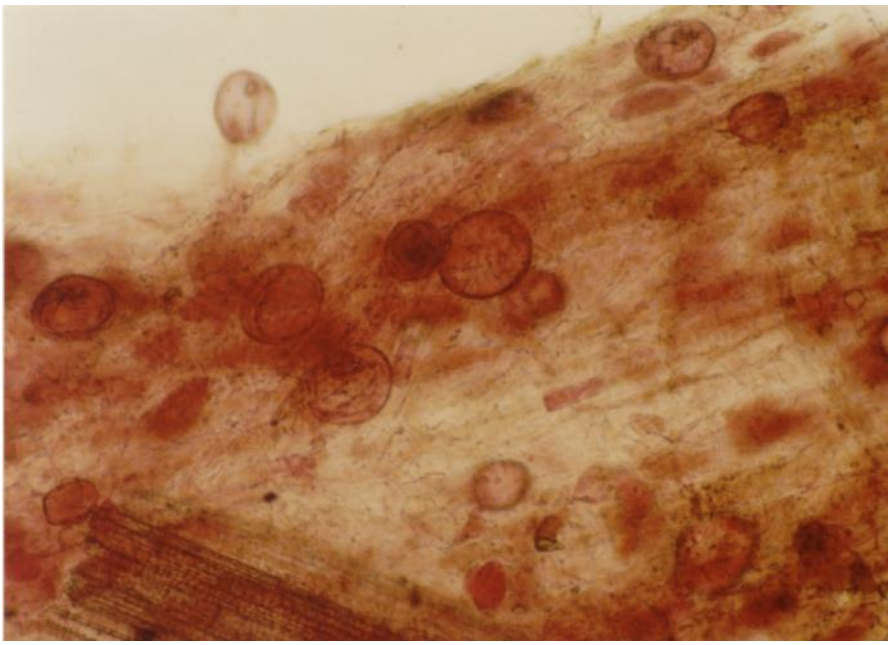

Fig. 1. VAM colonization in *Coriaria nepalensis* roots.

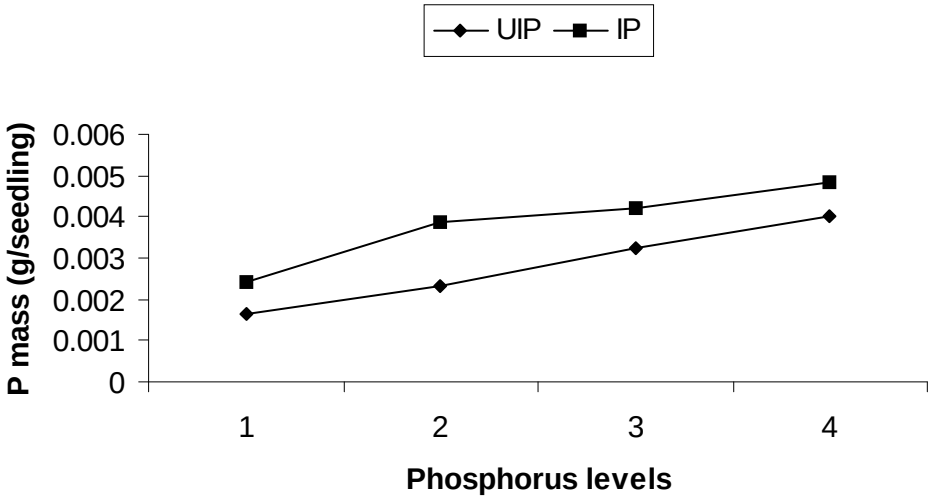

Fig.3. Phosphorus mass of inoculated and uninoculated seedlings of *C. nepalensis* seedlings at different P levels. UIP= Uninoculated plants; IP= Inoculated plants

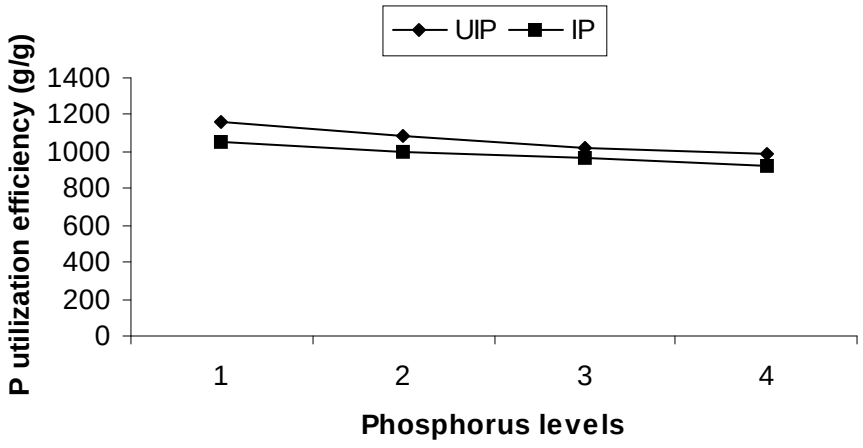

Fig. 4. Phosphorus utilization efficiency of C. nepalensis seedlings as affected by VAM inoculation and P levels. UIP = Uninoculated plants; IP= Inoculated plants

Conclusion

Our results underline the importance of considering VAM as an essential for optimising fertilizer efficiency in the growth increment of N_2- fixing plants like *C. nepalensis* in nutrient-poor soils. Use of VAM as a biofertilizer is a low cost technique and can be helpful in the establishment of plants in degraded lands.

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4/4/2009

Fertilization effects on Competitive abilities of *Quercus floribunda* and *Cupressus torulosa* seedlings

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Abstract: *Quercus floribunda* Lindl. (tilonj oak) and *Cupressus torulosa* D.Don (surai) form mixed oak-conifer forest in many areas of Central Himalayan region. In the present study, competitive abilities of *Q. floribunda*, and *C. torulosa*, were compared along a gradient of nutrient availability. For this, seedlings of the two species were grown in monoculture (intraspecific competition) and mixed culture (interspecific competition). Adding 0, 144, 264, 384, 504 and 624 mg of 20:20:20 NPK fertilizer per kg soil established a gradient of nutrient availability. In both the species the dry mass yield increased with increasing nutrient availability. At each treatment, the dry mass yield of *C. torulosa* was greater than that of *Q. floribunda* and the difference between the two species was greater in mixed culture. The Relative yield (RY), Relative Crowding coefficient (RCC), Relative competition intensity (RCI) and Absolute competition intensity (ACI) indicated that *C.torulosa* is a better competitor for nutrients than *Q. floribunda*. The relative replacement rates suggest that *C. torulosa* is gaining an advantage over *Q. floribunda* with increasing nutrient availability. [Nature and Science. 2009;7(6):25-29]. (ISSN: 1545-0740).

Keywords: *Quercus floribunda*, *Cupressus torulosa*, dry mass, competition, relative yield, relative competition intensity, relative replacement rate.

Introduction

Competition is an important factor affecting the composition of many plant communities (Tilman 1982, 1988). Relative competitive ability may also depend on the supply of the resource in question and it's relation to other resources (Tilman 1982). There are four possible outcomes of pair- wise interspecific competition (de Wit 1960, Harper 1977): i. Both species perform as they do in interspecific competition; ii. One species grows larger in interspecific competion than it does in intraspecific competition, while the other species grows larger in intraspecific competition; iii. Both species accumulate less biomass in interspecific competition than they do in intraspecific competition (mutual antagonism); iv. Both species grow larger in interspcific competition, than in intraspecific competition.

Quercus floribunda Lindl. (tilonj oak) and *Cupressus torulosa* D.Don (surai) form mixed oak-conifer forest at and around Nainital lying at an elevation of 2000 m (29^o 25' N latitude and 79^o 27' E longitude) in Kumaun Himalaya (Central Himalaya) (Rao 1988). While *Q. floribunda* is a climax species (Troup 1921; Champion and Seth 1968) forming extensive forests in many areas of Central Himalaya, *C. torulosa* has most restricted distribution among the Himalayan species (Champion and Seth 1868). The former is a late successional species and latter is regarded as the colonizer of barren area created by landslides, fire and cutting of forests (Dwivedi and Mathur 1978). In this paper the effect of nutrient availability on competitive abilities of these two contrasting species have been compared. Main objectives of this study were: i. to find out whether competition intensity is more in favourable (high nutrient availability) condition as predicted by Grime (1979); and to test whether early successional *C. torulosa* is a better competitor for nutrient as predicted by Tilman (1982, 1986)(as early successional conditions are relatively nutrient-poor than the late successional conditions).

Materials and Methods

Seedlings of *C.torulosa* and *Q. floribunda* were raised from healthy seeds collected from the seed crop of the same year. Seeds were sown in plastic bags (12 cm x 12 cm x 12 cm) holding 2 Kg of a soil-sand mixture. After germination seedlings were thinned to two seedlings per bag. In one set, each bag contained two individuals of one species only (monoculture). In this set there were 9 bags per species per treatment as described below. In the other set each bag contained one individual of each species (mixed culture) and there were 18 bags per treatment.

The soil material used in this experiment was collected from a *Q. floribunda* stand to a depth of 15 cm. The soil was air dried and sieved through a wire mesh screen (mesh size 1 mm x 1 mm) to remove roots and gravel. The sieved soil contained 0.38% N, 0.11% P and 0.14% K The soil was then mixed with washed commercial sand taken from a nearby river bank (containing negligible nutrient) in 1:3 ratio. This

mixture had a pH of 6.6 (measured in a 1:1 soil: water extract). Plastic bags were filled with this soil-sand mixture. A gradient of nutrient availability was produced by adding 0, 144, 264, 384, 504 and 624 mg 20:20:20 NPK fertilizer per kg of soil. Hereafter referred to as N_1, N_2, N_3, N_4, N_5 and N_6 nutrient level, respectively. Plants were watered regularly (at least three times a week). A layer of cotton gauge on the bottom of each bag prevented the soil from being washed away during watering. This experiment was carried out in a glass house at 2000 m a.s.l., where the mean maximum temperature was 1-5^{o}C higher than the air temperature. Bags were kept for apart from each other to minimize any shading.

Seedlings were harvested (three harvest were taken at 8 months interval), separated into leaves, stem and roots, and oven-dried at 80^{o}C to constant weight. Leaves shed were collected and weighed.

Statistical analysis of the data was carried out using SPSS(PC+) statistical package for analysis of variance (ANOVA). Means were compared using least significance difference test (LSD). Data were subjected to analysis of variance. Studentized Range Q. procedures (Snedecor and Cochran 1969) were used for discrimination of means where appropriate.

Relative Competition Intensity was calculated as given in Grace (1995):

$RCI = (P_{mono} - P_{mix}) / P_{mono}$

where, P_{mono} is dry mass yield (g seedling^{-1}) of a species in monoculture and P_{mix} is dry mass yield in mixed culture. The Absolute Competition Intensity was calculated following Grace (1995):

$ACI = P_{mono} - P_{mix}$

Relative Yield Totals (RYT) were calculated RYT = (mean yield of species i in mixed culture/mean yield of species i in monoculture) + (mean yield of species j in mixed culture / mean yield of species j in monoculture). An RYT > 1 indicates growth in mixture exceeds the average growth of each species growing alone i.e. niche differentiation with respect to growth; RYT = 1 indicates the use of identical amount of resource i.e. that competition is not occurring; RYT < 1 implies a mutually antagonistic relationship between the two species. Relative Crowding Coefficients (RCC) were calculated as a measure of competitive ability or aggressivity of one species toward the other: RCC = (mean yield of species i in mixed culture/mean yield of species j in mixed culture) / mean yield of species i in mono culture/mean yield of species j in monoculture). Values of RCC > 1 indicates that species i is competitively superior to species j. The opposite is true when RCC <1.

The relative replacement rates (Vander Bergh 1968; see Bargali 1992) were calculated as follows:

$$\frac{\text{Relative yield of species i at } n^{th} \text{ harvest}}{\text{Relative yield of species j at } n^{th} \text{ harvest}} \;\%\; \frac{\text{Relative yield of species i at } m^{th} \text{ harvest}}{\text{Relative yield of species j at } m^{th} \text{ harvest}}$$

Between harvests, a relative rate > 1 implies that species i is gaining an advantage over species j and values < 1 implies the opposite. In the present study the species i is *Q. leucotrichophora* and the species j is *C. torulosa*. All data are based on dry mass per seedling^{-1}.

Results and Discussion

Dry mass yield

In monoculture as well as mixed culture the drymass yield of both the species increased with increasing nutrient levels (Fig. 1). Analysis of variance indicated that dry mass of seedling was significantly affected by species, competition, nutrient treatment and all their interaction ($P< 0.05$ or 0.01). In monoculture the *Q. floribunda* seedlings has greater dry mass yield than *C.torulosa* particularly towards the lower nutrient gradient, while towards the higher nutrient level the drymass yield was greater for *C. torulosa*. This indicates that being an early successional species *C. torulosa* utilized nutrient according to their availability (Zangerl and Bazzaz 1983). The higher growth rate of *Q. floribunda* seedlings at initial stage was attributed to the higher food reserve in its seeds as compared to *C. torulosa* seeds. However, in mixed culture higher dry mass was reported for *C.torulosa* in all treatments.

When the data of drymass yield were visually analysed for similarity to competitive models given by de Wit (1960) and Harper (1977) then in general model 2 of competitive outcome was observed i.e. one species (*C.torulosa*) grows larger in interspecific competition than it does in intraspecific competition, while the other species (*Q. floribunda*) grows larger in intraspecific competition (Fig. 1). Parrish and Bazzaz (1982) and Bargali (1992) explained that in intraspecific competition, individuals very likely have similarity in genetic identity and consequent limitation in variation in capabilities of using a given resource. However, in interspecific competition individuals are of different species; it is likely that in competition

one will be considerably better than the other at obtaining resource. As a result a clear winner or loser may be expected

Relative competition intensity and Absolute competition intensity

The relative competition intensity and Absolute competition intensity data indicate that the growth of *C. torulosa* was increased in presence of *Q. floribunda* while the growth of Q. floribunda was suppressed in the presence of *C. torulosa.* This effect became more pronounced towards the higher nutrient levels (Table 1).

Relative performance of species in mixture

At each nutrient level as well as harvest the relative yield (the yield of a particular species in mixture over its yield in monoculture) of *C. torulosa* was higher than *Q. floribunda* (Table 2). The quotient of relative yield (*Q. floribunda/ C.torulosa*) showed inconsistent pattern along the nutrient gradient. In all treatments Relative yield total was > 1 indicating the niche differences with respect to growth (Table 2). The RCC values were < 1 in all nutrient treatments indicating that *C. torulosa* was a better competitor than *Q. floribunda* in obtaining nutrients. The relative replacement rate indicates that *C.torulosa* is gaining an advantage over *Q. floribunda* particularly towards higher nutrient levels (Table 2). These results indicate opportunistic behaviour of early successional species as they utilize resources according to availability. According to the R-C-S (Ruderal- Competitor- Stress tolerator) model (Grime 1979) competition intensity is presumed to intense with increasing habitat productivity. Zangerl and Bazzaz (1983) also reported competitive superiority of early successional species in resource- rich environments. In addition at low nutrient level, nutrients are present in low amount and species fail to manifest their genetic differences (Parrish and Bazzaz 1982).

To conclude, the result of this study support some previously observed difference between early and late successional species. Maximum production levels were greater in the early successional *C. torulosa* than they were in the later successional *Q. floribunda*. This is consistent with the trend toward decreased net primary production (Odum 1969) and lower net photosynthetic rates (Bazzaz 1979) as succession proceeds. The difference in dry mass of individuals of the two species was lowest at the lowest nutrient level and increased with increasing nutrient level. Competition between species has been noticed to be more intense at higher nutrient levels (Austin and Austin 1980). The early successional *C. torulosa* generally emerged as the more opportunistic of the two as evidenced by greater utilization of resource in proportion to the availability of that resource.

Table 1. Relative competition intensity (RCI) and Absolute competition intensity (ACI) of *Quercus floribunda* and *Cupressus torulosa* as affected by nutrient availability.

Nutrient level	**Harvest**	**RCI**		**ACI**	
		Q. floribunda	*C.torulosa*	*Q. floribunda*	*C.torulosa*
N_1	H_1	0.05	-0.54	0.10	-0.62
	H_2	0.003	-0.15	0.01	-0.43
	H_3	0.01	-0.14	0.06	-0.67
N_2	H_1	0.03	-0.27	0.07	-0.60
	H_2	0.20	-0.25	1.07	-1.13
	H_3	-0.07	-0.08	-0.44	-0.53
N_3	H_1	-0.25	-0.65	-0.77	-1.56
	H_2	0.14	-0.38	1.00	-2.40
	H_3	0.20	-0.33	1.77	-2.94
N_4	H_1	-0.03	-0.31	-0.17	-0.96
	H_2	0.14	-0.35	0.14	-2.90
	H_3	0.09	0.51	1.03	-5.28
N_5	H_1	0.11	-0.32	0.73	-1.39
	H_2	0.19	-0.41	1.97	-3.89
	H_3	0.10	-0.62	1.22	-7.20
N_6	H_1	0.04	-0.45	0.26	-2.91
	H_2	0.16	-0.32	1.87	-3.93
	H_3	0.14	-0.53	2.15	-7.13

Table 2. Relative yield (RY), quotient of relative yield (QRY) relative yield total (RYT), relative crowing coefficient (RCC) and relative replacement rates (RRR) of *Q. floribunda* and *C.torulosa* seedlings in different nutrient levels. H1, H2 and H3 refer to first, second and third harvest, respectively. All data are based on drymass per seedling (g). Nutrient level increases from N1 to N6.

Nutrient level	**Harvest**	**RY**		**QRY (*Q.flori*/*C.toru*)**	**RYT**	**RCC**	**RRR**
		Q. flori.	***C.toru.***				
N_1	H_1	0.95	1.54	0.61	2.49	0.61	-
	H_2	0.99	1.15	0.86	2.15	0.86	1.41
	H_3	0.99	1.14	0.86	1.13	1.00	1.00
N_2	H_1	0.97	1.27	0.77	2.24	0.77	-
	H_2	0.80	1.25	0.64	2.05	0.64	0.83
	H_3	0.98	1.08	0.91	2.06	0.91	1.42
N_3	H_1	1.25	1.65	0.76	2.90	0.76	-
	H_2	0.86	1.38	0.63	2.24	0.63	0.82
	H_3	0.79	1.33	0.59	1.96	0.59	0.95
N_4	H_1	1.03	1.31	0.79	2.34	0.79	-
	H_2	0.86	1.35	0.64	2.21	0.64	0.81
	H_3	0.90	1.51	0.59	2.22	0.59	0.93
N_5	H_1	0.88	1.32	0.67	2.19	0.67	-
	H_2	0.81	1.41	0.57	2.22	0.57	0.86
	H_3	0.90	1.62	0.56	2.15	0.56	0.97
N_6	H_1	0.96	1.45	0.66	2.41	0.66	-
	H_2	0.83	1.32	0.63	2.15	0.63	0.96
	H_3	0.86	1.53	0.56	2.14	0.56	0.89

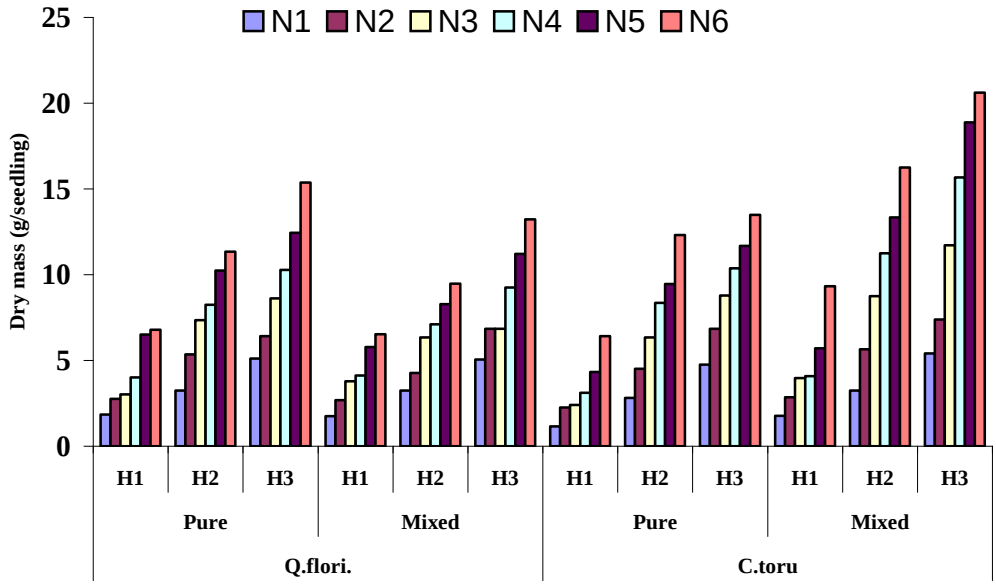

Fig.1. Dry mass of *Quercus floribunda* and *Cupressus torulosa* seedlings as affected by nutrient availabilityH1, H2, H3 denotes harvest 1,2 and 3 respectively; pure indicate monoculture and mixed indicate mixed culture (for detail see materials and methods).

Acknowledgement
Financial support from CSIR, New Delhi is gratefully acknowledged.

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4/4/2009

The allelopathic potential of bryophyte extract on seed germination and seedling growth of *Bidens biternata*

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Abstract: The present work embodies the germination behaviour of *Bidens biternata* in response to different bryophyte extracts. The bryophyte species used were: *Targionia hypophylla*, *Marchantia polymorpha*, *Plagiochasma appendiculatum*, *Brachythecium buchananii*., *Leucodon secundus*, *Timmiella anomala* , *Rhodobryum roseum* and *Plagiomnium integram*. The extracts of these bryophytes were prepared in water (aqueous extract), acetone and methanol (lipophilic extracts) in order to dissolve the bio-active principles present in their thalli. In control experiments seed germination was 100% whereas bryophyte extracts had different degrees of inhibitory effect in different solvents. Complete inhibition was recorded in 100 % acetone extracts of most of the bryophyte species and with the decrease in concentration of extracts, germination increased. Like the acetone extracts, seed germination was also retarded in methanolic extract with the increase in concentrations. The 100 percent lipophilic extract of *Targionia*, *Marchantia*, *Plagiochasma*, *Rhodobryum* and *Plagiomnium* were found most effective where the seed germination was completely checked. Initiation of germination was also adversely affected by the increase in concentration of extract in organic solvents but in aqueous extracts germination was not delayed significantly. Total time taken for the completion of germination varied between the bryophyte species, concentrations and the solvents used. In organic solvent extracts, in some cases, germination was completed if water is added periodically. [Nature and Science. 2009;7(6):30-38]. (ISSN: 1545-0740).

Keywords: Bryophyte, extract, germination, initiation, completion, inhibition.

INTRODUCTION

Bryophytes living on variety of habitats represented a group of plants, which were exposed to different environmental and biotic dangers by virtue of their miniature size. Perhaps, for this reason, many of the secondary metabolites especially terpenoids and phenolic compounds of quite numerous chemical structures are synthesised as defense systems (Herout, 1990). These chemical substances effectively deter herbivores and pathogens from attacking them. Possibly, it may be one of the reasons why so many bryophytes grow in more or less in pure turf and cushions or mats. These allelochemicals also known as allomones have an advantage to the plant that produces them by preventing the growth of other plant species that may compete for soil nutrients, CO_2 and sunlight.

Huneck and Schreiber (1972) reported that allelopathic chemicals obtained from bryophytes possess growth regulatory activities. Asakawa et al. (1976 b) suggested that higher plants sometimes do not grow in these places inhabited by certain bryophytes because some liverwort gives off allelopathic compounds. The crude extracts of bryophytes show inhibitory activity against germination, root elongation and second coleoptile growth of rice in husk, wheat, lettuce and radish. Asakawa (1981,) and Asakawa et al. (1979 a, 1982) reported that sesquiterpene lactones isolated from liverworts had plant growth inhibitory effects on the germination and root elongation of rice husks at concentration of 50-200 µg/ml, while sesquiterpene dial, polygodial and diterpene dials, perrottetianal A and B had a weak inhibitory effect (100-500 µg/ml). Contrary to liverworts, little is known about the secondary compounds from mosses. *Leucobryum glaucum* grows in cushions of a diameter up to 30 cm in forests. These cushions store water for a long time during dry periods and yet young seedlings of forest trees are very rarely found. Similarly, the mats of *Sphagnum* are almost free from other plants. These examples indicate some type of allelopathy between bryophytes and other plants. Gavrillova (1970) reported that aqueous extracts of *Polytrichum commune* and *Sphagnum* spp. inhibited the growth of *Pinus* and *Picea* seedlings, but stimulated the growth of *Larix* seedlings.

The present research was carried out to study the allelopathic effect of different bryophyte extracts on the seed germination and early seedling growth behaviour of *Bidens biternata* (Lour.) Merr & Sherff. was examined. The *B. biternata* is a common weed species of the Kumaun Himalayan region which occupy disturbed as well as undisturbed habitats of the region. It produces a large number of seeds with 100% germination capacity which is resulting in rapid spread of this species over a large area. The objective of this experiment was to find natural herbicides for the biological control of *B. biternata* to reduce the risk of manufactured herbicides.

MATERIAL AND METHODS

Collection and identification of bryophytes

Three liverworts viz., *Marchantia polymorpha* L., *Targionia hypophylla*, and *Plagiochasma appendiculatum* Lehm. et Lindeb. and five mosses viz. *Leucodon secundus*. Schwaegr., *Rhodobryum roseum* (Schimp.) Limpr., *Plagiomnium* integrum Hedw., *Timmiella anomala* (De Not.) Limpr. and *Brachythecium buchananii* have been selected to study their effect on germination behaviour of *B. biternata* seeds.

The bryophytes were collected by regular and repeated local field trips at different localities of Nainital. The plants were collected in the first week of August. Fresh plants, devoid of dead tissue and with proper reproductive organs (to aid in identification), were collected. The plants were freed from contaminant parts of other plants, if present, and were carefully scooped out. Plants thus collected were kept in separate polyethylene bags and sealed immediately. The collected plant material was sorted out on the basis of their characters and identified following Kashyap (1929) and Gangulee (1972, 1977, 1980).

Collection of *Bidens biternata* seeds

The seeds of *B. biternata* were collected in July from monospecific stand of *B.biternata* situated at Ayarpatta hill at Nainital. Seeds were packed in polyethylene bags, brought to the laboratory, air dried and healthy seeds were sorted out.

Preparation of bryophyte extract

The bryophyte material was washed to remove adhering soil particles and blotted. As specimens were small and were not collected in large amounts due to conservation viewpoint, extracts were prepared from entire green part of the thallus. Water, methanol and acetone were used as extracting solvents. For preparing extract 5 g fresh material of bryophyte was ground with a pinch of sand in mortar to yield a pulp and dissolve in 50 ml of solvent and shaked in rotary shaker (200 r.p.m.) for 1 h, and filtered with Whatman No. 1 filter paper. Final volume of the extract was made upto 100 cc by adding respective solvent and considered as full concentration (100%). Then this extract was diluted to 70%, 50% and 20% concentration.

Experimental design

For each bryophyte species and for each extract the petri plates were prepared in triplicate. The petri plates were lined with a thin layer of cotton and filter paper and sterilised. In each petri plate 20 seeds of *Bidens* were placed to observe the germination behaviour in various concentrations of bryophyte extracts in different solvents. Control plates were also prepared in the same manner for each test. In each Petri plate 10 cc of extract was poured. For organic solvent after pouring 10 cc extract in Petri plate the solvent was evaporated aseptically at 35 ^{o} C and then 20 seeds were placed and 5 cc of distil water was added. It was assumed that active principles dissolved in organic solvent were absorbed in filter paper. In control experiment of each concentration solvents of same concentration but without bryophyte extract were used. The experiments were done at room temperature (20^{o} – 22^{o} C) and were carried out for 30 days. The seeds were considered germinated if the radical exceeded 3 mm in length.

RESULTS

The allelopathic potential of different bryophytic extracts on germination of *Bidens biternata* is shown Table 1.The germination of *Bidens biternata* seeds was hundred percent in each of the control experiments. However, different degrees of germination were found in various concentrations of

bryophytic extracts. The hundred percent lipophilic (acetone and methanol) extracts of the species of *Targionia*, *Plagiochasma*, *Brachythecium*, *Rhodobryum*, *Marchatia* and *Plagiomnium* checked the germination completely, while in the extracts of the remaining species percent germination ranged between 32% (methanolic extract of *Rhodobryum*) and 100% (acetone extract of *Plagiomnium* and methanol extract of *Leucodon* and *Brachythecium*). The 70 and 50 percent methanolic extract of liverworts was as effective in inhibiting the seed germination as 100 percent extract. The methanolic extract of *Brachythecium* had no inhibitory effect on seed germination. Further, 20 percent extract of *Targionia* was effective in checking the seed germination while the extracts of rest of the species were totally ineffective. Irrespective of the concentrations, the water extracts of all bryophyte species except *Rhodobryum* were ineffective in controlling the seed germination.

In controlled conditions, germination was initiated within 24 hours, while it was delayed by 1 (*Marchantia*) to 18 days (*Plagiochasma*) in lipophilic extracts of liverworts. For the extracts of mosses, radicle initiation occurred between 5 and 14 days (Table 2). The methanolic extracts of *Brachythecium* delayed the germination by 5 days, irrespective of the concentration. In aqueous extracts, initiation was delayed from 1 day (*Rhodobryum* 70%) to 8 days (*Marchantia* 100%).

In controlled condition, the germination was completed within 48 h. However, for liverworts in most of the cases, irrespective of concentration, germination was not completed in lipophilic extracts till the culmination of the experiments (Table 3). The exception was *Plagiochasma*, where germination was completed between 3 and 7 days in acetone extract. For mosses, the effect of acetone extracts of *Leucodon*, *Timmiella* and *Brachythecium* was most promising with regard to the inhibition of germination, while in methanolic extract of *Plagiomnium* and *Rhodobryum* germination was not completed. Contrary to this, in the aqueous extracts of bryophytes germination of *B,biternata* seeds was completed between 10 and 4 days (*Marchantia* and *Targionia*) in liverworts and between 3 (*Plagiomnium*) and 9 (*Rhodobryum*) days in mosses.

Table 1. Percent seed germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent											
	Acetone (%)				Methanol (%)				Water (%)			
	100	70	50	20	100	70	50	20	100	70	50	20
Liverworts												
T.hypophylla	-	-	56	76	-	-	-	-	92	80	60	80
M.polymorpha	88	88	92	96	-	-	-	96	100	100	100	100
P.appendiculatum	-	100	100	100	-	-	80	88	100	100	100	100
Mosses												
L. secundus	-	-	-	84	100	60	100	100	100	100	100	100
T. anomala	56*	64	76	100	32	-	-	100	100	100	100	100
R. roseum	-	100*	100*	100	32	-	-	28	100	100	100	64
P. integrum	100*	44*	72*	100	-	-	-	28	76	84	80	84
B. buchananii	-	-	-	80	100	100	100	100	100	100	100	100

* = After the addition of sufficient water (10 cc)

Table 2. Number of days taken for initiation of seed germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent used											
	Acetone				Methanol				Water			
	100	70	50	20	100	70	50	20	100	70	50	20
Liverworts												
T.hypophylla	-	-	07	07	-	-	-	-	04	04	03	03
M.polymorpha	11	08	01	01	-	-	01	01	08	02	05	04
P.appendiculatum	-	18	03	03	-	-	04	04	03	03	03	03
Mosses												
L. secundus	-	-	05	05	05	05	05	05	04	04	04	03
T. anomala	-	-	08	08	06	05	05	05	05	04	04	04
R. roseaum	11	11	09	09	07	-	09	09	04	01	04	04
P. integrum	-	09	07	07	-	-	14	08	07	07	07	07
B. buchananii	-	-	-	05	05	05	05	05	04	04	04	03

Table 3. Actual number of days taken for completion of germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent used											
	Acetone				Methanol				Water			
	100 %	70%	50%	20%	100 %	70%	50%	20%	100 %	70%	50%	20%
Liverworts												
T.hypophylla	-	-	-	-	-	-	-	-	04	07	05	-
M.polymorpha	05	-	-	-	-	-	-	-	10	08	06	-
P.appendiculatum	-	03	03	07	-	-	-	-	07	06	07	-
Mosses												
L. secundus	-	-	-	-	06	06	06	06	06	06	05	06
T. anomala	-	-	-	-	08	-	04	06	06	07	07	06
R. roseaum	-	-	-	05	-	-	-	09	08	09	08	09
P. integrum	-	05	06	04	-	-	-	-	03	04	05	-
B. buchananii	-	-	-	08	06	06	06	06	06	06	05	06

Table 4. Relative performance of seed germination in *Bidens biternata* in different bryophyte extracts as compared to control.

Species	SOLVENTS			
	Concentration of extract (%)	Acetone	Methanol	Water
T.hypophylla	100	-	-	0.92
	70	-	-	0.80
	50	0.56	-	0.60
	20	0.76	-	0.80
M.polymorpha	100	0.88	-	1.00
	70	0.88	-	1.00
	50	0.92	-	1.00
	20	0.96	0.96	1.00
P.appendiculatum	100	-	-	1.00
	70	1.00	-	1.00
	50	1.00	0.80	1.00
	20	1.00	0.88	1.00
L. secundus	100	-	1.00	1.00
	70	-	0.60	1.00
	50	-	1.00	1.00
	20	0.84	1.00	1.00
T. anomala	100	0.56	0.32	1.00
	70	0.64	-	1.00
	50	0.76	-	1.00
	20	1.00	1.00	1.00
R. roseaum	100	-	-	0.84
	70	1.00	-	0.80
	50	1.00	-	0.60
	20	1.00	0.28	0.64
P. integrum	100	1.00	-	0.76
	70	0.44	-	0.84
	50	0.72	-	0.80
	20	1.00	0.28	0.84
B. buchananii	100	-	1.00	1.00
	70	-	1.00	1.00
	50	-	1.00	1.00
	20	0.80	1.00	1.00

Table 5. Seedling size of *Bidens biternata* as compared to control in different bryophytic extracts.

Species	SOLVENTS			
	Concentration of extract (%)	Acetone	Methanol	Water
T.hypophylla	100	-	-	1.09
	70	-	-	1.17
	50	0.46	-	1.01
	20	0.64	-	1.02
M.polymorpha	100	1.09	-	1.27
	70	1.09	-	1.27
	50	1.10	-	1.27
	20	0.81	0.86	1.28
P.appendiculatum	100	-	-	0.98
	70	0.84	-	1.00
	50	0.71	0.87	0.98
	20	0.51	0.84	1.02
L. secundus	100	-	1.24	1.37
	70	-	1.17	1.38
	50	-	1.09	1.29
	20	1.28	1.09	1.29
T. anomala	100	1.11	1.07	1.43
	70	1.12	-	1.50
	50	1.18	-	1.42
	20	1.11	1.24	1.42
R. roseaum	100	-	-	1.24
	70	1.05	-	1.24
	50	1.05	-	1.19
	20	1.21	0.88	1.17
P. integrum	100	0.91	-	1.33
	70	0.87	-	1.10
	50	0.89	-	1.33
	20	0.84	0.85	1.27
B. buchananii	100	-	1.07	1.25
	70	-	1.08	1.56
	50	-	1.08	1.56
	20	1.05	1.08	1.33

DISCUSSION

The present work examines the growth regulatory activities of three liverworts (*T. hypophylla, M. polymorpha* and *P. appendiculatum*) and five mosses (*L. secundus, T. anomala, R. roseum* and *P integrum B. buchananii*) on germination behaviour of *B. biternata* seeds. Irrespective of the concentration, in aqueous extracts of all bryophyte species germination was approximately 100 %. However, in lilophilic (acetone and methanol) extracts germination of *B. biternata* exhibited marked variations and declined with the increase in concentration of lilophilic extracts of bryophyte. This has indicated that allelochemicals (terpenoids and phenols) present in the tissues of bryophytes were not soluble in water. In present study, methanolic extract appeared to be more effective than the acetone extract. The methanolic extract of

Targionia was the most effective where germination was completely inhibited. Chemicals with allelopathic activity are present in many plants and in various organs and have potential as either herbicides or templates for new herbicide classes (Duke et al 2000).

At higher concentration of acetone soluble extracts (100 %) germination percentage was generally low and germination was accomplished only in *Marchantia*, *Timmiella* and *Rhodobryum*. In rest of the extracts, seeds failed to germinate. In *Timmiella*, *Rhodobryum* and *Plagiomnium*, germination was brought out only after the addition of sufficient water at regular intervals. On the other hand, methanolic (100 %) extracts of *Targionia*, *Marchantia* and *Plagiochasma* exhibited complete inhibition. The results indicate that these mosses contain the terpenoids, phenols or other compounds which are more readily soluble in acetone rather than methanol. Asakawa (1990) reported a group of terpenoids isolated from liverworts that exhibited complete inhibition of germination in rice in husk. Exceptional seed germination was observed in 100 percent methanol and acetone extracts of *Timmiella*, *Rhodobryum* and *Plagiomnium* possibly due to the dilution of the inhibitory effect of the active principles by addition of water at regular intervals. Mosses usually lack mono- and sesqui- terpenoids in their tissues but they are rich in steroids and flavonoids (phenolic) and it is likely that these compounds may somehow be responsible for the allelopathic effects observed in the present study. Time taken for the initiation of germination of seeds increased with the decrease in concentration of extracts. In aqueous extracts of all the experimental species, no significant difference was observed in time for the initiation of germination. At higher concentration of lipophilic extract, germination was maximally delayed up to 15 days (*Plagiochasma*, 70 % alcohol extract) compared to the water extract. In *Rhodobryum* and *Plagiomnium* extracts, germination was initiated only after the addition of water.

Total time taken for the completion of germination of seeds varied according to the type of the extracts. Variation in total germination period appears to be independent of the concentration of the moss extract. Some other factors like size of the seeds may be responsible for delaying the completion of germination in certain extracts. Among liverworts, maximum time was taken by *Marchantia* (10 days in 20 percent aqueous extract).

Relative performance of *B. biternata* seed germination in different bryophyte extracts as compared to control was mainly inhibitory (Table 4). Among the extracts of liverworts, maximum inhibition was shown by *Targionia* as it checked the seed germination completely in 70 and 100 percent acetone extracts, 20 to 100 percent inhibition in methanol extract and about 10 percent inhibition in aqueous extract (Table 1). The allelopathic effect of *Targionia* on seed germination may be attributed to the allomones present in their thalli. Sharma (1999) reported about 22 terpenoids in *Targionia hypophylla*, collected from Nainital. Some of the major components are α-pinene, bi- cyclogermacrene, plagiochiline N, Vitranal, eucarvone etc. Also, *Targionia* generally grows as a pure patch in nature. Sometimes few mosses and rarely angiosperms are intermingled with it thus, indicating the presence of volatile allelochemicals in their tissues which appeared to be unfavourable for the growth of other plants in near vicinity of this plant. In addition to *Targionia*, *Brachythecium* and *Leucodon* exhibited complete inhibition in 50, 70 and 100 % acetone extracts. *Rhodobryum* and *Plagiomnium* showed 20 to 40 % inhibition in seed germination in water extracts.

Effect of bryophyte extract (as compared to control) on seedling size is given in Table 5. Irrespective of the concentration, the extracts of liverworts retarded the seedling size more in comparison to the moss extracts. Among all bryophytes, *Plagiochasma* and *Targionia* extracts were most effective in reducing the seedling growth. This may be due to the presence of large number (27) of terpenoids in the tissues of *Plagiochasma appendiculatum* of this locality (Joshi 1999). The major compounds are β –pinene α-elemene, thujopsene, bicyclogermacrene, Cuparene etc. Several modes of action attributed to allelopathic compounds include effect on cell elongation and ultrastructure of roots by inhibition of cell division (Rice 1984). Among solvents, generally the acetone extract was more effective in reducing the seedling growth. However, no significant effect of concentration of extract was observed.

Struggle for space and nutrients for propagation, continuity and university is the most powerful law of nature. In this trend, some plants have allelopathic potential by releasing allelochemicals to their surrounding that have deleterious or beneficial effects on other plants. The compounds are released to the environment by mean of volatilization, leaching, decomposition of residues and root exudation. These compounds inhibited plant growth by affecting many physiological processes. The degree of inhibition depends on their concentration. Results of present experiment indicate that the bryophyte extracts had

inhibitory potential in reducing and checking seed germination of *B. biternata* and can be used in biological control of this weed species.

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4/4/2009

Conservation and Management Study of High Altitude Nainital Zoo (Uttarakhand) With Special Reference to Ecotourism

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Abstract: The conservation and management of wild species in Zoo need a proper planning and expenditure, which provides us the information of our nature gift of wild life and promotes the ecotourism activities. The Zoo management and wild life requires new research planning for their better management and sustainability. [Nature and Science. 2009;7(6):39-42]. (ISSN: 1545-0740).

Key Words: Wildlife, Management, Conservation and Planning

Introduction:

Many wild species both plant and animals have become extinct from their natural habitats, because of destruction of forests as well as the other natural unit areas by the anthropogenic pressure. Therefore the remaining species are not in a position to cope up with the changing conditions of the environment. Expanding human population resulted in to expanding needs of man with the scientific progress and technology development; man started utilizing natural resources at a much larger scale. He also the main source for its recovery, such as significant resources of energy and cause minimum disturbance to the different elements of the life support system. Conservation and management of wildlife not only preserving the remaining flora and but also helping in promoting economic activities that brings money through tourism. It also contributes towards maintenance of biodiversity of landscape. Many wild life such colorful birds, animals and other forms of life in the forest are the important in maintaining the ecosystem. Distraction of forests will cause disappearance of much wild life. Therefore it imperative to conserve and manage the forests as well as wildlife, which are in endangered and threatened condition, this also needs both conservation and management programme. The ministry of environment and forests of India has setup the separate wild life department for coordination between states and the central for speedy and faithful implementation of the steps to be taken in programme of wild life management in the country (Gopal 1992). As far as the concern of Indian region it has total area of 32 million hectares with rich in biological diversity. As we know that India is one of the12 mega biodiversity centers in the world. It is estimated that about 45,000 species of plants comprise 15,000 species of which several hundred species are endemic to India. In 1976 the 42nd constitutional amendment acts (CAA) the forestry and wildlife are put in on the concurrent list. This has enabled the government of India to directly take the decision on the forest and wildlife conservation practices (Khanna, 1994). The origin of Zoo may be said to have commenced with the opening of the London Zoo in 1828, most of the older Zoos in North America and Europe were founded in the later part of the 19th centaury after 1870. During that period animal species were being regularly discovered and the various Zoo were keen to collect as many different kinds of animals as possible for public display. These animals were housed in cages behind or in small pits or in- enclosure having compounds, which did not differ much from the old menageries of the mediaeval times (Sharma, 2000).The Zoo movement in India is also one of the oldest in the world. The first Zoo was setup in Madras in the year 1855, which was seen followed by Trivandrum (1887), Bombay (1863), Hyderabad (1959) and Assam state at Guwahati (1960). During the last 30 years modern building material have been extensively used in the construction of animal enclosure. This is resulted in better ventilation and living space. Apart from improving the public viewing with the advancement of scientific knowledge pertaining to the biological requirements of captive animals. So as to facilitate captive breeding an ideal enclosure should enable on animal to freely display its various behavioral patterns. The nature based tourism involves education and interpretation of the ecologically sustainable natural environment. Ecotourism is viewed as a means of protecting natural areas through the generation of revenues, environmental education and the participation of local people. In such a ways, both conservation and management would be promoted in sustainable forms. The main objectives of study were given below. (i) To study the diversity of animals & birds (ii) to study their conservation and management procedure (iii) to study the relation between Zoo, ecotourism, education and awareness programme among the people.

Study area and Methodology:

Nainital is situated at 1938 m from sea level in central Himalaya of Uttarakhand state. Mr. P.

Barren of Shajapur known as 'Pilgrim' was the first man who discovered the Nainital. The well known Nainital lake comes in the category of National lake. The area of the lake is 487639.40 m^2 and its perimeter is 3429 m. The length is 1372 m and breadth is 366 m. (Rawat, 1998). The Nainital zoo is situated at an elevation of 2100 m above mean sea level on the hill of Sher – ka – Danda where the mountain quail was last seen in 1876. this is also known as Pandit Govind Ballabh Pant high altitude Nainital zoo.The zoo was established in 1984 and is spread over an area of 4.69 ha. The zoo was declared open to public on 1st June 1995 and is managed by "The Bharat Ratn Pandit Govind Ballabah Pant High Altitude zoo Management Society" from 1st March 2002. The main objectives of zoo are to conserve the high altitude Himalayan birds and animals which all endemic and endangered and to create awareness about our rich Himalayan fauna among the journal people. The zoo has also been created to facilitate the research and coordinate breeding of endemic and endangered Himalayan fauna and flora of many species of trees, shrubs and herbs.

Methodology:

According to study plan we visited weekly and collected the respective data based our objectives. Thereafter we developed the data tables for the finding obtained from the zoo like animals and birds details, expenditure for each animal and birds, category of tourists and their numbers, amounts earned from the tourists. Apart from this, we also studied the persons and organizations who adopted the zoo animals and birds for their annual expenditures.

Results: Conservation and Management

Zoo is an important ex – situ conservation procedure. It is for not only the conservation and management of endangered, rare and threatened wild animals and birds but also play a very crucial role in the ecotourism, education and public awareness about the wildlife. As far as Nainital zoo is concerned, it is an only zoo many species of birds and animals belongs to the high Himalayan regions is being conserved. The following animals and birds are being managed by zoo are:

1. **Carnivorous**
   - Siberian Tiger *(Panthera tigris)*, Snow leopard *(Panthera unica)*, Leopard (Panthera pardus), Leopard Cat *(Felis bengalensis)*, Tibetan Wolf *(Canis lupuschanco)* and Hill fox *(Valpus vulpus Montana).*
2. **Omnivorous**: Himalayan Black Bear *(Selenarctos hribetanus)*, Palm Civet Cat *(Pagumo larvata).*
3. **Primates**: Japanese Macaque *(Macaca fuscota)* and Bonnet macaque *(Macaca radiata).*
4. **Herbivorous**: Sambar *(Cervus unicolar)*, Sika Deer *(Muntiacus muntijak)*, Goral (Nemorhaedus *goral)*, Serow *(Crpricornis sumatraensis)*, and Rodents.
5. **Birds:** (a) Pheasants: Lady Amherst pheasant *(Chrysodophys amherstae)*, Silver pheasant *(Pucrasia macrolopha)*, Kalij pheasant *(Lophura leucomelanos)*, Japanese Green pheasant *(Phasianus versicolar versicolar)*, Chinese Ring Neck pheasant *(Phasianus colenicus)*, Glden Pheasant *(Chrysolophus pictus)*, Silver Golden Pheasant *(Syrmaticus humiae humiae)*, Brown eared pheasant, Bornean Fireback *(Lophura lgnita)*, Linnneated Kalij pheasant *(Lophura hycthemira lineate)*, Edwards Pheasant *(Lophura Edwards)*, White Pea Fowl *(Pavo mutant)*, red Jungle Fowl *(Pavo cristatus)* and Swinhoe.

   (b) Parakeets: Rose Ringed Parakeet *(Psittacula krameri)* and Blossom Headed Parakeet *(Psittacula cyanace phala).*

   (c) Prey Birds: Steppe Eagle *(Aquiola napalensis)*, Indian Great Horned Owl *(Bubo bubo)* and Spotted bellied Eagle owl.

   (d) Partridges Small Birds: Hill partridge *(Arborophila torqueala)*, Scaly Brested munia *(Lonchura punctulata)* and Red Advadat.

Zoo Management:

Zoo management is a very complicated task. It needs continuous watch and supervision of zoo animals and birds in the context of their diets and health care and sanitation works. The zoo authority and concerned zoo staff performed all these tasks regularly. Certain works performed daily by the zoo staff are mentioned below.

1. **Checking of Flesh (Meat) for carnivores and food for Herbivores and omnivores:**
   Before providing flesh to carnivores, it is examined time to time by the experts. Available flesh should be odorless, proper in color and un- chopped. Food material like fruits, vegetables, green vegetables and millets are given to the herbivores and omnivores. The detail of animals and birds diet is given the following table.

Diet Status of Different Animals in Nainital Zoo

S.No.	Animal	Food Material	Amount (Kg.,gm./ Day)	State
A. Carnivores				
1.	Siberian Tiger	Beef	11kg.	Un - Chopped
2.	Leopard	Mutton	3 to5 kg.	Un - Chopped
3.	Leopard Cat	Mutton	3 to 5 kg.	Un - Chopped
B. Omnivores				
4.	Cicvet	Beef& Banana	250 & 200 gm.	Boiled & Fresh
5.	Wolf	Flesh	2 to 2.8 kg.	Boiled
6.	Hill Fox	Flesh	600 gm.	Boiled
C. Herbivores				
7.	Bear	Vegetables, Fruits, Gram	Twice in a day	Fresh
8.	Monkey		Morning & vening	Fresh
9.	Sambhar, SikaDeer, Goral, Barking Deer & Serow		Every day	Fresh
D. Birds				
10.	Pheasants	Chopped vegetables, wet wheat, cereals and chopped onion garlic	200 gm.	Fresh
11.	Munia, Eagle & Great Horned Owl		200gm. Everyday	

2. **Sanitation of cages:** Cleaning of cages, management of drinking water, cleaning of animal sheds and proper sanitation of bird's cages is very necessary.
3. **Facilities regarding to animal health:** Health facilities are considered to be the backbone of the zoo. The wild animal kept in zoo are mostly rare and threatened, therefore protection is given to them and their progeny in the zoo.
4. **Disease:** The disease are spread by the inhalation, food bite of animals and cuts in the skin, contaminated instruments, via coital and polluted water.
5. **Quarantine:** Whenever a new animal is brought in the zoo, it is kept alone 20-25 days.
6. **Preventive measures:** Vaccination for rabies and foot and mouth diseases should be done every year**.**
   - Injection for hoof disease should be given before monsoon.
   - Anti – worm medicines should be given to the animals in the 3-4 months time. Their fecal matter should also be checked from time to time.
   - Blood test for blood protozoa should be done before the on set of monsoon, and the injection is given.

7. **Tranquilizing of Wild Animals:** It is very difficult to control an injured wild animal because they feel helpless and become aggressive. Therefore, medicines used for the control of wild animals by the giving the injections of Kitamin and xylazine. They should be given in the ratio of 1:1 or 1:2. The injections completely paralyze the animal for 10 – 15 minutes. During this period the possible treatment is given.

Eco – Tourism Activities:

Among tourists visited the, children (5-12 years age) and adult (>12 years) were 20.75 % and 79.3% respectively. Total numbers of tourists visited in the zoo during 2004 – 2005 were 94,884. Out of them maximum tourists (26.6%) were visited in the month of June, 2005 and followed by the month May, 2005 (15.9%). However, very small number of tourists came in the month of February 2006.

As far the economy is concerned, zoo authority earned total rupees seventeen lakh and one

thousands forty only (Rs. 17, 01040=00). Of this amount received from adult and children tourists were 88.4 and 11.6% respectively. Of this total amount, about 26.3 % were earned during June 2005. The amount, 29.8 and 25.8 % came from children and adult tourists, respectively.

Conclusion

As our findings showed that the Zoo animals and birds needs proper management planning because the zoo has such species, which are rare, endangered and threatened in the natural habitats. Therefore such species either they belongs to the animals or to the birds category requires lot of investment for their look after for diet treatment from breeding activities. Managing the wild life species in Zoo also aware us and encourage our education and economy through the activity of tourism. Our findings indicate that the annual expenditure for the management of each species was very high. Therefore the zoo authority has invited the people and organizations are interested in the wildlife conservation and management for the adoption of the wild species kept in the Zoo for their annual expenditure. The money was also collected individually from tourists visited in the Zoo. This first afford to study the Zoo conservation and management programme in high altitude Nainital zoo. We studied the interest of people and other organizations about the wild life species and their relationship in the upliftment of our economy through tourist's activities.

Acknowledgements:
Author is thankful to Department of Forestry, Kumaon University, Nainital for the help and support.

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4/5/2009

Geological Significance of Bouguer Anomalies in Harir Plain, Northwest Zagros Folds-Thrust Belt (Iraqi Kurdistan Region)

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Abstract: Gravity measurements of about 500 points were made along the paved and some unpaved roads of Harir plain in the northwestern part of the Zagros Fold-Thrust Belt (ZFTB). Necessary corrections were carried out and a complete Bouguer map was constructed. The Bouguer anomaly map shows regular patterns, which was compatible with the surface geological structures. Regional, residual and second vertical derivative maps were also constructed to give better qualitative interpretations. Considerable density contrast of 0.3gm/cm^3 was observed between the Cretaceous and Tertiary rocks which thought to be the marker contact for most of the gravity anomalies, as calculated by samples collected from outcrops. This density contrast was used to carry out two-dimensional modeling along seven NE-SW profiles and one NW-SE profile in order to show the topography of the top of Cretaceous rocks. The model shows that there is a good correlation between the folds (± thrusts) at the Tertiary-Cretaceous boundary and the surface structures (Harir, Safin and Shakrok anticlines). However, this correlation is not one-to-one since Harir plain is underlain by an unexposed anticline in the Tertiary-Cretaceous contacts. [Nature and Science. 2009; 7(6):43-51]. (ISSN: 1545-0740).

Key words: Harir-Kurdistan-Gravity Prospecting

Introduction

Harir plain, which covers an area about 300km^2 is located some 40km east of the city of Erbil, in the northwestern most part of the Zagros fold-thrust belt of Iraqi Kurdistan Region (Fig. 1). The studied area is represented by a hummocky plain bounded from northeast and southwest by the Harir and Mirawa Anticlines respectively (Fig. 1 and 2). Some local and limited-length gravity traverses were carried out [1, 2, and 3]. Those studies did not describe the geophysical signature of the area. The data of the first author was re-interpreted later by [4 and 5] and yet did not give a detailed picture of the subsurface. The present work is therefore a necessary step towards understanding the structural pattern in this part of the High-Folded Zone of Iraq using gravity data. In this study, gravity anomaly data is used to investigate the subsurface structural features of the Harir area and compare them to the surface data in order to outline the geometric configuration of the structures at depth and if they have or not any relationship with the basement.

General Geologic setting

Harir area is located within the Zagros High-Folded Zone (Fig. 1), which is characterized by relatively long and high-amplitude anticlines. These anticlines, most of which are asymmetrical, are associated with thrust/reverse faults along their southern or southwestern limbs and could be either fault-propagation or fault-bend folds. They also represent the last NW-SE trending structures in the Zagros Fold-Thrust Belt before the belt changes trend to E-W. The High-Folded Zone affected by transversal faults system which reactivated from late Jurassic time onward resulting in the formation of transversal blocks. Some faults of this system underwent sinstral strike slip movement in Quaternary time along Ana-Qalat-Dizah fault. Many anticlines are segmented into separate domes and their fold axis is bent at the intersection with these transversal faults [6].

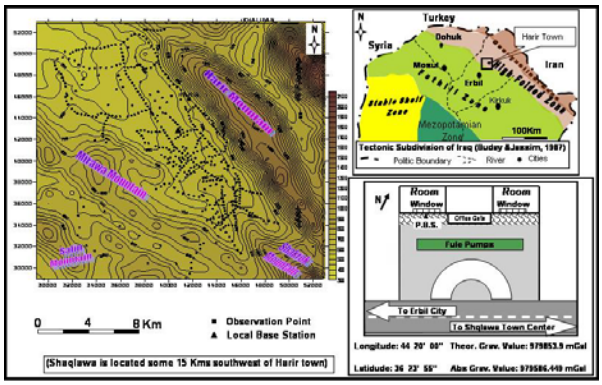

Fig. 1: Topographic Map of Harir Area Showing the Observation Stations (Left)
Simplified Tectonic Map of Northern Part of Iraq (Up Right)
A sketch for the Location of the Primary Base Station (Down Right)

	Age	Formation	Thickness (m)	Lithology	Brief Description
Tertiary	Late Miocene	Upper Fars	150		Bedded or massive sandstone &
	Middle Miocene	Lower Fars	130		Mudstone & layered limestone
	Late Eocen	Pila Spi	53		Chalky & Dolomitic limestone
	Middle Eocene	Gercus	130		Mudstone, shale, & limestone
	Early Eocene	Kolos	165		Mudstone, shale sandstone & fossil.
Cretace.	Early Maastric	Shiranish	150		Shale, marl & marly limestone
	Late Campan.	Bekhme	110		Dolomitic & Bituminous limestone

Fig. 3: General Stratigraphic Column of Harir Area
(Al-Nabawi, 2002)

Harir plain is bounded from the northeast by Harir anticline and Mirawa anticline from the southwest (Fig. 2). The northwestern plunge of Shakrok anticline bounds the area from the southeast diverging underneath the Harir syncline in an en-echelon configuration with the other anticlines of Safin, Harir and Mirawa

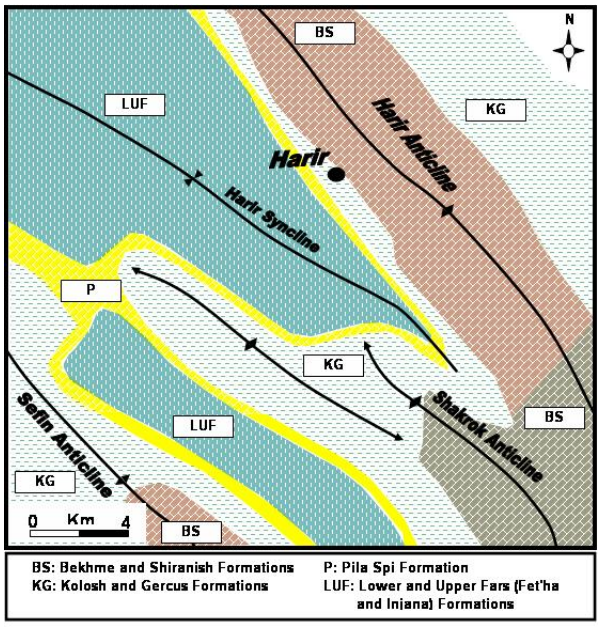

Fig. 2: Geological Map of Harir Area
(After Surdashy and Aqrawi, 2001)

The oldest rocks cropping out in the area belong to the Bekhme Formation (Late Cretaceous) (Figs. 2 and 3).

This formation makes up the core of the Harir and Shakrok Anticlines and is composed of massive or layered dolomitic limestone. Qemchuqa Formation (M. Cretaceus) dolometic limestone however underlies the Bekhme Formation, it crops out in the bottom of some valleys. Bekhme Formation is followed stratigraphically by the Shiranish Formation (Late Cretaceus), which is composed of marl and marly limestone and occupies the limbs of the Shakrok Anticline. It is followed by the clastic rocks of Kolosh and Gercus Formations (M.Paleocene and L.Eocene). These are composed of mudstone, shale, sandstone and marlstone. The Khurmala Limestone Formation (L. Eocene), which is present as tongues within the upper part of the Kolosh Formation [7], consists of well-bedded dolomitic or clayey limestone. The Pila Spi Limestone Formation (L.Eocene) is composed of dolomitic and chalky limestone. The formation is well bedded and fractured [8 and 9]. The Fat'ha (Lower Fars) and Injana (Upper Fars) Formations respectively follow the Pila Spi Formation. The former is composed of alternating beds of mudstone, limestone, shale and sometimes-thin layers of evaporate. The latter is composed of alternating fractured beds of sandstone and mudstone, which are less competent than the former.

Bekhme Formation, is the main aquifer in the Harir and surrounding areas. Problems have been faced in the search for groundwater in this area. As such, depicting the depth, geometry and structural pattern of this formation is of great significance both structurally and hydrogeologically.

Data Collection and Reductions:

The standard formula for the calculation of simple gravity anomalies assumes a flat earth surface at the observation point. Gravity stations should be sited on a flat area with at least 200m clearance from sharp changes in ground elevation. Moreover, surveys designated to solve geological problems should contain profiles of variable station spacing [10]. These two concepts were taken in mind when 499 gravity measurements (Fig.1), spaced in average about 300m, were conducted in the Harir plain along the paved and accessible unpaved roads. Locations and elevations were determined using a Global Positioning System (GPS) since a differential GPS unit was not present at the time of surveying and spirit level techniques were so expensive. Gravity data were collected using LaCoste and Romberg gravity meter model G. All data were tied to a gravity base station established at a central part of the study area [11] (Fig. 1). This station has an absolute value of 979655.48 mGal based on tying to a primary base station in the nearby Shaqlawa town (about 15 km SW of Harir), which in turn has an absolute gravity value of 979586.449mGal [3] (Fig. 1).

Gravity data were reduced using the International Association Geodesy formula (IAG), which reduces the actual gravity values at sea level within 0.1mGal [12]. Bouguer, Free air and Terrain corrections were carried out relative to sea level. Thirty-five Cretaceous and Tertiary rock samples were collected for density measurements (Table 1). The manner of calculating the density contrast between Tertiary and Cretaceous rocks are shown in Table (1). A contrast of 0.34gm/cm^3 was estimated and used in our interpretation of the data. Densities were weighed relative to the thickness of each formation and an average surface density was calculated to be 2.4gm/cm^3. This value was used in both Bouguer and Terrain corrections.

Qualitative Interpretation of gravity maps

The total uncertainty in the final Bouguer value based on uncertainties in observed gravity, horizontal position, elevation and terrain correction was estimated to be about 0.35 mGal. The reduced gravity data were plotted to construct the Bouguer anomaly map (Fig. 4I). This map is characterized by the presence of several two-dimensional positive and negative anomalies. The majority of them are trending in NW-SE direction, which is the main trend of the structures in this area. Most of positive and negative anomalies are consistent with the surface structures. Main gravity gradients are towards northeast and southwest across the main trend of the Zagros Fold-Thrust Belt**.**

Table 1: Calculation of density contrast between Tertiary and Cretaceous rocks.

Formation Name	Mean density (d) (g/cm^3)	Mean thickness (T) (m)	T *d	Age	Group Density (g/cm^3)
Injana (U.Fars)	2.25	150	337.5	Tertiary	2.32
Fat'ha (L.Fars)	2.28	130	296.4		
Pila Spi	2.55	52	132.6		
Gercus	2.23	130	289.9		
Kolosh	2.41	165	397.7		
		=627	=1454		
Shiranish	2.58	150	387	Cretaceous	2.62
Bekhma	2.67	110	293.7		
		=260	=680.7		
		Contrast = 0.3 g/cm^3			

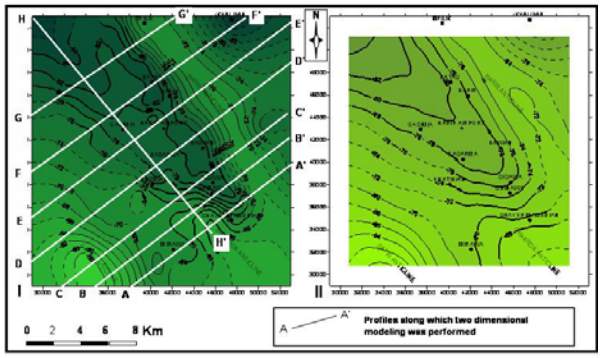

Fig. 4: Gravity Maps of Harir Area;
I : Bouguer Anomaly Map
II : Regional Anomaly Map

The maximum gradient value is observed on the southwestern limb of the Harir anticline and reaches about 5mGal/km. This steep gradient, which is represented by densely spaced linear contour lines to the east of Harir town, may indicate the steep southwestern limb of the Harir anticline. The crestal areas of the Harir anticline are represented by a longitudinal positive anomaly having a maximum value of –70 mGal. The central part of the studied area is occupied by a main negative anomaly with a minimum value of -82mGal. The presence of several irregularities within this anomaly exhibited by contour patterns, particularly in the form of plunging patterns give impressions that the gravity field in the speculated synclinal area has been influenced by relatively shallow anomalous masses within the trough. This central part of the studied area could easily be outlined by the dense contour lines bounding it from northeast, southwest and southeast.

The northwestern plunge of Shakrok Anticline is shown as a positive anomaly in the southeastern corner of the area. The dense contour nose trending towards southeast is actually representing the narrow plunge of the syncline between Harir and Shakrok Anticlines diminishing towards southeast. A major shallow subsurface reverse fault was detected at this region [13] using resistivity method.

The southwestern corner of the area on the other hand is represented by a positive anomaly that plunges northwestward. This anomaly represents the northwestern plunge of Safin anticline, southwest of the area (See Fig. 2).

Trials with different grid spacing were made to compute and construct regional-, residual- and second vertical derivative maps. Among many, the radius $3s\sqrt{5}$ was found to be most suitable (where s is the grid space). Griffin's [14] and Elkins's [15] methods were applied to calculate the transformed gravity values.

The general view of the regional anomaly map (Fig. 4II) shows quite regular anomalies trending in the NW-SE direction reflecting the main structural units of the area. An average gradient of about 0.4mGal/km was observed towards northeast.

The residual anomaly map (Fig. 5I) is of special interest since it reflects the presence of many relatively near surface anomalous masses represented by detailed gravity lows and highs. The central part of the studied area is not as simple as expected. Many positive anomalies are present within the main gravity low. The

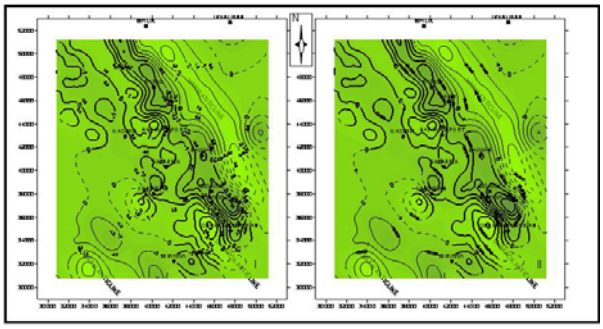

Fig. 5: Gravity Maps of Harir Area;
I : Residual Anomaly Map
II : Second Vertical Anomaly Map

most conspicuous one is the longitudinal NW-SE positive anomaly located southwest of Harir town possibly representing a local positive structure within the synclinal area. Moreover, plunges of both Shakrok and Safin Anticlines as well as Mirawa Anticline have their imprints.

The inspection of the second vertical derivative map (Fig. 5II) shows that the major and minor anomalies shown on the residual anomaly map are emphasized clearly and the zero contours of both maps coincide. Since these second vertical derivative anomalies are proportional with the residual anomalies, noises, which are often amplified by the second vertical derivative, are nearly absent and most anomalies should be of geological significance [16].

Bouguer and residual anomaly maps were used to infer many faults of major and minor

characters. The northeastern set is following the southwestern limb of Harir anticline. According to some structural models, the NW-SE trending faults, which are displacing the southwestern overturned limb of Harir Anticline, are most likely thrusts that have formed during the folding of the layers [17]. It is not possible at this stage to conclude whether the anticlines are fault propagating folds or detachment folds.

Some faults of this set and the southwest-Harir Anticline set (dashed lines in figure 6A) were also detected by resistivity data [18 and 13]. On the other hand, many other faults were detected from the residual anomaly map (Fig. 6A).

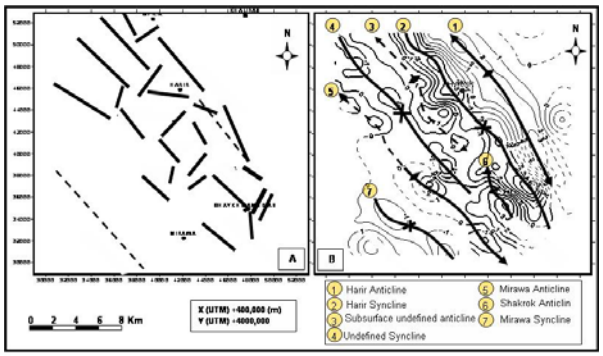

Fig. 6: Structural Features as Inferred From Gravity Data
A : Inferred Faults B : Inferred Fold Axes
Superimposed on the Residual Anomalies

The majority of these faults trend in the NW-SE direction, parallel to the main trend of the Zagros belt structures, while some of them trend in the NE-SW direction (i.e. transversal faults). These faults are possibly related with the rejuvenation of the main Zab fault which its trace is located some 12km to the north of Harir area trending NE-SW and following the Greater Zab River. This major fault is the boundary between the Mosul basement block in the north and Kirkuk basement block in the south [24]. Harir area is located within the second block. This rejuvenation was possibly occured occurred due to the second phase of the Alpine Orogeny during Tertiary.

Axes of longitudinal positive and negative anomalies have been traced from the residual anomaly map (Fig. 6B). Most of these axes coincided well with anticlinal and synclinal axes on the geological map (Fig. 2). A minor positive gravity axis interpreted as an anticlinal signature (number 3 in Fig. 6B) is observed in the northern central part of the area. This structure within the Harir syncline has no surface indications other than a slight ground elevation.

Quantitative Interpretations by Modeling

The following two aspects make the interpretation of the data difficult. The first is that the gravity value at a certain point is the summation of the gravitational attraction of all subsurface sources detected by the instrument. The second reason is the lack of unique solution for a certain gravity anomaly [19 and 20].

The modeling procedure involves the use of suitable residual gravity anomaly and density contrast between the body of interest and surrounding rocks. The modeling approach in relatively rugged terrain is well stated by [21 and 22]. There points were carefully taken into account in constructing the final shapes of the geological models in Harir plain depending upon the formula of Talwani et. al [23].

Geophysical and geological models for profiles AA' through HH' (Fig. 4I) are given in Figures (7 and 8).

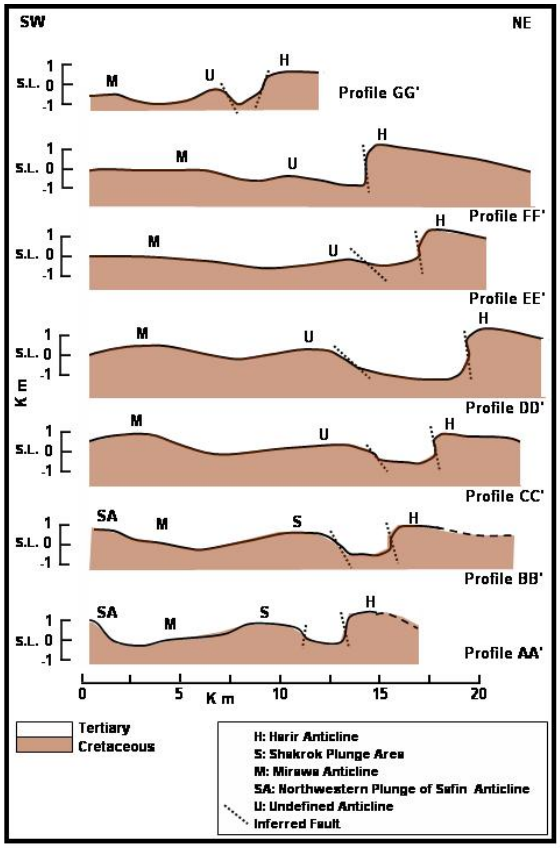

Fig. 7: Geological Models Along the NE-SW Gravity Profiles Showing the Cretaceous-Tertiar Rocks Boundary (CTB) Relative to Sea Level

Surface outcrops of Cretaceous rocks were of great importance as controls for constructing the models since one of the targets is defining the contact between Tertiary formations collectively with Cretaceous rocks.

A considerable density contrast of 0.3gm/cm^3 was observed between the Cretaceous and Tertiary rocks (Table 1). In our geophysical approach we have assumed that there is no lateral variation of the lithologies in these two groups of formations and hence the densities used are generally applicable throughout the area.

Two negative and two positive features characterize profiles AA' and BB' (Fig. 7). From the northeast they represent Harir Anticline, Harir syncline, Shakrok Anticline and Mirawa syncline. The plunge of the Shakrok Anticline does not appear in profile CC' through GG' (Fig. 7). In profile CC' the Mirawa Anticline effect is present instead of Shakrok plunge. The effect of Harir Syncline on the other hand shows progressive widening towards northwest as shown in profile DD', EE' and FF' and tightening again in profile GG'. In profiles EE', FF' and GG' a small positive structure is coming to be existing with a small syncline between it and the Harir Anticline ("U" in Figure 7). In all these models, one or two sets of faults parallel with structural trends are suggested. Numan and Al-Azzawi [17] suggested normal faults bounding most of the northeastern limbs of the Iraqi anticlines beside the thrust faults in their southwestern limbs.

Figure 8 (profile HH'), shows how the geophysical models were constructed for the purpose of geological modeling along all profiles. It is the only profile which was taken along the structural trend (NW-SE direction). It suggests that the Tertiary-Cretaceous boundary dips towards northwest. This change in the depth could be structurally induced. However, we do not have enough subsurface data to verify the reason for the depth change.

This map shows that depth of the Cretaceous rocks increases generally towards northwest. It is elevated about 800m above sea level in the area around Shakrok and on the limbs of Harir anticline while it is about 800m below sea level (i.e. about 1200m below ground surface) in the northwestern parts of the area. This map could serve as a base for improving the hydrogeologic framework of Harir area since a lot of problems face geologists in drilling water wells in the plain. No chance is present in the plain to drain water from the Bekhme Formation (the main aquifer in the region) because of the great depth.

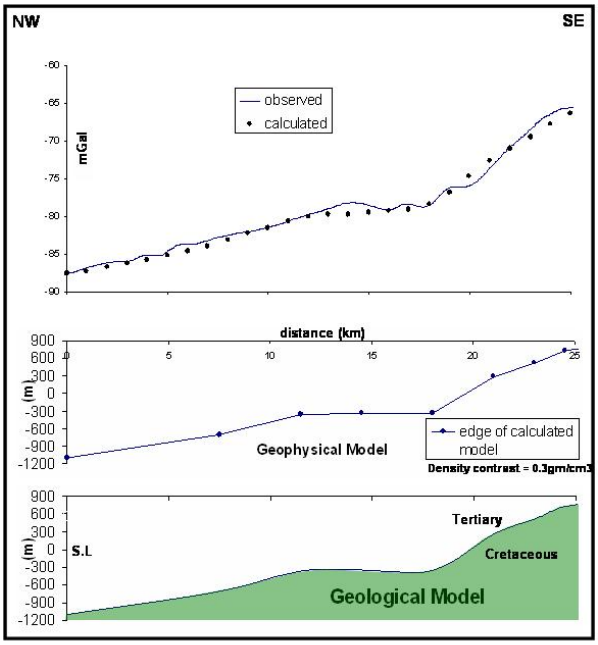

Fig. 8: Geophysical and geological models along profile HH'.

Using the geological models estimated in Figures (7 and 8) a map for the top of Cretaceous rocks relative to sea level has been constructed (Figs. 9).

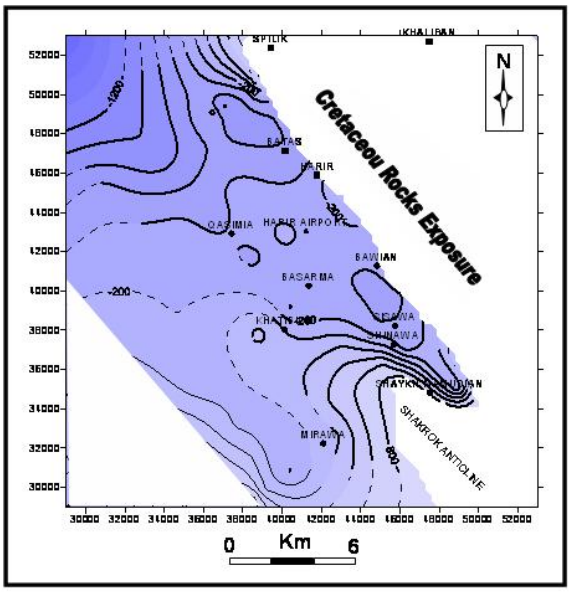

Fig. 9: Top of Cretaceous Rocks Relative to Sea Level.

This depth ranges from 9km in the southwestern part to about 11.3km underneath the Harir syncline. The contact between the basement and the sedimentary cover is deepening towards northeast approximately by a rate of 1km/15km. The topographic changes in the shape of the basement surface through a short distance (about 25Km) reveals that the major faults have their sources within the basement rocks.

Discussion

Numan [24] used landsat images to give descriptions for Harir, Shakrok and Safin Anticlines. He mentioned that there is a bend in the axis of Shakrok anticline at the northwestern plunge without giving explanations. Al-Shaikh et. al.[1] considered the part of this anticline, which lies to the northwest of this bend, as a separate anticline. Surdashy and Aqrawi [25] considered that the apparent extension of this part of the anticline to be a separate structure called Mirawa double plunging Anticline. This consideration is in agreement with the present work.

However, the present study shows that the Shakrok Anticline axis (Fig. 6B) has a relatively sharp bend at the northwestern part presently considered plunge and possibly extends farther more to include the so called (undefined anticline, number 3 in the figure) as reflected gravimetrically, which seems to be a decoupled subsurface structure with no corresponding structural equivalent on the surface.

The authors believe that the synclinal area between the Harir Anticline and the Mirawa Anticline (Fig. 2) is never a simple one. Instead a subsurface anticline and two sub-synclines are present within.

We have used the profiles, which were constructed from the gravity data, to prepare a three dimensional image of the structures at Tertiary-Cretaceous boundary (Fig. 9). This three dimensional construction depicts the structural configuration of the Cretaceous-Tertiary contact (CTC) and shows that; 1, folds are non-cylindrical and change along strike (NW-SE), 2, the folds are asymmetrical with their steepest limb on their southwestern side, and 3, the syncline located between the Harir and Shakrok anticlines widens northwestwards to include another inferred structure. The 3D image (Fig. 10) constructed from the figure 7

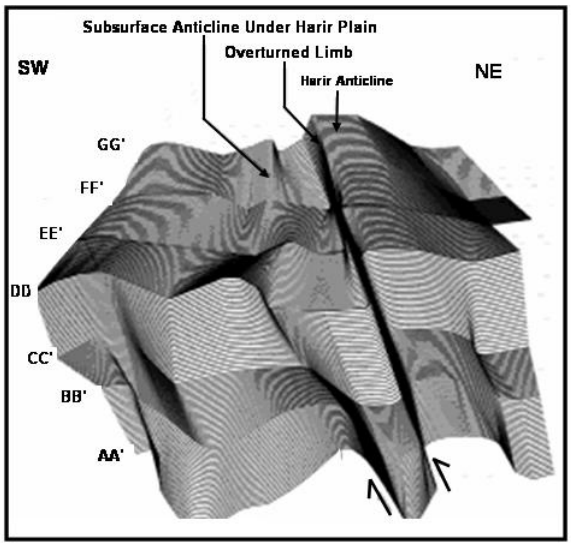

Fig. 10: A three dimensional image of the structure depending upon the CTB sections.

shows that the southwestern limb of the Harir anticline remains steep and changes dip to become overturned along strike towards the NW. This suggests that the thrust that cuts this limb is continuous along strike. This scenario is quite different in the Shakrok anticline, whose northeastern limb is overturned suggesting that here; the back-thrust is more active than the fore-thrust. Towards the NW along strike, the northeastern limb is not overturned, suggesting that the back-thrust that cuts this limb is either discontinuous or change dip.

Comparison of the 3D image of the Tertiary-Cretaceous boundary with the surface geology reveals some significant differences. One of these features is the clear unfit between some of the structures displayed by the Tertiary-Cretaceous contact and those seen on the surface. Although, Harir, the southeastern part of Shakrok and Safin anticlines are visible on the surface, the Tertiary-Cretaceous contact shows additional structures. One of these structures is the anticline which underlies the Harir plain and does not have a corresponding structure at the surface. The presence of this anticline, which seems to be smaller both in amplitude and size, indicates a structural decoupling between the deeper anticlines and the shallow ones.

In areas where the adjacent southwestern limb of Harir anticline and the northeastern limb of Shakrok anticline are overturned, a pop-down syncline has developed (Fig. 10). This pop-

down syncline is bounded on either side by thrust faults. Normally, in this area, a narrow syncline is formed.

In order to roughly estimate the amount of shortening in this area, the geological sections of figure 7 were restored by bed-length balancing assuming area conservation (Fig. 11). The restored sections show that the area has shortened by 14-29.5 % by folding and thrusting, during the Zagros Orogeny. Since it is not possible to deduce the amount of displacement along the thrusts from the gravity data, it is not possible to distinguish the amount of shortening by thrusting from the shortening by folding. In addition, it is not possible to estimate the amount of shortening by penetrative strain, i.e., tectonic compaction [26]. As such, the calculated value (14-29.5 %) is a lower estimate. However, the value provided here fits well with estimates produced for other parts of Zagros [27-30].

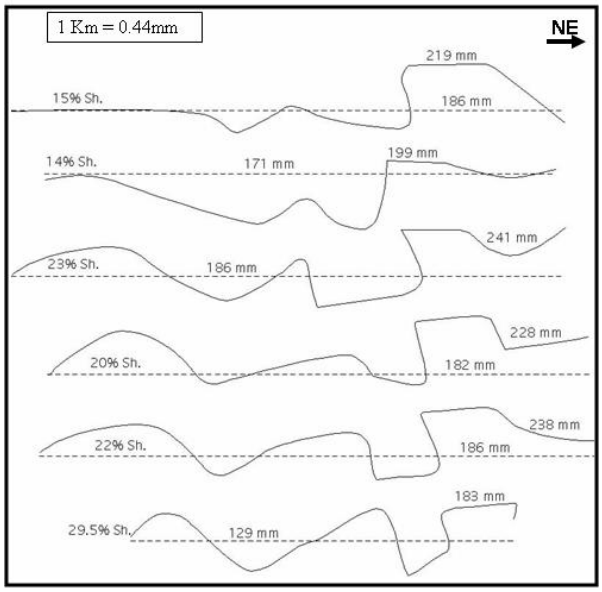

Fig. 11: Restored sections used for shortening calculations

Hydrogeologists face problems when drilling water wells in the Harir plain. These problems according to this study may stem from two reasons; the first is that the most important aquifer yielding good amounts of water, which is the aim of the drillings, is being the karstified Bekhme Formation. This study shows that this formation is too deep to be reached for that purpose in addition to its rough and sharply undulated surface. The second reason is the presence of at least two sets of minor relatively shallow faults. These faults, if leaking, may destroy some of the present aquifers in the area. However, even though these faults may render some of the aquifers obsolete, the anticlinal crests, which take the Bakhme formation to shallower level, may still act as aquifers and host significant amount of water. This is of course of great significance for petroleum exploration in the area as well.

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Pre and Post Monsoon Variation of Heavy Metals Concentration in Ground Water of Angul-Talcher Region of Orissa, India

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ABSTRACT: *This study was undertaken to assess the portability of ground water with the reference to heavy metals in Angul-Talcher region of Orissa, India. The groundwater samples from 19 different locations from industrial as well as domestic areas were collected in pre and post monsoon season. The Standard methods (APHA, 1998) were adopted for heavy metal analysis of these samples and the results were compared with the Indian Standards (IS: 10500) for potable water. The study reflects the presence of some heavy metals in few groundwater samples but all were within the limits except cadmium. Mercury (Hg) could not be detected in any of the samples in the study area.* [Nature and Science. 2009;7(6):52-56]. (ISSN: 1545-0740).

Key Words: Groundwater quality, heavy metals, drinking water

INTRODUCTION:

In India almost 80% of the rural population depends on untreated ground water for potable water supplies (Sudhkar at el., 2004). It is well known fact that potable safe water is absolutely essential for healthy living. Adequate supply of fresh and safe drinking water is a basic need for all human beings on the earth. Drinking water is a potential source of human exposure to toxic substances. Contamination of drinking water may occur by, percolation of toxics through the soil to ground water that is used as a source of drinking water (Sargaonkar and Deshpande, 2003).

In recent years, because of continuous growing of population, urbanization and rapid industrialization, the rate of discharge of pollutants into the environment is far higher than the rate of purification. Groundwater contamination and its management has become the need of hour because of far reaching impact on human health. Contamination of ground water resources occurs through surface discharge as well as naturally due to geochemical activities (Sudhaker at el., 2004). Open dug wells are generally considered as one of the worst type of drinking water source (Adekunle, 2008). Usually in unaffected environments the concentration of most metals is very low and is mostly determined by the mineralogy and the weathering (Jinwal and Dixit, 2008). The water composition varies in the groundwater reservoir through percolation and the reactions with minerals present in the rock that may modify the water composition. There are a few examples of metal pollution through natural weathering but in most of the cases metals become an environmental and health issue because of anthropogenic activity.

STUDY AREA

Angul-Talcher is situated at an average height of 139 m above mean sea level (MSL) and about 160 km from the Bhubaneswar, the state capital of Orissa, India. The area lies between 20°37’ to 21° 10’ N latitude and 84°53’ to 85° 28’ E longitude. Angul-Talcher region is economically very important and has also been identified as a critically polluted area (CPCB, 2007). Many small, medium and large scale industries such as coal mines, super talcher thermal power plant (Kaniha), Talcher Thermal Power Station, Nalco smelter and its captive power plant and other iron & steel industries are situated in the region. These industries are polluting the surrounding areas including groundwater resources.

The present study on heavy metals concentrations in ground water of Angul-Talcher area is necessitated because of coal mining and coal based heavy industries. Large number of motor vehicles may also contribute in the release of heavy metals into surrounding environment. In this region the heavy rain fall occur during June to September, so there is possibility that heavy metals present in the atmosphere may also contaminate shallow ground water resources. Hence it is extremely important to assess the groundwater quality in respect of heavy metals. Geographical location of study area is shown in the Figure 1.

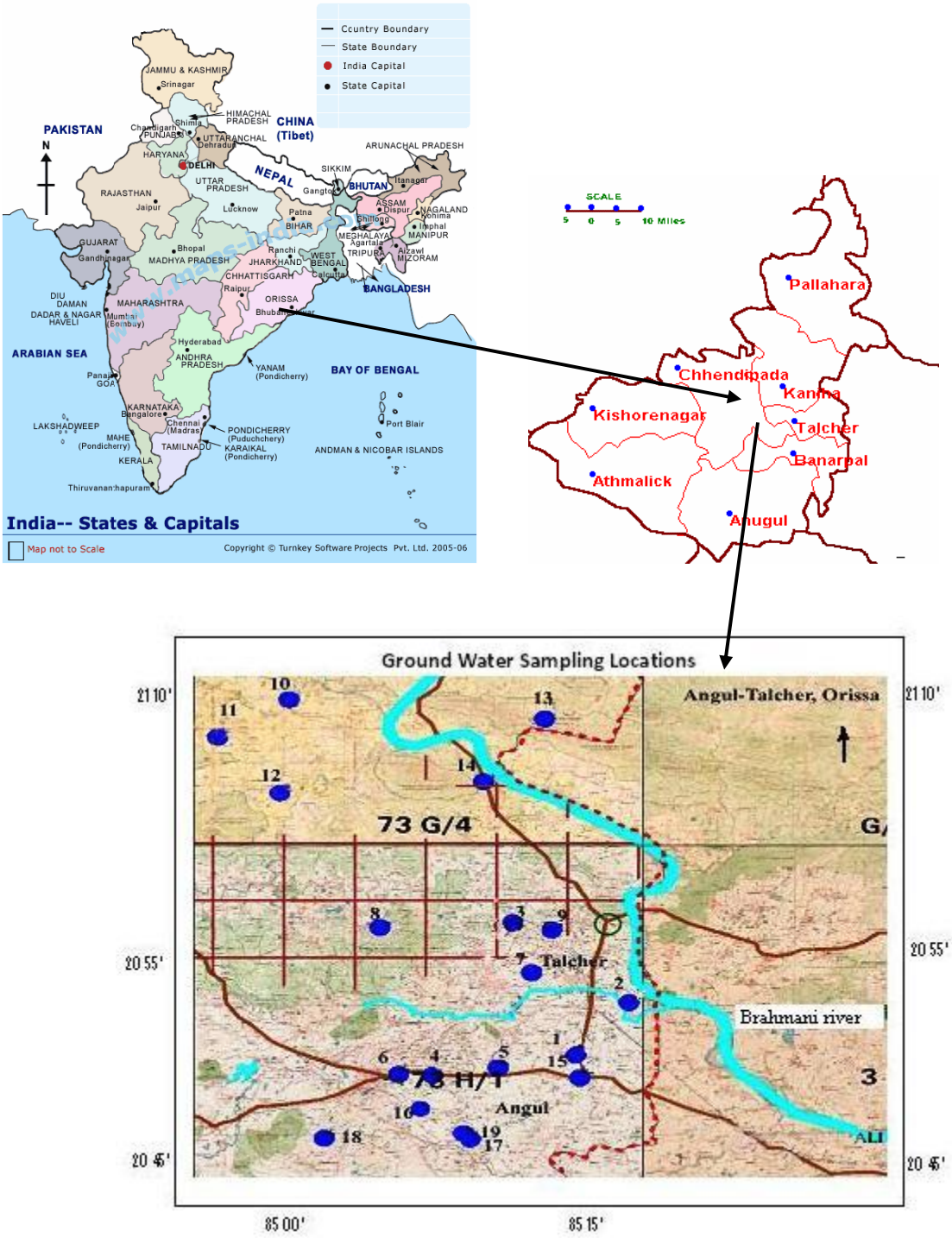

Figure 1: Study Area

SAMPLING

The sampling locations consist of industrial as well residential area. Nineteen no. of ground water samples were collected from tube well and open well during pre and post monsoon period. Details of sampling locations are illustrated in Table 1. Samples were collected in plastic containers to avoid unpredictable changes in characteristic as per standard procedures. (APHA, 1998).

ANALYSIS

The collected water samples were pretreated and preserved by concentrated HNO_3. Metal concentrations in the water samples were determined using the Atomic Absorption Spectrophotometer (Avanta) for major heavy metals such as Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Iron (Fe), Cobalt (Co), Mercury (Hg), Arsenic (As) as per to the standard procedure prescribed by APHA (1998) and the results were compared with the Indian Standards (IS: 10500) for potable water.

Table 1. Ground water Sampling Stations within the study area.

Code	Sampling Locations	Latitude	Longitude
GW1	Gotamara village, tube well water	20º 51’ 21”	85º 12’ 46”
GW2	Dasnala village, open well water	20º 53’ 33”	85º 14’ 33”
GW3	Jagannath village tube well water	$20^0$56’50”	$85^0$10’40”
GW4	Kandasar village, open well water	20º 50’ 33”	85º 07’ 58”
GW5	Girang village, open well water	20º 50’ 52”	85º 10’ 08”
GW6	Hingula- Gopal Prasad village, Tube well water	20º 50’ 30”	85º 06’ 50”
GW7	Sharma Chak, open well water	20º 54’ 44”	85º 11’ 15”
GW8	Donara village, Open well water	20º 56’ 36”	85º 06’ 12”
GW9	Hingula tube well water	20º 56’ 32”	85º 11’ 57”
GW10	Takua village, open well water	21º 06’ 04”	85º 03’ 10”
GW11	BarhaGundari village, open well water	21º 04’ 47”	85º 00’ 02”
GW12	Kamarel village open well water	$21^0$02’10”	$85^0$02’50”
GW13	Blinda village open well water	$21^0$05’20”	$85^0$11’40”
GW14	Ekgharia village, open well water	$21^0$02’38”	$85^0$09’39”
GW15	Near Banarpal junction, tube well water	20º 50’ 28”	85º 12’ 55”
GW16	Nuashahi village, open well water	$20^0$48’10”	$85^0$09’00”
GW17	Tulsipal village,open well water	$20^0$49’00”	$85^0$07’40
GW18	Longibeda village, tube well water	$20^0$47’50”	$85^0$04’20”
GW19	Gadrak khai village, open well water	$20^0$48’20”	$85^0$09’30”

RESULTS AND DISCUSSION

The range of heavy metals concentrations of various groundwater samples are shown in Table 2 along with statistical variations. In most of the cases the concentration of Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Iron (Fe), Cobalt (Co), Mercury (Hg), Arsenic (As) from groundwater samples were below detection limit. In other samples such as GW3, GW4, GW5, GW7, GW9 and GW15, the concentrations were found within the prescribed limit for drinking water (IS: 10500) except cadmium. The level of cadmium was found slightly above than permissible limits in sample of Jagannathpur village tube well water (GW3), Sharma Chhak open well water (GW7), Hingula tube well water (GW9), Kandasar village open well water (GW4) and Banarpal junction tube well water (GW15) in pre-monsoon season. In sample GW9 and GW4, Cd levels were above the prescribed limits in post monsoon season as well. Mercury (Hg) could not be detected anywhere in the study area. Distribution of various heavy metals during pre and post monsoon season is shown in figure 2 & 3 respectively.

Cadmium is natural constituent of ground water and it may found with in organic and inorganic forms. Cadmium in ground water may arise from industrial discharge, mining activities, weathering and erosion of bed rock (Stanley, 1993).

Table 2. Statistical analysis of heavy metals at various locations.

Sl. No.	Heavy metals	Pre-Monsoon			Post Monsoon			IS: 10500 (ppm)
		N	Range	Mean ± SD	N	Range	Mean ± SD	
1	Cadmium (Cd)	9	0.005-0.031	0.015 ± 0.010	8	0.001-0.02	0.007 ± 0.006	0.01
2	Copper (Cu)	10	0.001-0.01	0.004 ± 0.003	5	0.001-0.04	0.011 ± 0.016	0.5
3	Lead (Pb)	8	0.01-0.035	0.021 ± 0.010	10	0.01-0.04	0.022 ± 0.012	0.5
4	Nickel (Ni)	6	0.01-0.12	0.030 ± 0.044	8	0.009-0.08	0.027 ± 0.023	--
5	Zinc (Zn)	9	0.01-0.026	0.016 ± 0.007	8	0.001-0.021	0.007 ± 0.007	5
6	Iron (Fe)	14	0.009-0.15	0.041 ± 0.040	9	0.002-0.11	0.034 ± 0.033	0.3
7	Cobalt (Co)	2	0.04-0.09	0.065 ± 0.035	3	0.004-0.01	0.007 ± 0.003	--
8	Arsenic (As)*	4	0.13-0.068	0.049 ± 0.025	3	0.05-0.09	0.067 ± 0.021	0.5

(* = ppb) (N= No. of samples (out of 19) in which heavy metals occurred.)

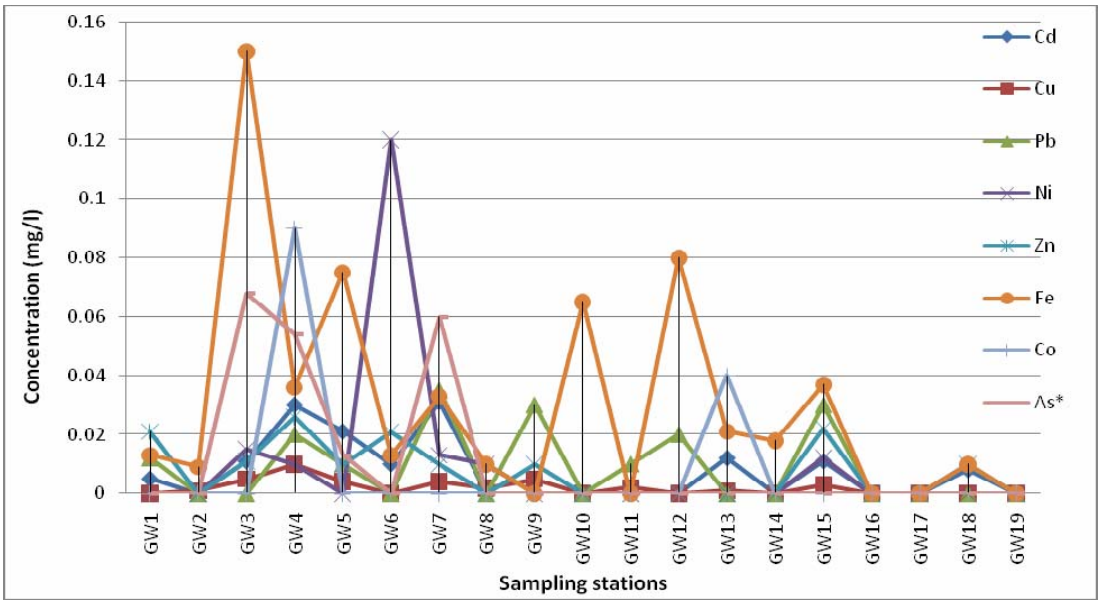

Figure 2. Distribution of various heavy metals at different locations during pre- monsoon season.

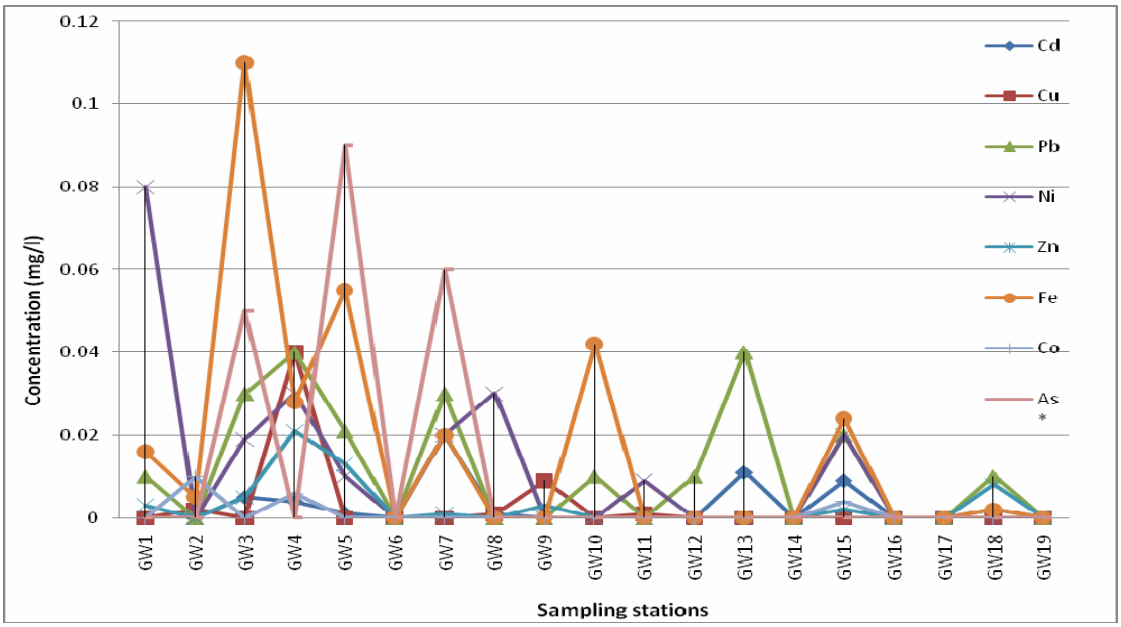

Figure 3. Distribution of various heavy metals at different locations during post- monsoon season.

CONCLUSIONS

The metal concentrations at all the locations were within permissible limit (IS: 10500) during both seasons. It was observed that the metals concentrations were slightly higher in pre monsoon that post monsoon The concentration of these metals may increase in near future due to heavy industrialisation and uncontrolled mining and related activities. Precautionary measures have to be adopted by upcoming various industries to protect heavy metal concentration in the ground water.

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Eco-Friendly Production of *Agaricus bisporus* (Lange) Imbach (White Button Mushroom)

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Abstract: For the profitable eco-friendly bioconversion of lingo-cellulosic wastes of agro-industry, the production of mushroom is regarded as the second most commercial microbial technology next to the yeast. With the view point of eco-friendly production of *Agaricus bisporus* (Lange) Imbach, the study conducted upon the yield of different strains of *A. bisporus* in which, strain P1 and NCS 5 were found superior over the other strains. The yield of strains varied from 11.0 kg to 13.75 kg per quintal compost. The strains NCS 5 (13.75 kg) and P1 (13.10 kg) were statistically at par over the check. [Nature and Science. 2009;7(6):57-60]. (ISSN: 1545-0740).

Key words: *Agaricus bisporus*, Eco-friendly production, White Button Mushroom

Introduction:

Mushroom has been defined as a "Macro-fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand'' (Chang and Miles, 1993). Mushrooms are non-conventional sources of human food. These are delicious, nutritionally rich and have their own importance as medicines. The widespread use of mushrooms in ancient times is also confirmed by the hypothesis of Wason (1971) that the "Soma" of Rig-Veda was a preparing of mushroom, *Amanita muscaria.* Taxonomically (Alexopoulas et al, 1996) these are species of phylum Basidiomycota and Ascomycota.

The cultivation of white button mushroom is mostly confined to small, seasonal growing units which are mostly unpasteurised compost and producing 10-14 kg mushroom/qt. compost. However, 18-22 kg mushroom/qt. compost had been harvested under controlled conditions using the pasteurised compost by a limited number of commercial growing units but in the developed countries the production is 25-30 kg mushroom/qt. compost (Chaddha and Sharma, 1995). Introduction of better quality strains from other countries cannot solve the problem of better yield in our country due to the altogether different conditions of growing mushrooms than those of Europe and America. Therefore we need to develop better performing strain in terms of yield, quality and wider adaptability under diverse growing conditions.

Jin (1990) studied the yield performance of fluffy and appressed type mycelium of the same strain of *A. bisporus*, he reported that fluffy type tended to give high yield whereas, the appressed type showed good quality. Mehta and Dhar (1991) evaluated 9 strains of *A. bisporus* for yield performance. The strains NCS 14, NCS 5, NCS 11, NCS 6 and NCS 15 were at par in terms of yield and yielded significantly higher than S 1, MS 39, P2 and NCS 12. Singh (1990, 1991) conducted the yield evaluation trials of different strains of *A. bisporus.* He reported that strains S 11 was introduced in sixties while strains RRL 89, S 22 and S 649 were introduced during 1975- 1983. The strain S 11 and S 310 were good yielders, while TM 7 and L 20 were identified as moderate yielders.

In view of the above background, present study was undertaken with the objective of studying the yield of selected strains of *A. bisporus.*

Methodology:

The experiment was conducted at **"**Mushroom Research and Training Centre**",** Centre of Advanced Studies in Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, district-U.S. Nagar (UK), India.

Selection of the strains:

Four strains of *A. bisporus* namely P1, NCS5, NCS12 and S11 (check) selected based on their yield performance under All India Coordinated Mushroom Improvement Project at G. B. Pant University of Agriculture and Technology, Pantnagar (UK).

The yield of strains was assessed by growing them on pasteurized synthetic compost. The synthetic compost of 2.2% nitrogen level was prepared as per following formulation:

Wheat straw : 1000 kg
Chicken manure : 600 kg
Urea : 14.5 kg
Wheat bran : 100 kg
Gypsum : 50 kg

For compost making, short composting method was employed giving 7 days out and 6 days indoor composting period. Casing mixture (FYM+ soil) 3:1 sterilized with 4 % Formaldehyde was used. 10kg compost was filled in polythene bags of 70 X 45 cm in size and spawning was done using 0.75% grain spawn of compost weight. Each treatment was replicated 3 times and bags were kept in crop room at prevailing temperature of 20 ± 2°C. The 3.5 cm thick casing was done on 17^{th} day from the date of spawning. The yield of strains obtained from 30 days harvesting period were compared with each other.

Statistical analysis:

Statistical analysis of the data was done as per the requirement of the experiment. Critical differences (CD) were calculated at 5% level of significance for comparison of differences between the treatment means.

Results and discussion:

The yield performance of different strains was recorded using pasteurized compost. The experimentation was done on prevailing room temperature during January, 2006 onwards. The humidity in crop room was maintained by sprinkling of water on walls, floor and beds. The yields obtained from different strains are summarized in the table given below.

Yields performance of different strains of *A. bisporus* in 30 days cropping period

S. No.	Strains	Average yield in kg/qt. Compost		
		Number	Weight	Weight / Fruit body (gm)
1.	P1	2280	13.10	5.74
2.	NCS 5	2482	13.75	5.53
3.	NCS 12	2230	12.50	5.60
4.	S 11 (check)	1920	11.00	5.72
	CD at 5 %	263.72	1.140	-

It is evident from the data in the above table that yield of strains varied from 11.00 kg to 13.75kg. The strains P1 (13.10 kg), NCS 5 (13. 75 kg) and NCS 12 (12.50 kg) were statistically at par in terms of yield. The yield obtained from these strains was significantly higher than strain S 11 (check, 11.0 kg).

The number of fruit bodies obtained from the strains NCS5, P1 and NCS 12 were significantly higher as compared to strain S 11(1920, check). It is interesting to record that the maximum weight per fruit body harvested was from the strain S 11 (check) followed by P1, a poor and a moderate yielders, respectively.

The environment, substrate and strain are equally important factors for the mushroom production. Since, the present studies were evaluated for their yield. The rest of the strains NCS12 were at par with that of check (S 11). Earlier workers Mehta and Dhar (1991); Singh (1990, 1991) found NCS 5 to be one of the best yielders.

It may be concluded from the foregoing discussions that the strain NCS 5 and P1 have superiority over the other strains studied in present investigations.

Acknowledgement:
The author has expressed her gratitude towards Centre of Advanced Studies in Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, District. –U.S.Nagar (UK), India

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4/10/2009

Bioadhesive alginate copolymers as platforms for oral delivery of insulin

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Abstract: The objective of this study is to utilize the pH sensitivity of modified alginate for oral delivery of insulin. The chemical modification of natural polymers by grafting has received considerable attention in recent years because of the wide variety of monomers available. Acrylic-type polymeric prodrugs were synthesized by free radical copolymerization of acrylic acid, poly (ethyleneglycol monomethyl ether methacrylate) (PEGMA) and alginate in the presence of bis-acrylamide as a cross-linking agent and persulfate as an initiator. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). Insulin was entrapped in these gels and the in vitro release profiles were established separately in both (SGF, pH 1) and (SIF, pH 7.4). Drug releases, in both SGF and SIF media from polymer bonded drugs containing alginate with or without calcium ions were significantly different from each other. The drug release rates from polymer bonded drugs prepared without ions and in the presence of sodium ions were faster. Incorporation of calcium ions into the graft copolymers led to a significant decrease in swelling as well as a substantial retardation of drug release. [Nature and Science. 2009;7(6):61-69]. (ISSN: 1545-0740).

Key words: Alginate, poly (ethylene glycol), pH-sensitive hydrogel, oral insulin delivery.

Introduction

The development of bioadhesive controlled-release systems has been the subject of many studies in recent years (1-9). The ideal drug delivery system should be inert, biocompatible, bioadhesive, comfortable for the patient and capable of achieving high drug loading.

Alginic acid (Alg), a natural anionic polysaccharide, has been used as a medicine for stomach ulcers as well as a food additive because of the protective effect for the gastric mucosa on per oral administration. The solution of sodium alginate immediately forms a cured gel matrix in the presence of a divalent cation and this characteristic has been utilized practically as a bioreactor. Alginate is a linear copolymer composed of 2 monomeric units, D-mannuronic acid (M blocks) and L-guluronic acid (G blocks). Because of the particular shapes of the monomers and their modes of linkage in the polymer, the geometries of the G-block regions, M-block regions, and alternating regions are substantially different, as shown in Figure 1. The G blocks are buckled while the M blocks have a shape referred to as an extended ribbon. If 2 G-block regions are aligned side by side, a diamondshaped hole results. This hole has dimensions that are ideal for the cooperative binding of calcium ions. When calcium ions are added to a sodium alginate solution, such an alignment of the G blocks occurs, and the calcium ions are bound between the 2 chains like eggs in an egg box. Thus, the calcium reactivity of alginate is the result of a calcium induced dimeric association of the G-block regions. Depending on the amount of calcium present in the system, these interchain associations can be either temporary or permanent. With low levels of calcium, temporary associations are obtained, giving rise to highly viscous, thixotropic solutions. At higher calcium levels, precipitation or gelation results from permanent associations of the chains. The pH sensitive nature and its ability to control gel permeability means; alginate based polymers have significant potential for drug delivery applications. However the bioadhesive potential of alginate is not sufficient to make suitable for prolonged contact with the intestinal mucosal surface in case of oral drug delivery of poorly absorbable agents. Because, it may be need to be further modified for some special applications. Among diverse approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties.

Polyacrylic acid (PAA) polymers with pH-sensitive properties have been shown to have good mucoadhesive properties, but their tendency to cause irritation has limited their broad application as buccal bioadhesives. Polysaccharides, such as starch, alginate, cellulose and cellulose derivatives, have been used

in buccal drug delivery systems due to their high biocompatibility and hydrophilicity (10-15). However, their application is limited by their low bioadhesive properties.

Grafting of PAA into alginate has been considered as an alternative procedure to produce nonirritant delivery systems in tablet form with good bioadhesion and controlled-release properties for buccal application. The usual procedure for preparation of alginate graft copolymers is to initiate a free radical on the alginate backbone and then allow the radical to initiate acrylate polymerization.

Existence of polar functionally groups as carboxylic acid need not only for bioadhesive properties but also for pH-sensitive properties of polymer (16-18). Then the incorporation of polyacrylates into biopolymers and, specifically, the grafting of acrylic monomers onto alginate could result in combined properties such as biocompatability, nontoxicity, and higher bioadhesion, which would confer attractive characteristics on the newly prepared composite materials (19).

Poly(ethylene glycol) (PEG) is widely used in the drug delivery system (DDS) for many reasons, with one being their low toxicity to cells (20). Another reason is that PEGs bind relatively little with proteins (21), thereby enabling the long chain of the PEGs to protect the proteins and peptides in the DDS that are used to target the cells from reacting with other sites in the body (22). PEGs may also increase the chances of the DDS carriers to reach the desired cells (3), as the large molecular weight of the PEGs have been reported to increase the circulation time of the DDS carriers in the bloodstream (23).

In this study our aim was to utilize the pH sensitivity of alginate, (Alginate is stable in acidic pH of stomach, but it swells and starts dissolving slowly in the intestinal alkaline pH) which can be used for protecting insulin in stomach and the bioadhesivity of PAA to make prolonged contact with the intestinal mucosae, so as to increase the absorption of insulin. The free radical graft copolymerization poly acrylic acid (PAA) and poly (ethyleneglycol monomethyl ether methacrylate) PEGMA onto alginate was carried out at 70 °C**,** bis-acrylamide as a cross-linking agent and persulfate as an initiator. Insulin was entrapped in these gels and the in vitro release profiles and stability of insulin in contact with these hydrogels during the release were studied. Influences of different factors, such as polymer composition, cross-linking, swelling, effect of the amount of calcium ions on drug released and bioadhesion properties were studied.

Experimental

Materials

The insulin used was recombinant human insulin (AK2U Nobel France; lot # 821156, Batch L-00023822). Poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) was prepared by the method described in the literature (24). Poly (ethylene glycol) monomethyl ether (PEGME) was purchased from Aldrich (France) (Mn = 1000, 2000). Dicyclohexylcarbodiimide (DCC) were purchased from Merck Co. 4-dimethylaminopyridine (DMAP) and reagents were obtained from Fluka Co. Sodium alginate of medium viscosity (3500cps for a 2% solution at 250 °C) was obtained from Sigma chemicals Co. Acrylic acid (AA) and bis-acrylamide were purchased from Merck Co. All the other chemicals used were of analytical reagent grade.

The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released drug was analyzed using a high-performance liquid chromatography-ultraviolet (HPLC-UV) Waters bus SAT/IN Module at 210 nm. Isocratic elution was performed using 30% acetonitrile and 70% buffer containing 0.1M KH_2PO_4 and 1% triethylamine adjusted to pH 3.0 with phosphoric acid. The column used was Nulcleosil-C185-m PHASE SEPARATIONS 4.6-250 mm Analytical Cartridge (part no. psl841020) equipped with a precolumn.

Methods

Preparation of graft copolymers of alginate with acrylic acid: General Procedure

Polymer bonded drugs (PBDs) were synthesized by graft copolymerization of alginate, PAA, PEGMA (variable feed ratio as shown in Table 1) and bis-acrylamide as a cross-linking agent in water as the solvent (50 mL). Copolymerization was carried out in the presence of persulfate as an initiator ([I] = 0.02 M) at 60-70 ºC in a thermostatic water bath. All experiments were carried out in Pyrex glass ampoules. After the desired time (48 h) the precipitated hydrogels was collected, washed with deionized water for 1 week and the water was changed every 12 hours in order to remove any unreacted monomers. After washing, the samples were dried in air and stored in desiccators until use. The values are given in Table1. IR (KBr): 3450-2500 (broadened, -COOH group), 1730, 1650, 1220, 1210 cm^{-1}.

Buffer Solutions

Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia (25). For the study of the influence of calcium ion on the properties of the graft copolymers, the buffers SGF (pH 1) or SIF (pH 7.4) were modified by adding calcium oxide. The pH change due to the addition of calcium oxide was corrected with either 1.0 N HCl or 1.0 N NaOH and then used for the drug release experiments.

Swelling ratio

Grafting of acrylic acid monomer onto biopolymers is usually performed to prepare materials with high absorbency for water. To measure the swelling, preweighed dry drug-free hydrogels were immersed in various buffer solutions (pH 7.4 and pH 1) at 37 ºC. After excess water on the surface was removed with the filter paper, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling was calculated according the relation:

$$SW\ (\%) = [(W_s - W_d)/ W_d] \times 100$$

Where, W_s and W_d represent the weight of swollen and dry samples, respectively. The study of swelling shows that swelling of hydrogels increases with time, first rapidly and then slowly, reaching maximum constant swelling (mass equilibrium swelling, MES). The swelling value of cross-linked polymers in pH 1 and pH 7.4 at 37 ºC are given in Table 2.

Insulin stability during release studies from hydrogels

In order to study the stability of insulin in contact with hydrogels, two different conditions were chosen: 37 ºC and darkness, 37 ºC and light. Insulin was loaded in hydrogels as described and then the peptide stability was investigated during release under the above mentioned conditions at two different pH values of 1 and 7.4. Samples were analyzed under each condition after 24 and 48 h. In this condition insulin remained fairly stable at both pH values during the course of experiments, indicating that adsorption of the peptide to the hydrogels and their release afterwards did not substantially influence the stability of this peptide drug.

To investigate the protective ability of the hydrogel for insulin in the harsh environment of the stomach, insulin and insulin-incorporated were treated with a simulated gastric solution that contained endopesidase pepsin. After the treatment in gastric solution, the biological activity of insulin was determined with HPLC. These results indicated that all insulin was degraded immediately after insulin was in contact with gastric fluid and the main cause of degradation was the proteolytic enzyme, pepsin. After being treated with gastric fluid, all of hydrogels demonstrated a protective effect on insulin and the biological activity remained after the treatment with gastric fluid of hydrogels. Studies of hydrogel showed that when the PAA content increased, degradation of insulin decreased.

Insulin release from hydrogels

Insulin release from the delivery systems was tested in the pyrex glasses. The powdered hydrogel (10 mg) was poured in 5ml of aqueous buffer solution (pH=7.4 & pH=1) at 37 ºC. The rotation speed was adjusted with stirrer. Samples were measured using HPLC-UV at 210 nm. The flow-rate and injection volume were 1 ml/min and 60 µL, respectively. Insulin was detected at a retention time of 5.5 min and the detection limit was 0.3 µg/mL. Triplicate samples were used. The amounts of insulin released from hydrogels was collected by taking 60-µL samples at predetermined time intervals and analyzed by HPLC.

Quantitative analysis of insulin

Three milligrams of polymer-drug adduct was dispersed in 3 mL of mobile phase solution. The reaction mixture was maintained at 37 ºC. After 4 h the hydrolysis solution filtered and analyzed by HPLC for the determination of total insulin in hydrogels. The results obtained are presented in Table 2.

In situ Bioadhesivity Studies

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri (26). A freshly cut 5-6cm long piece of small intestine of rat was obtained and cleaned by washing with

isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere hydrogels. The values are given in Table 3.

Results and Discussion

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium.

When an aqueous solution of sodium alginate is added to an aqueous solution of calcium ions, spherical alginate beads with regular shape and size are produced, since an insoluble calcium alginate matrix is formed by the cation exchange between Na^+ and Ca^{2+} (27). Alginate beads have the following advantages: 1) Alginate is known to be nontoxic as taken orally and to protect the mucous membrane of the upper gastrointestinal tract from the irritation of chemicals (28). 2) Since dried alginate beads have the property of reswelling, they can act as a controlled-release system. 3) Since the property of reswelling is susceptible to environmental pH, acid-sensitive drugs incorporated into beads would be protected from gastric juice (29). In addition, the presence of divalent calcium ions was found to affect the drug release and mucoadhesion properties of PAA polymers (8, 9).

In general, PEG has good biocompatibility and has been approved for a wide range of biomedical applications (30). Especially, the presence of grafted PEG chains in these hydrogels plays an important role. At low pH, the oxygen groups in the grafted PEG chain form hydrogen-bonded complexes by interacting with carboxylic groups of PAA. These hydrogen bonds lead to more collapsed polymer networks resulting in protection of drug incorporated in the hydrogels. Moreover, several studies have shown that these grafted PEG chains promote mucoadhesion by chain interpenetration leading to increased drug absorption through the intestinal wall.

All the matrices with the presence of PEG and increase in the content of AA had shown increased bioadhesivity (Table 3). However, in the graft copolymers, especially those with incorporated calcium ions, had better bioadhesive properties than without calcium ions and were shown to be promising buccal drug carriers for systemic delivery. The binding of those with sialic acid residues make prolonged contact of the drug with the epithelium, also it was assumed that opening of the intercellular junctions by PEG could lead to the enhancement of insulin absorption across the mucosa.

The swelling value shows that, an increase in the content of AA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. The loading numbers in Table 2 shows existence of polar functionally groups as carboxylic acid need not only for loading insulin on the polymer but also for pH-sensitive properties of polymer. Insulin molecules have a tendency to attach to polar groups due to hydrogen-bonding. Hydrogen bonding is a key contributor to the specificity of intramolecular and intermolecular interactions in biological systems. Because the increase of AA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

In vitro release of insulin

Drug releases, in both SGF and SIF media with or without calcium ions were significantly different from each other. For these hydrogels, the released insulin in the acid media increased with the molecular weight of the grafted PEG in the network. At the incorporating pH of 7.0, the carboxylic acid groups in the networks, as well as the insulin, (pI of 5), were negatively charged resulting in repulsion. Thus, the negatively charged insulin was mainly distributed in the neutral PEG chain domains. A researcher shows that insulin appeared to partition into the PEG phase in hydrogels containing PEG and a negatively charged component (31). When insulin-incorporated polymer bonded drugs were placed in acidic media, particles with longer PEG chains, where more insulin was distributed, had more chance to contact the outer aqueous environment, and as a result insulin was released by a concentration gradient at low pH. However,

there was no significant difference of insulin release at high pHs from systems with different PEG molecular weights.

The degree of hydrolysis of the hydrogels containing insulin as a function of time is shown in figure 2. It appears that the degree of hydrolysis network polymers depends on their degree of swelling and reticulated degree. With increased cross-linking and an increase in the reticulated degree of the polymer, diffusion of the hydrolyzing agents in the networks polymer is reduced and the hydrolysis rate is slower. A high different hydrolysis rate for polymers at pH 1 and pH 7.4 can be related to the number of carboxylic acid groups units along the polymer chain. Existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged $–COO^-$ groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer is increased and the hydrolysis rate increased (32).

The calcium cation can act as chelating agents and cross-linkers. Incorporation of the (Ca^{2+}) resulted in a remarkable retardation of drug release. Evidently, the divalent ions may have acted as simple cross-linking agents by interacting with carboxyl groups and forming bridges between polymer chains. This interaction may in turn reduce swelling and drug release. The effect of the amount of calcium added on drug release in SGF and SIF is shown in Table 4. At pH 7.4, the drug release decreased apparently when 3 mg of calcium ions was added into the 300 mg of Polymer Bonded Drugs (PBDs). The calcium ions may have acted as simple cross-linking agents by interacting with carboxyl groups and forming bridges between polymer chains. Furthermore, the dissolved calcium ions interact with alginate, thus forming a calcium alginate gel matrix. Therefore, the slower drug release from PBDs with calcium ions was due to the retention of the drug in the calcium alginate gel matrix. However, a large amount of added calcium 30 mg produced a faster drug release (Table 4). This can be explained by the influence of calcium on the gel formation. The gel strength increases with the addition of calcium up to a critical concentration. Above this concentration, the gel strength weakens. This weakening is due to excessive cross-linking by the calcium and hence formation of a nonhomogeneous gel matrix. The PBDs disintegrated partially, and the larger surface area created resulted in the faster drug release.

This result was consistent with findings from previous studies (33, 34). In pH 1, the drug release was insignificantly decreased in the alginate-based matrix containing a small amount of calcium ions 3 mg as shown in Table 4, probably because the added calcium ions were replaced by protons in the medium. Increasing the calcium amount in the formulations to 30 mg clearly increased the release rate. The complete disintegration of matrices would cause the faster drug release in an acidic medium.

Table 1. Composition of alginate based copolymers

Polymers	Molar composition of monomers in the feed				
	Alginate	$PEGMA^{1000}$	$PEGMA^{2000}$	MAA	CA(%)
P-1	1	1	---	3	5
P-2	1	1	---	3	10
P-3	1	1	---	5	5
P-4	1	1	---	5	10
P-5	1	---	1	3	5
P-6	1	---	1	3	10
P-7	1	---	1	5	5
P-8	1	---	1	5	10

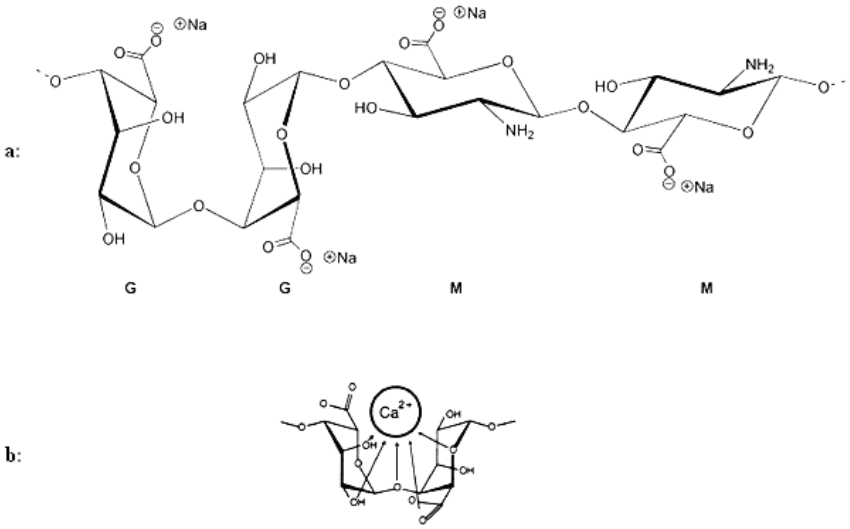

Figure 1. A schematic illustration of the principal structure of alginate: (a) the alginate chain (b) calcium alginate matrix.

Table 2. Percent of swelling and drug loading numbers

Polymers	Maximum constant swelling (%)		Percent of Insulin loading (%)
	pH 1	pH 7.4	
P-1	200	800	94
P-2	130	600	88
P-3	190	1050	99
P-4	100	950	95
P-5	250	900	98
P-6	180	750	95
P-7	240	1200	99
P-8	160	1100	99

Table 3. Percentage of particles adhered onto rat intestine

Polymers	Percentage adherence	Percentage adherence containing Ca^{2+} (3 mg)	Percentage adherence containing Ca^{2+} (30 mg)
P-1	55	58	63
P-2	50	58	54
P-3	59	60	65
P-4	55	59	65
P-5	60	62	68
P-6	55	60	65
P-7	65	65	72
P-8	60	63	68

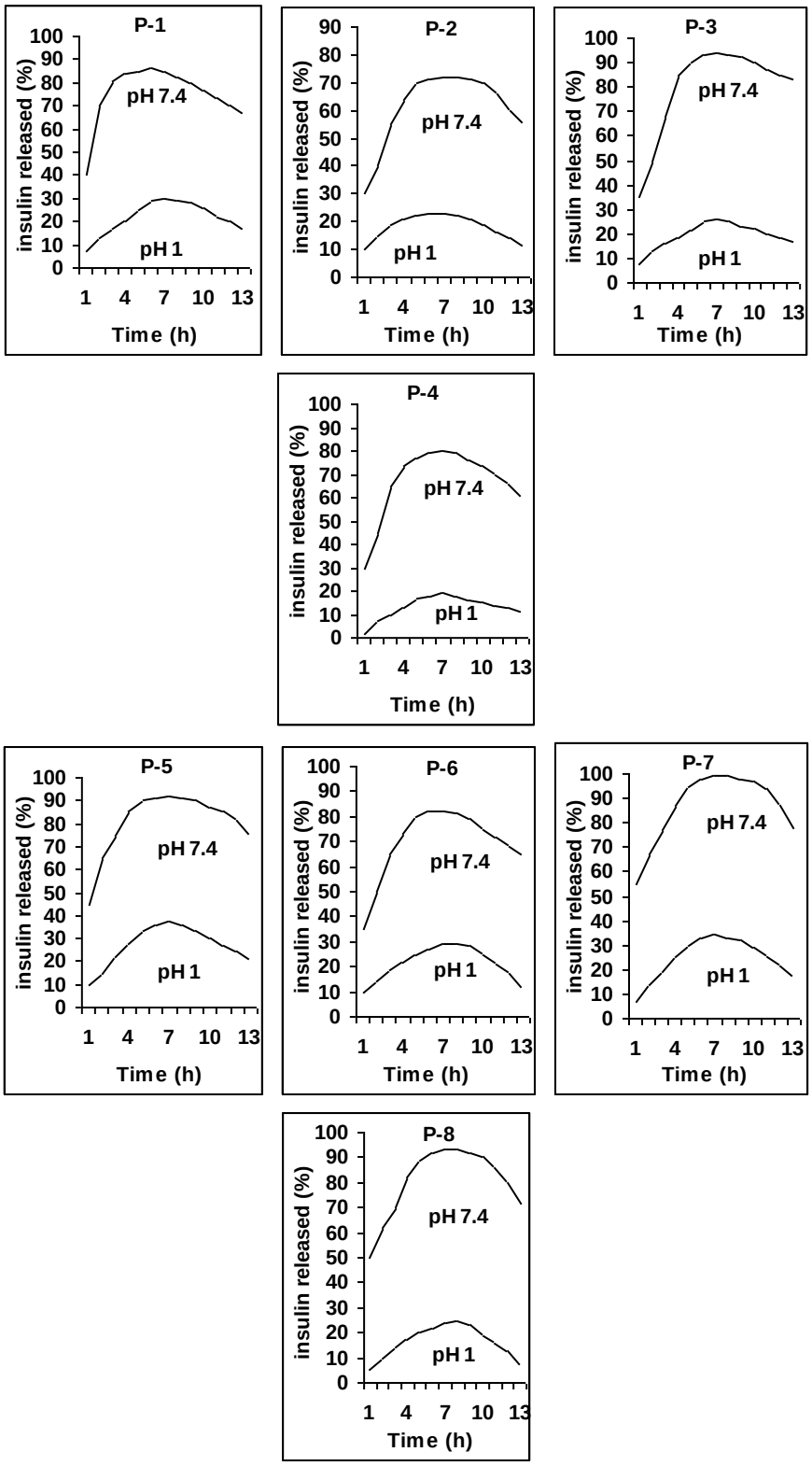

Figure 2. Release of insulin from polymeric carriers as a function of time at 37ºC.

Table 4. Effect of amount of calcium added on insulin released from polymeric carriers at 37 ºC.

Polymers	Maximum constant of release (%) at 7 hour		Maximum constant of release (%) containing Ca^{2+} (3 mg) at 7 hour		Maximum constant of release (%) containing Ca^{2+} (30 mg) at 7 hour	
	pH 1	pH 7.4	pH 1	pH 7.4	pH 1	pH 7.4
P-1	30	86	10	25	25	60
P-2	23	72	8	20	20	50
P-3	26	94	8	27	25	75
P-4	19	80	7	22	15	67
P-5	37	92	15	25	35	65
P-6	29	82	8	20	25	57
P-7	34	99	12	33	30	80
P-8	25	93	8	30	23	75

Conclusion

A new nonirritating buccal adhesive system for the controlled-release of insulin was developed using graft copolymers of alginate and acrylic acid in various compositions. Novel bioadhesive and pH-responsive hydrogels containing pendent alginate (Poly(alginate-co-AA-co-MEG)) were synthesized by free-radical crosslinked copolymerization. By regulating the crosslinking percentage of the AA copolymers, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. The hydrolysis of the drug-polymer conjugates were performed at pH 1 and 7.4 at 37 °C. The drug-release profiles of PBDs indicated that the amount of drug released depended on the amount of calcium ions.

Acknowledgement: The office of research vice chancellor Azarbaijan University of Tarbiat Moallem has supported this work.

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4/12/2009

Biochemical Changes in *Parasarcophaga. aegyptiaca* and *Argas (persicargas) persicus* Haemolymph Infected With Entomopathogenic Nematode

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Abstract: The physiological changes in *Parasarchofaga aegyptica* larvae and *Argas (persicargas) persicus* adult haemolymph had been investigated resulting in entomopathogenic nematode infection. It was dramatically declined in total protein and total lipids in both *P.sarchophaga* and *A.persicus*. The Amino acid was fluctuated between increase in aspertine, glutamine, serine and decrease in glycine, histadine, argnine, porline, tyrosine, valine, isoleucine, leucine, methonine, and phenylalanine. Also, there was a significant increase in protease and lipase activity in larval and Adult haemolymph of both studied hosts after nematode infection. [Nature and Science. 2009;7(6):70-81]. (ISSN: 1545-0740).

Key words: Entomopathogenic nematodes; Steinernema; Heterorhabditits; Parasarchophaga; Argas; Ticks; Biochemical.

INTRODUCTION

With the exception of some lipids and carbohydrates, information on biosynthetic processes in entomopathogenic nematodes is extremely limited(Wright and Perry, 2002). Knowledge of which amino acids cannot be synthesized by nematodes ('essential' amino acids) has been based partly on nutritional studies on species that have been grown axenically in fully defined culture media (Vanfleteren,1980). For example, *Caenorhabditis elegans* and *C.briggsae* require arginine pluse nine amino acids that are usually essential in mammals. The requirement for entomopathogenic nematodes to be produced monoxenically has precluded such studies. Like most organisms, nematodes can synthesize purine and pyrimidine bases but studies on the biosynthesis on nucleoside monophosphates are lacking. Anderson and Kimble (1997). have reviewed the translational mechanisms involved in protein synthesis in *C.elegans*. Nutrial lipids include triacylglycerols, diacylglycerols, free fatty acids, sterol esters of fatty acids, and free sterols. Phospholipids, which provide the major proportion of cellular membranes, are usually derived from a molecule of glycerol, two fatty acids, phosphoric acid and and asecond alcohol. In freshly-emerged infective juveniles of *Steinernema* species (Patel and Wright, 1997). Other lipids include glycolipids, lipoproteins and proteolipids (Chitwood,1998). For entomopathogenic nematodes, the quality and quantity of lipids in infective juveniles produced on a commercial scale is paramount since this has a critical influence on nematode viability and infectivity (Wright and Perry, 2002).

The physiological information of the entomopathogenic nematode pathogenisity on veterinary insects are lacked.So, this investigation aims to study the evaluation of the nematodes *Steinernema riobrave* and *Heterorhabditis bacteriophora* Hp88 role in degradation haemolmph proteins, amino acids and lipids in infected flesh-fly larvae *Parasarcophaga. aegyptiaca* and chicken ticks *Argas (persicargas) persicus* through quantitatively determination of protease and lipase enzymes present in the haemolymph of control and infected hosts.

MATERIAL AND METHODS

Biochemical studies: Five larvae of *Parasarcophaga aegyptiaca* and five adult *Argas (persicargas) persicus* were homogenized separately as a whole body in 5 ml of extraction solvents. The samples were centrifuged for 20 minutes at 10,000 rpm, and then the supernatant was collected for the biochemical

studies. The experiment was done at 10, 20, and 40 hr post injection of *P. aegyptiaca* and 10, 20, and 30 hr after infection of *A.(P) persicus*.

***P. aegyptiaca*:** The third instar larvae were divided into 4 groups ;
1)Control negative group. 2) Control positive group, injected with 10µ water.3) Injected group with 40 IJs/larva of *Heterorhabditits bacteriophara.* 4) Injected group with 40 IJs/larva of *Steinernema riobrave*.Every group contain 10 larvae.

***A. persicus*:** In this case adult were frankly exposed to 500 infected juveniles (IJs) which were suspended in 1.5 ml water and sprayed on 15gm clean sand. The used plastic pots were 25cm^3 .This experiment divided into 3 groups.1) Control group. 2) Infected group with *H. bacteriophara*. 3) Infected group with *S. riobrave.* Every group contains 10 pots every pot contains 3 adults.

Determination of total protein: Total protein was determined using kit supplied by Diamond, according to Doumas (1975) at 546 nm wave length.

Sample preparation: Protein was extracted from the haemolymph by the homogenization the hall body in NaCl 0.15 Molar according to Marion (1976).

Free amino acid analysis:
HPLC determination of the free amino acid content in the haemolymph:
Haemolymph free amino acids were detected by high performance liquid chromatography (HPLC) using the precolumn PTC derivatization technique according to the method of Heinrikson and Meredith (1984). The HPLC system of Perkin- Elmer consisted of quaternary pump; a column oven, Rheodine injector and 20µ / loop, UV variable wavelength detector. The report and chromatogram taken from data acquisition program purchased from Perkin- Elmer. PICO- TAG column (Waters) for free-amino acid analysis 3.9 × 30 cm.; Eluent (1) and Eluent (2), Phenylisothiocyanate (PITC), Triethylamin, *Amino acids standard. (standards and eluents are Waters chemistry package for free amino acids). 46 ^{0}C; wave-length: 254 nm; flow rate: 1ml/min.

Preparation of the sample: *Parasarcophaga aegyptiaca* larvae were injected with 40 IJs /larva and *Argas (p) persicus* was infected with 500 IJs / one, and then let them for 40 hr post infection. Control samples were considered in all experiments. The first step in determination of amino acids by HPLC method involved weighting and homogenization of the haemolymph in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was spun at 3000 r.p.m. for 10 min and the supernatant was divided into two halves; the first was dried using vacuum (70 millitore) at room temperature, whereas the second half was used for monoamine determination.

Derivatization procedure: The derivatization started by re drying the sample under test using drying solution consisted of 2:2:1 mixture (by volume) of methanol: 1M sodium acetate trihydrate: triethylamine (TEA). The drying solution was added to the dry sample, shook well and then put under vacuum till complete dryness.

The derivatizing agent consisted of 7:1:1:1 mixture (by volume) of methanol: TEA: water: PITC (Phenylisothiocyanate). The derivatizing solution was added to the redried sample, shook well and left to stand at room temperature for 20 min, then applied to vacuum (70 millitore) till dryness. The dry sample was then diluted by a sample dilution composed of 0.71-g di sodium-hydrogen phosphate adjusted to a pH of 7.4 by 10% phosphoric acid. Acetonitrile was then mixed, as 5% by volume with the resulting solution.
Derivatized amino acids standard and derivatized sample were injected, (the injected volume is 20µl), into the column for separation by HPLC. The resulting chromatogram identified each amino acid position and concentration from the sample as compared to that of the amino acids standard and finally the determination of the µ mole content of each amino acid per gram brain tissue was achieved.

Determination of total lipid: Total lipid was determined using kit supplied by Diamond, according to Knight *et al*., (1972), at 525 nm wave length.

Sample preparation: Lipids were extracted from the haemolymph according to Abu-Hatab and Gaugler (1997) as homogenization in 2:1 chloroform : methanol (v/v).

Determination of protease enzyme: The protease activity of *A.(P) persicus* adult or *P. aegyptiaca* larva was determined by the casein digestion method at 280 nm wave length according to Birk, ***et al***., (1962).

Sample preparation: The enzyme solution was prepared by the extraction method of Abu-Hatab ***et al***., (1995). The samples were homogenized in phosphate buffer 0.1 Molar at PH 7.6. The method is based on casein substrate since it is considered to be supplemented protein.

Procedures: The reaction mixture was prepared by addition of 0.2 ml glycine-NaOH buffer (0.075 Molar, PH 10) and 0.4 ml 1.5 % casein solution, to 0.2 ml enzyme solution. After 60 minute incubation at 37 C°, the enzymatic activity was determined by adding 1.2 ml of 5% trichloroacetic acid solution. The reaction mixture was centrifuged at 18,000 rpm for 15 minute. The supernatant was taken for enzymatic activity evaluation.

Determination of lipase enzyme:

Sample preparation: The enzyme solution was prepared by the extraction method of Abu-Hatab ***et al***., (1995)**.** The samples were homogenized in phosphate buffer 0.1 Molar at PH 7.6. The method depends on titration of liberating fatty acids with alkaline reagent.

Procedures: Lipase activity was measured according to the method of Tietz and Fiereck (1966, 1972). Substrate emulsion were prepared by mixing 0.2 gm sodium benzoate with 7 gm gum acacia in 100 ml water in mechanical blender at slow speed, and then 1.3 gm triolin was added slowly and mixed for 10 minute at maximum speed. The 3 ml assay reaction mixture contained 2 ml of substrate emulsion, 0.4 ml 20 mM Tris-HCl, PH 8.0, 0.4 ml 5 mM $CaCl_2$, and 0.2 ml enzyme solution. The reaction mixture was incubator 1 hr at 37 C°. The reaction was stopped by addition of 0.6 ml 96% ethanol. Then titrated with 5 mM NaOH in presence of an indicator (phenolphthalein).

Statistical Analysis: The data were subjected to statistical analysis using T. test and F test (one way classification least significant differences "L.S.D.") according to (Snedecor and Cochron, 1967). The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P<0.05$, 0.01). All analysis was made using a software package "Costat", a product of Cohort Software Inc. Berkeley, California.

Results

Total protein content: There were dramatically declined in total protein content of *P. aegyptiaca* larvae 10hr and 20hr post injection. It was also found that, the lowest decline was recorded at 40 hr post injection with *H. bacteriophora* and *S. riobrave* 0.12±0.08 and 0.142±0.01 respectively (Table 1). Results of *A.persicus* haemolymph gave highly significant declined in total protein content in the same intervals time. The data declared that, the infection with *S. riobrave* induce more decrease in total protein content than *H. bacteriophora* (Table 2).

Amino acids:

The data indicated that, the free amino acids aspertine, glutamine, and serine increased 35.5, 19, and 82% respectively in infected larval haemolymph of *P. aegyptiaca*, but the other amino acids glycine, histadine, argnine, porline, tyrosine, valine, isoleucine, leucine, methonine, and phenylalanine decreased -65.8, -36, -73,
-8.9, -66.4, -97, -24, -15.7, -94.4, and -63.5 % respectively in infected larval haemolymph of *P. aegyptiaca* (Table 3). There were clearly changes in free amino acids concentration in control and infected haemolymph of *A. (p) persicus*. These changes demonstrated as increasing in concentrations of aspartine, glutamine, and serine 57.6, 16, and 92.9% respectively post infection of *S. riobrave* nematode, and sharply decline in concentrations of glycine, histadine, theronine, alanine, valine, isoleucine, and leucine, -48.2, -96.3, -92.4, -87.2, -75.2, -82.1,and -69% respectively in infected haemolymph of *A.(p) persicus*. But argnine and phenylalanine were disappeared in infected groups (Table 4).

Total lipid content: The results obtained in table (5) showed that, there was reduction in total lipid content of the 3rd instar larvae of *P. aegyptiaca* after 10 hr post injection with *H. bacteriophora* and *S. riobrave* 3.83±0.09 and 3.62±0.03 respectively as compared with the two types of control 4.61±0.12 and 4.3±0.17**.** The lowest decrease in total lipid content was recorded at 40 hr post injection. From the present data we noticed that, the injection with *S. riobrave* induced more reduction in total lipid content. The reduction in total lipid was clearly observed in the infected groups of *A. (p) persicus* which measured at10 and 20 hr after infection. The lowest decrease was recorded at 30 hr post infection with *H. bacteriophora* and *S. riobrave* as compared with control group. The present data represented that, the infection with *S. riobrave* induce more decrease in lipid content than *H. bacteriophora* (Table 6).

Protease activity: The results revealed that, there was significant increase ($P \leq 0.001$) in protease activity after 10 hr of injected groups of *H.bacteriophora* and *S.riobrave* 363.26±2.107 and 345.56±2.8 respectively, compared with control groups 214.3±4.1 and positive control groups 289±2.It also observed that, the protease activity of *P. aegyptiaca* was increased by the increasing of the time post injection (Table7). The results in table (8) revealed that, there was significant increase ($P \leq 0.001$) in protease activity of *A. persicus* after 10 hr post infection with *H. bacteriophora* and *S. riobrave* 167.53±2.83 and 252.43±1.5 respectively compared with control group 70.46 ±.38. The same results were obtained in the infected groups at 20 hr post infection. Thus the highest increase in protease activity was recorded at 30 hr post infection with the two strains of nematodes *H. bacteriophora* and *S. riobrave* 586.1± 6.65 and 564.43±7.17 respectively as compared with control group 80.8±0.18.

– Lipase activity: The data demonstrated that *S. riobrave* more increase in lipase activity as compared with *H. bacteriophora* in both two studied hosts. Table (9) revealed that, there was a significant increase ($P \leq 0.001$) in lipase activity of 3rd instar larvae of *P. aegyptiaca* after 10 hr of injected groups with *H. bacteriophora* and *S. riobrave* 33.5±0.28 and 35.66±0.166 respectively as compared to the two control groups. Such changes were clearly observed in infected *P. aegyptiaca* estimated at 20 and 30 hr post injection. Similar changes were clearly observed in infected *A.(p) persicus* estimated at10 and 20 hr post infection. The highest increase in lipase activity was recorded at 30 hr post infection with *H. bacteriophora* 586±6.65 and *S. riobrave* 564.43±7.17 compared with the control group 80.8±0.18 (Table10).

Table (1): Effect of *H. bacteriophora* and *S. riobrave* on total protein content (gm/dl)of non infected and infected *P. aegyptiaca*, n = 5

Time (hours) Post infection	Contro Negative	Control Positive	*H bacteriophora*	*S. riobrave*
10	1.58±0.03	1.49±0.02	0.9±0.02 ***	1.05±0.089 ***
20	1.49±0.01	1.43±0.02	0.43±0.02 ***	0.45±0.058 ***
40	1.35±0.01	1.34±0.03	0.12±0.08 ***	0.142±0.01 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 highly significance**

Table (2): Effect of *H. bacteriophora* and *S. riobrave* on total protein content (gm/dl) of non- infected and infected *A. (p) persicus.* n=*5*

Time (hours) post infection	Control	*H. bacteriophora*	*S. riobrave*
10	**2.17±0.03**	**1.68±0.02 *****	**1.29±0.26 *****
20	**2.02±0.02**	**1.37±0.037 *****	**1.004±0.005 *****
30	**2.01±0.02**	**0.84±0.039 *****	**0.91±0.0134 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (3): Effect of *Steinernema riobrave* on the concentrations of free amino acids in the haemolymph of the 3rd instar larvae of *Parasarcophaga aegyptiaca*.

Free amino Acids	Control mg /dl	Treated mg /dl	% of change from control
Aspertine	14	18.97	35.5
Glutamine	314	373.66	19
Serine	24	43.68	82
Glycine	5	1.71	-65.8
Histadine	1	0.64	-36
Argnine	8	2.16	-73
Proline	16	14.58	-8.9
Tyrosine	23	7.73	-66.4
Valine	15	0.45	-97
Isoleucine	8	6.1	-24
Leucine	7	5.9	-15.7
Methonine	7	0.39	-94.4
Phenlalanine	7	2.56	-63.5

Table (4): Effect of *Steinernema riobrave* on the concentration of free amino acids in the haemolymph of the adult *A. (p) persicus.*

Free amino Acids	Control (mg /dl)	Treated (mg /dl)	% of change from control
Aspertine	6.34	10	57.6
Glutamine	80.24	93.16	16
Serine	8.12	15.66	92.9
Glycine	7.01	3.6	-48.2
Histadine	8.43	0.31	-96.3
Theronine	7.5	0.57	-92.4
Argnine	Trace	-	-
Alanine	4.01	0.5	-87.2
Valine	24.92	6.2	-75.2
Isoleucine	7.44	1.33	-82.1
Leucine	5.35	1.66	-69
Phenylalanine	Trace	-	-

Table (5): Effect of *H. bacteriophora* and *S. riobrave* on total lipid content (gm/dl) of non infected and infected *P. aegyptiaca*, n = 5

Time (hours) Post infection	Control negative	Control Positive	*H. bacteriophora*	*S. riobrave*
10	4.61±0.12	4.3±0.17	3.83±0.09 ***	3.62±0.03 ***
20	3.97±0.04	3.9±0.05	3.48±0.1 ***	3.25±0.02 ***
40	3.67±0.01	3.6±0.02	1.8±0.07 ***	1.36±0.15 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (6): Effect of *H. bacteriophora* and *S. riobrave* on total lipids content of non infected and infected *A. (p) persicus* n=5

Time (hours) Post infection	Control	*H. bacteriophora*	*S. riobrave*
10	**2.97±0.029**	**1.25±0.06 *****	**1.11±0.052 *****
20	**2.96±0.035**	**0.983±0.02 *****	**0.88±0.014 *****
30	**2.72±0.15**	**0.761±0.031 *****	**0.671±0.031 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (7): Effect of *H. bacteriophora* and *S. riobrave* on protease activity units 10^3x no of non infected and infected *P. aegyptiaca* n=5

Time (hours) Post infection	Control negative	Control Positive	*H bacteriophora*	*S.riobrave*
10	241.3±4.1	289±2.1	363.26±2.107 ***	345.6±2.8 ***
20	274.8±3.5	319.7±3.1 *	544.2±3.1 ***	590.5±4.9 ***
40	315.7±2.9	387.4±3.2 *	686.7±7.8 ***	811.03±5.8 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (8): Effect of *H. bacteriophora* and *S. riobrave* on protease activity units x 10^3/no. of non infected and infected *A. (p) persicus.* n=*5*

Time (hours) Post infection	Control	*H.bacteriophora*	*S. riobrave*
10	**70.46±0.38**	**167.53±2.83 *****	**252.43±1.5 *****
20	**71.7±0.46**	**325.2±11.76 *****	**416.1±8.68 *****
30	**80.8±0.18**	**586.1±6.65 *****	**564.43±7.17 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (9): Effect of *H.bacteriophora* and *S. riobrave* on lipase activity units no. of Mm of fatty acid/hr of non infected and infected *P. aegyptiaca* n = 5.

Time (hours) Post infection	Control Negative	Control Positive	*H.bacteriophora*	*S.riobrave*
10	25±0.0	25.7±0.2	33.5±0.28 ***	35.7±0.2 ***
20	25.66±0.2	26.5±0.3	37±0.6 ***	38.8±0.16 ***
40	26.5±0.5	27.2±0.2	44.16±0.6 ***	47.2±0.16 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (10): Effect of *H. bacteriophora* and *S. riobrave* on lipase activity unit (no of Mm fatty acid/hr of non infected and infected *A. (p) persicus* n=5.

Time (hours) Post infection	Control	*H.bacteriophora*	*S. riobrave*
10	**70.46±0.4**	**165.53±2.83 *****	**252.43±1.5 *****
20	**71.7±0.46**	**325.2±11.76 *****	**416.1±8.68 *****
30	**80.8±0.18**	**586.1±6.65 *****	**564.43±7.17 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

DISSCUSION

The present study has been shown that, the nematodes *H. bacteriophora* and *S. riobrave* severely deplete proteins in the haemolymph of larval *P. aegyptiaca* and adult *A. (p) persicus.* This finding agree with that obtained by Mckinstry and Steinaus (1970) who were able to separate electrophoretically septicaemic and fresh plasma of *G. mellonella* infected with *pseudomonas aeurginosa* and detected a reduction in the amount of protein. Also, Pare *et al.,* (1977) studied the effect of *Bacillus thuringiensis* on the plasma protein of *Choristeneura fumiferana* and reported reduction in number of slow moving proteins.

Similar results were observed by Abdel- Kawy (1981 & 1985), El-Bishry (1989) who reported that, *N. carpocapsae* caused a reduction and or disappearance of haemolymph slow moving protein fractions in both *Schistocerca gregaria* and *S. littoralis*. Johns *et al.,* (1998) found that, the bacterial infection in the hard tick *Dermacentor variabilis* reduced the protein content non significantly by 24 hr, but the mean protein concentration was even lower at 48 hr which was highly significant. Moreover Gillespie *et al.,* (2000) observed that, there was reduction in total protein content of the haemolymph of desert locust *Schistocerca gregaria* during the course of infection with the entomopathogenic fungus, *Mertarhizium anisopliae* var *acridum*.

The losses of soluble protein from the host' haemolymph during parasitism may be explained in three ways. (1) The parasite may secrete proteolytic enzemes into the haemocoel of the insect and hydrolyze the host's proteins. Muller (1931) suggested that, mermithids produce a secretion that serves for the predigestion of the host haemolymph. In addution, Gordon and Webster (1971) could not find proteolytic enzyme activity in homogenates of *Mermis nigrescens* and concluded that this nematode affects the protein metabolism directly or indirectly in the fat body of the host. (2) Protein metabolism in the host fat body may be altered by nutritional stress or endocrine manipulation brought on by parasitism. Protein metabolism, transport of amino acids, and excretion are all regulated hormonally (Highnam and Hill, 1969) and disturbance of the host's endocrine balance by the parasite could be responsible for reduced protein concentrations in the host haemolumph. (3) The nematodes may absorb intact protein directly from the hosts' haemolymph; however, research by Gordon and Webster (1972) showed that. *Mermis nigrescens* was unable to incorporate a dipeptide, tritiated L-histihyl-L-leucine, or tritiated haemolymph proteins of *Schistocerca gregaria* into its protein, whereas labeled amino acids were rapidly utilized.

On the other hand our results do not agree with Andreadis and Hall (1976) who studied the defense reaction of *Aedes aegyptii* against the nematode *Neoaplactana carpocapsae* elactrophoretically and showed a shift in certain bands, a reduction in intensity of others and him presence of an additional protein fraction. They also found that, some proteins released by the host or by the parasite in response to parasitism, and were unable to understand the function of this protein fraction in the defense reaction. Hulbert *et al.,* (1985); Spies and Spence, (1985); Dunn, (1986); Spies *et al.,* (1986); Dimarcq *et al*., (1990) found that, the injection of forgein molecules into the insect haemolymph induce the synthesis of immune protein, these finding confirmed with Ayaad *et al.*, (2001).They observed that, the stimulated newly protein bands particularly at 40 hr post injection of *P. surcoufi* with *H. bacteriophora* are probably immune proteins.

Amino acids play an important role in maintaining the proper osmotic balance in haemolymph of arthropods (Sutcliffe, 1963). The effect of parasitism on the total and individual amino compounds varies depending on the host and parasite considered.

The entomopathogenic nematodes parasitism caused sharply changes in free amino acids of *P. aegyptiaca* and *A. (p) persicus* haemolymph. In this study, results induced dramatically increased in 3 amino acids in *P. aegyptiaca* and *A. (p) persicus* haemolymph after infection with *S. riobrave* nematode. On the other hand there was depletion in 10 amino acids in both hosts after infection. Our results confirmed with Gordon *et al.*, (1978) who reported variable results between two black fly species. In *Prosimulium mixtum / fuscum* most amino compound concentrations were reduced by parasitism, while nematode infections of *Simulium venustum* induced increases in the concentration of almost half of the ninhydrin positive substances. However, these infected and control black flies. Moreover, Rutherford and Webster (1978) found that parasitism of *Schistocerca gregaria* by *Mermis nigrescens* caused the haemolymph levels of 10 amino acids to increase and 3 to decrease significantly.

Rutherford *et al.,* (1977) explain the variable effects on amino acids encountered in nematode infections reflect the dynamic relationship between host and parasite. The importance of maintaining an osmotic balance in the insect haemolymph necessitates an efficient system of amino acids regulation. Hormones are responsible for control of excretion of nitrogenous compounds, transport of amino acids into the fat body, protein synthesis, and proteolysis. A simpler alternative is that the mermthid absorbs the amino acids selectively across the cuticle. Schmidt and Platzer (1980) reported that, the reduction in the

haemolymph amino acids are replenished by increased host proteolysis, decreased excretion or transport into the fat body, or reduced protein synthesis, these explanation agree with our results in determination of total protein and protease activity of *P. aegyptiaca* and *A. (p) persicus* post infection with nematode, where we showed sharply decrease in total protein which were replenished by increasing in protease activity in both hosts *P. aegyptiaca* and *A. (p) persicus*.

In our study the entomopathogenic nematodes *S. riobravus* belongs to the family Steinernematidae have symbiotic bacteria *Xenorhabdus spp*. This bacteria play an important role in enhance and proliferate the host haemolymph for the nematode reproduction. So, we can explain the sharply changes in the free amino acids of the two studied hosts *P. aegyptiaca* and *A. persicus* haemolymph, that, it may be due to the interference between the nematode and symbiotic bacteria inside the host. In addition knowledge of which amino acids can not be synthesized by nematodes (essential amino acids) has been partly on nutritional studies on species that have been grown axenically in fully defined culture media (Vanfleteren, 1980). For example, *Caenorhabditis elegans* and *Caenorhabditis briggsae* require arginine plus nine amino acids that are usually essential in mammals. Rutherford and Webster (1978) reported changes in the individual carbohydrates and amino acids in the haemolymph of infected locusts. It has been also noticed from this present study that, there was actual decrease in total lipid of infected hosts after 10, 20, 30, and 40 hr from infection with the nematodes. These results were agreed with Milstead (1979) while studied the pathophysiological influences of nematode *H. bacteriophora* complex on the sixth instar larvae of *G. mellonella*, he reported that, shortly after the nematode penetration into the haemocoel of the larvae begin feeding upon the fat body. Thompson and Barlow (1983) reported that, an extreme depression of glyceride synthesis would allow the parasite to use its host's fat after partial digestive hydrolysis and its own fatty acids for rapid triglyceride synthesis, thereby minimizing the energy cost of fat synthesis. Moreover, our results were agreed with Ghally *et al.,* (1988) who observed that, the lowest decline in total lipids was after 18 hr post infection of *Ceratitis capitata Wiedmann* with *Steinernema feltiae* Filipjev nematode. This finding is in consort with the findings of Hawlitzky and Boulay (1986) who reported that, the significant declines in lipids of parasitized larvae *Anagasta kuehniella* Zell by *Phanerotoma flavitestacea* are a normal feature with the general scope of endoparasite action on host chemical composition.

The higher level of protease enzyme in infected hosts compared with control may be due to the parasite may secrete proteolytic enzymes into the haemocoel of the insect and hydrolyzed the hosts proteins once hydrolyzed the free amino acids may be absorbed by the nematode and utilized for protein synthesis Rubstov (1967), who suggested that the parasitic stage released proteases for the digestion of the host fat body and absorbed the partially dissolved products through cuticle. Production of a toxins by axenic nematodes was discussed also by Goetz et al. (1981), Burman (1982), Goetz and Guelzon (1982), Boeman et al., (1983) and El-Bishry (1989). Poinar (1979) stated that, insect death probably arises from production of proteolytic enzymes which explain the relative lack of resistance to *Achromobacter nematophilus* when just one to three cell are injected in the body cavity of *G. mellonella* larvae Abdel Kawy (1985) mentioned that proteinase may produced by *X. nematophilus* since the addition of potato extract as proteinase inhibitor to infected haemolymph filtrate decreased the lethal effect of that filtrate when injected in healthy *Spodoptera* larvae. But protease may be released from disrupted fat body tissue and in part may account for the substantial proteolytic activity found in otherwise protein-depleted haemolymph of *Culex pipiens*. Digestion of proteins in the haemolymph by proteases present in the haemolymph may provide additional amino acids (Schmidt and Platzer,1980). However Gordon and Werbster (1971), reported that, proteolytic enzymes were absent in homogenates of *M. nigrescens*.

It is well obvious from the present work that, lipase enzyme of parasitized hosts *P. aegyptiaca* and *A. (p) persicus* is higher than controls; this finding may be provided by the reduction of total lipids. Spasski *et al.*, (1977) determined the lipase, pepsin, trypsine, pectinase and invertase activity in Hydromermis and observed that, the enzymic activity was much higher in pre parasitic than in post parasitic larvae, these enzymes are important in the extra intestinal digestion of the mermithids and probably also effect the lyses of the chitinous covering the host.

Metabolite depletion by the parasite could cause physiological imbalances in the host that lead to a reduction in haemolymph protein and lipid concentrations, and increase in protease and lipase enzymes activity. Mechanisms by which the nematode parasite could bring about such changes are unknown.

Generally, the purpose of this study was to evaluate the role of nematodes *H. bacteriophora* and *S. riobrave* in physiological imbalances in the hosts *P. aegyptiaca* and *A. (p) persicus*. This imbalance causes homolysis of haemolymph of the host and then death of the host. Thus we can use the entomopathogenic

nematodes as biological control agent against some veterinary insect pest which spend all or part from their life in soil.

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4/14/2009

Kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside from *Hymenophyllum crispatum*

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Abstract: *Hymenophyllum crispatum*, Wall (family Hymenophyllaceae), is a Filicinae fern of leptosporangiate group. It is widely distributed in sub-alpine regions of Kumaun Himalaya. For the present chemical investigation, *H. crispatum* was collected from the forest vegetation near to the timber line of Pindari glacier of Kumaun, Uttarakhand (India). Various members of the genus *Hymenophyllum* have previously been screened for anti-microbial activities. *H. crispatum*, a species native to Himalaya has still not been investigated for various biological activities and active constituents. Present communication reveals the isolation of a flavonol-di-O-glycoside from the fern fronds of *H. crispatum*. About 1kg air dried and powdered sample of *H. crispatum* was extracted sequentially with 80% aq. MeOH and 50% aq. MeOH. Both the extracts were combined and concentrated under reduced pressure until only small H_2O layer (approx.50ml) remained. It was partitioned with CH_2Cl_2 and BuOH successively. The BuOH fraction was chromatographed on Whatman No. 3 PC using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. After inspecting dried and developed chromatograms with UV light (360nm) a broad dark purple fluorescent band was observed. It was cut and eluted separately. The residue of the band was further chromatographed on Sephadex LH-20 column using 50% aq. MeOH as an eluent. Three flavonol-3-O-glycosides were isolated and identified. Out of these three glycosides, quercetin-3-O-ά-L-rhamnoside and quercetin-3-O-rutinoside were identified by CoPC with their standards and kaempferol-3-O-ά-L-glucosyl (1→2) rhamnoside was identified by color reactions, UV, ^{1}HNMR and hydrolytic methods. [Nature and Science. 2009;7(6):82-85]. (ISSN: 1545-0740).

Keywords: *Hymenophyllum crispatum,* flavonol-di-O-glycoside, active constituents.

Introduction

Hymenophyllum, genera of Filicinae fern of leptosporangiate group, is well known as a filmy ferns of family Hymenophyllaceae. It is a native to sub-alpine regions of Kumaun Himalaya and is widely distributed in moist shady places from 2000m-3800m. *H. crispatum* is a tribal folk medicinal plant of Kumaun Himalaya and various tribal inhabitants of the region use the plant extract for curing cough, bronchitis, asthma, wound healing and ulcers (Pande, 1992). Looking on the traditional medicinal significances of fern, the BuOH fraction of the fern was investigated chemically for flavonol-3-O-oligosacharide. The extracts derived from other medicinal plants have widely been investigated for various biological activities (Khetwal and Verma, 1983, 1984, 1986, 1990; Khetwal *et al.*, 1985, 1986; Mishra and Verma). Flavonoidal compounds, polyphenolic heterocyclic compounds, form a major family of natural products. Such compounds isolated from plants have widely been used for curing cancer, coronary dysfunction, inflammation, rheumatic arthritis, immune system decline, brain dysfunction and cataracts (Middleton *et al.*, 2000; Havsteen, 2002).

Material and methods

1. Authentification of fern species: *H. crispatum* Wall was collected from Kaphani and Sunderdhunga glaciers of Kumaun in the month of August. The Voucher specimen of fern was identified by Prof. P. C. Pande, Department of Botany, Kumaun University, SSJ campus, Almora (Uttarakhand) India, and deposited in the Department of Chemistry (Vouch. Sp. No.42).

2. Method of extraction: About 1kg air dried and powdered sample of *H. crispatum* was extracted sequentially with 80% aq. MeOH and 50% aq. MeOH. Both the extracts were combined and concentrated under reduced pressure in Rota-evaporator at 40^0C. The residue was fractionated between 50% CH_2Cl_2. The CH_2Cl_2 layer was separated and H_2O layer partitioned with BuOH. The BuOH Soluble was evaporated to dryness and residue was dissolved in MeOH and it was banded on Whatman No. 3 PC (10 sheets) using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. After three times repeated development

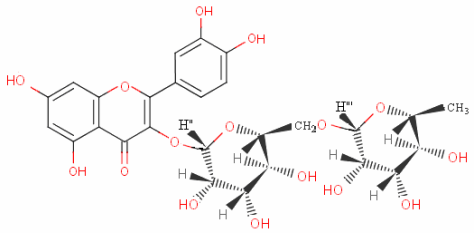
of PC with BAW, a dark purple fluorescent band was observed between two blue fluorescent bands on Pc with UV light. The broad dark purple fluorescent band was cut and eluted separately with 70% aq. EtOH. The aq. ethanolic elute was evaporated to dryness and residue was chromatographed on sephadex LH-20 CC using 20% aq. MeOH as an eluent. On eluting CC with 20% aq MeOH, three purple fluorescent bands were observed on PC with UV light of which the middle band was eluted separately from CC by monitoring with UV light. The aq. methanolic elute was evaporated to dryness. The residue was dissolved in 80% aq. MeOH and it was chromatographed on whatman No.1 PC using 30% HOAc as an eluent. Two purple fluorescent bands were observed on PC with UV light and were eluted with 70% aq. MeOH separately by monitoring PC with UV light.

Results and discussion

The slower moving compound was identified as quercetin-3-O-rutinoside (A) by CoPC with its standard using three solvent systems, BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer), 30% HOAc and BEW (n-BuOH-EtOH-H_2O, 4:1:2.2, upper layer). It was finally confirmed by ^{1}HNMR in DMSO-d_6, 500 MHz (table [1]):

Table [1]: ^{1}HNMR of compound (A) in DMSO-d_6, 500MHz

Shift (δ)	Multiplicity	Identification
6.20	1H, d, J=2.0Hz	H-6
6.40	1H, d, J=2.0Hz	H-8
6.89	1H, d, J=8.5Hz	H-5'
7.20	1H, dd, J=8.5 and 2.0Hz	H-6'
7.35	1H, d, J=2.0Hz	H-2'
5.28	1H, d, J=7.5Hz	Anomeric proton glucose
4.38	1H, d, J=1.0Hz	rhamnosyl anomeric proton
3.0-4.20	10H, m	For remaining sugar protons of gluco.&rham.



(A) Quercetin-3-O-rutinoside

The faster moving compound (B) gave an amorphous grey powder, mp 210^{0}C. The molecular formula of compound was deduced as $C_{27}H_{29}O_{15}$ from FABMS (-ve). The compound gave positive tests with $FeCl_3$, Mg+HCl and α-naphthol, indicating a polyphenolic heterocyclic glycoside (Harborne, 1967). It appeared as a dark purple fluorescent on PC with UV light and changed to yellow-green with NH_3 vapours, indicating presence of hydroxyls at C-4' and C-5 (Mabry *et al.*, 1970).

When cellulose TLC of the compound was sprayed with methanolic solution of NA reagent, the purple fluorescence of compound turned to yellow, indicating absence of ortho-di-hydroxyl group in B-ring and presence of 4'-OH group (Homberg and Geiger, 1960; Geiger and Homberg, 1963). The purple fluorescence of compound changed into fluorescent yellow-green with $AlCl_3$ and $ZrOCl_2$, indicating presence of 5-OH group in the A-ring (Feigl, 1960). Thus, on the basis of color reactions, the flavone has free hydroxyls at C-4' and C-5.

Acid hydrolysis of the compound with 2N-HCl at 100^{0}C for an hour, gave an aglycone, kaempferol and glucose and rhamnose.

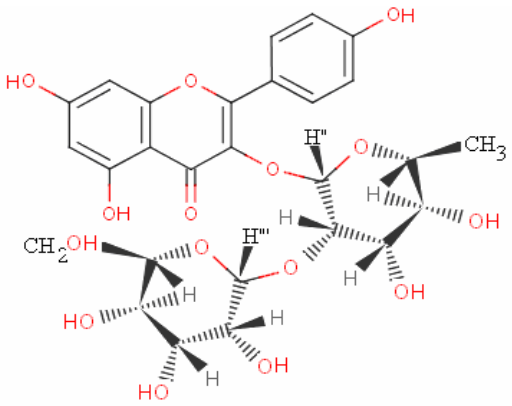
The aglycone was identified by ^{1}HNMR (DMSO -d_6, 400Hz): ^{1}HNMR showed two meta coupled doublets at δ 6.20 (1H, d, J=2.0Hz) and δ 6.33 (1H, d, J=2.0Hz) assignable to H-6 and H-8 of A-ring. Two symmetrical doublets, each with J=9.0Hz, appeared at δ 6.94 and δ 8.02 representing H-3', 5' and H-2', 6' of B-ring. A broad singlet at δ 12.5 indicated presence of free OH at C-5. Thus, the aglycone was identified as kaempferol. The acid hydrolysed sugars, glucose and rhamnose were identified by respecttive CoPC with their standards. UV spectra of compound (B) in MeOH at (λ_{max}, nm) at 268, 366 and shifts obtained with diagnostic reagents indicated the compound is a kaempferol-3, 7-di-O-glycoside.

The compound (B) was hydrolysed with the emulsin derived from almonds, gave a compound, kaempferol-3-O-α-L-rhamnoside (CoPC). The compound (B) gave dark purple fluorescence on PC with UV light while its acid hydrolysed aglycone gave dull yellow fluorescence on PC, indicating release of sugar moiety from C-3. Further the compound (B) was identified by ^{1}HNMR spectra in DMSO-d_6, 400MHz (Table [2]):

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

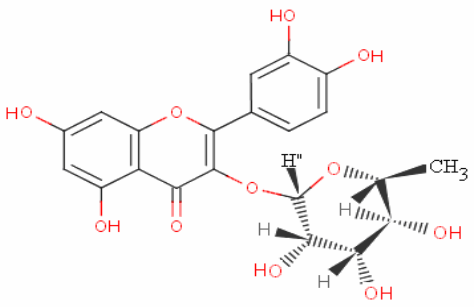
Shift (δ)	Multiplicity	Identification
6.22	1H, d, J=2.0Hz	H-6
6.43	1H, d, J=2.0Hz	H-8
6.86	2H, d, J=9.0Hz	H-3', H-5'
8.05	2H, d, J=9.0Hz	H-2', H-6'
5.56	1H, d, J=7.5Hz	H-1"rham.
4.19	1H, d, J=7.5Hz	H-1"' gluco.
4.10	1H, d, J=2.0Hz	H-2"rham
3.0-3.56	10H, m	For remaining sugar protons of gluco. & rham.
12.50	1H, s	5-OH

The downfield shift of H-2''of rhamnose indicated the terminal glucose and is attached with C-2''of rhamnose. Further, the compound (B) was identified as kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside by CoPC with its authentic isolated from the leaves of *Ginko biloba* (Markham and Geiger, 1992).



(B) Kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside

The slower moving component on PC, a dark purple fluorescent band derived from the fractionation of BuOH soluble on PC (Whatman No. 3) with BAW (4:1:5, V/V, upper layer) solvent gave a single compound (C). It was purified on Sephadex LH-20 column using H_2O-MeOH (60: 40) and identified as quercetin-3-O-α-L-rhamnoside by CoPC with its authentic.



(C) Quercetin-3-O-α-L-rhamnoside

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

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4/20/2009

Pretty Algebra

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Abstract: The sum of the interior angles of a number triangles were transformed into linear algebraic equations.The analysis of these equations without assuming the fifth Euclidean postulate established the following theorem: There exists a triangle whose interior angle sum is equal to two right angles. [Nature and Science. 2009;7(6):86-89]. (ISSN: 1545-0740).

Key Words: Euclid,elements,postulates,triangles,angles, algebraic applications
MSC: 08C99, 51 M04
PACS: 02.40.Dr

1.Introduction

It's hard to add to the fame and glory of Euclid who managed to write an all-time bestseller, a classic book read and scrutinized for the last 23 centuries. However insignificant the following point might be, I'd like to give him additional credit for just stating the Fifth Postulate without trying to prove it. For attempts to prove it were many and all had failed. By the end of the last century, it was also shown that the fifth postulate is *independent* of the remaining axioms, i.e., all the attempts at proving it had been doomed from the outset. Did Euclid sense that the task was impossible?

The earliest source of information on attempts to prove the fifth postulate is the commentary of Proclus on Euclid's *Elements*. Proclus, who taught at the Neoplatonic Academy in Athens in the fifth century, lived more than 700 years after Euclid. Although an invaluable source for the history of mathematics, the *Commentary* is unlikely to be complete. Proclus mentions Ptolemy's (2nd century) attempts to prove the postulate and demonstrates that Ptolemy had unwittingly assumed what in later years became known as Playfair's axiom. Proclus left a proof of his own, but the latter rests on the assumption that parallel lines are always a bounded distance apart, and this assumption can be shown to be equivalent to the fifth postulate.

al-Gauhary (9th century) deduced the fifth postulate from the proposition that through any point interior to an angle it is possible to draw a line that intersects both sides of the angle. He deduced the proposition from an implicit assumption that if the alternating angles determined by a line cutting two other lines are equal, then the same will be true for all lines cutting the given two. The proposition was implicitly used by A.M.Legendre (1800) in his proof of the fifth postulate.

al-Haytham's (10th century) kinematic method was criticized by Omar Khayyam (11th century) whose own proof was published for the first time in 1936. To Omar's credit he thought up a figure that was later named after Gerolamo Saccheri (1667-1733). Nasir ad-Din at-Tusi (13th century) was more fortunate. A Latin edition of his work appeared in Europe in 1657. at-Tusi critically analyzed the works of al-Gauhary, al-Haytham and Omar Khayyam. In one of his own attempts, at-Tusi tried to prove the postulate by a *reductio ad absurdum*. This appears to be the first attempt to prove the postulate by deriving a contradiction from the assumption that the fifth postulate is wrong.

John Wallis has been inspired by the work of at-Tusi and delivered a lecture at Oxford on July 11, 1663. To prove the postulate he made an explicit assumption that for every figure there is a similar one of arbitrary size. Unlike many (even later) mathematicians, John Wallis realized that his proof was based on an assumption (more natural in his view but still) equivalent to the postulate.

The line of reasoning of at-Tusi had been taken up by a professor of rhetoric, theology and philosophy at a Jesuit college in Milan, Girolamo Saccheri. In 1733, Saccheri published a two-volume work titled *Euclid Freed of Every Flaw*. Given a line and a point not on the line, there are exactly three possibility with regard of the number of lines through the point:

A. there is exactly one parallel;
B. there are no parallels;
C. there are more than one parallel.

The three hypotheses are known as hypotheses of the *right*, *obtuse*, and, respectively, *acute* angles. The first one is Playfair's axiom and, thus, is equivalent to Euclid's fifth postulate. Assuming that Euclid's second postulate (*A piece of straight line may be extended indefinitely*.) requires straight lines to be infinitely long, he showed that (B) indeed leads to a contradiction. Based on (C), he proved several counterintuitive statements but couldn't formally obtain a logical contradiction. Probably to justify the title of the work he stated

The hypothesis of acute angle is absolutely false; because repugnant to the nature of the straight line.

Saccheri's work attracted little attention and was virtually unknown until 1899 when it was republished by his compatriot, Eugenio Beltrami (1835-1900). In 1766, Heinrich Lambert (1728-1777) published a similar investigation. He also observed that results derived under the hypothesis (B) resemble those known for spherical geometry and suggested that, geometry following from (C) might be visualized on a sphere of imaginary radius.

Adrien-Marie Legendre (1752-1833) was preoccupied with the fifth postulate for decades. His work appeared in successive additions of his very popular *Éléments de Géométry* (1794-1823). The small book was translated into English first in 1819 and, then, by Thomas Carlyle, in 1822. Carlyle's translation ran through 33 American editions (from H.Eves, MAA, 1983). Legendre succeeded in popularizing geometry and the question of the fifth postulate but, of course, failed to prove it. His last article on parallels saw light in 1833, the year of Legendre's death, four years after publication by the Russian mathematician N.Lobachevsky of his paper on non-Euclidean geometry and a year after a similar publication by the Hungarian János Bolyai**.**

2.Construction

In the Euclidean construction as shown in figure 1,x,y,z and m denote the sum of the interior angles of triangles AOB,AOC,DOC and DOBE respectively.Also let a,b,c and d respectively refer to the sum of the interior angles in triangles ABC,ADC,EBC and AED.

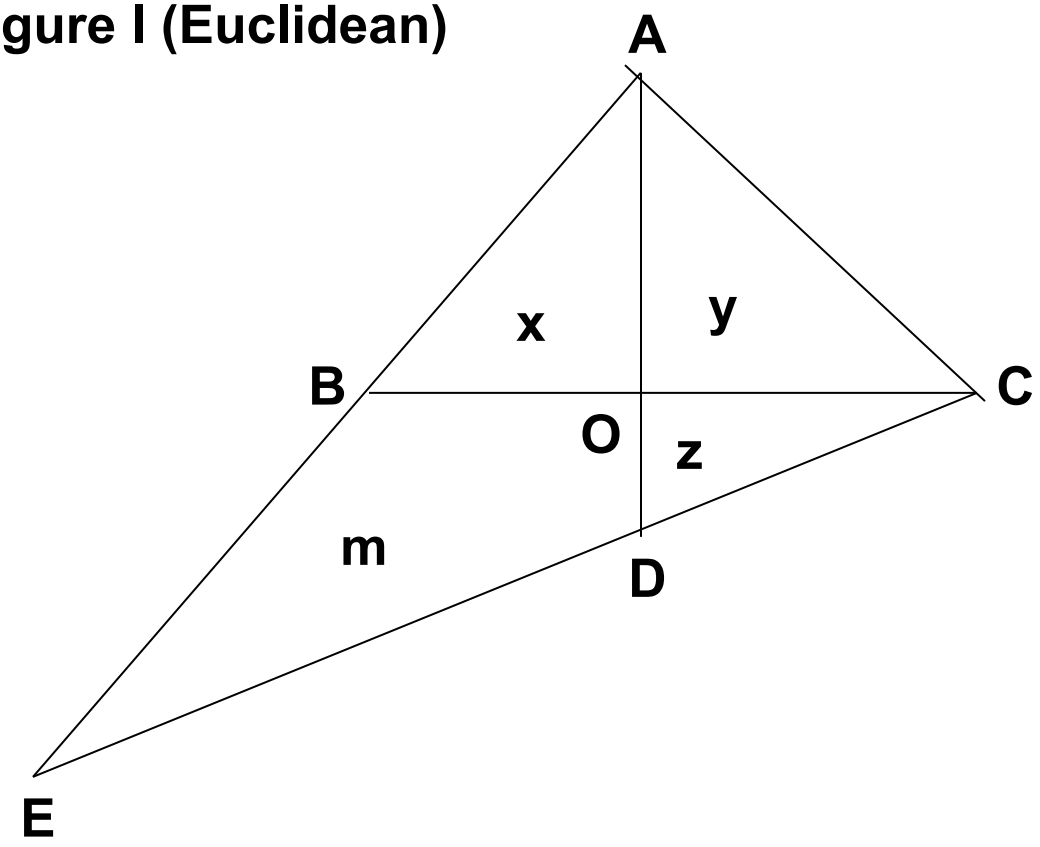
Sides OB and OC are equal (1)
Side OA is greater than side OD (2)
The angles AOD and BOC are straight angles and so their measures are equal to 180 degrees.Let v be the value of this 180 degree (3)

3.Results

Using (3), $x + y = v + a$ (4)
$y + z = v + b$ (5)
$z + m = 2v + c$ (6)
$m + x = 2v + d$ (7)
(4) - (7) gives,$m + a = y + v + d$ (8)
(5) - (6) gives,$m + b = y + v + c$ (9)
Squaring (8), $m^2+a^2+2ma = y^2+v^2+d^2+2yv+2yd+2vd$ (8a)
Squaring(9), $m^2+b^2+2mb = y^2+v^2+c^2+2yv+2yc+2vc$ (9a)

(4) + (6) = (5) + (7) = a+c = b+d (10)
Squaring (7), $a^2+c^2+2ca = b^2+d^2+2bd$ (10a)
(8a) - (9a) given, $a^2-b^2+2ma - 2mb = d^2-c^2 + 2v(d-c)+2y(d-c)$
$a^2-b^2+2ma - 2mb = d^2-c^2 + 2(d-c)[y + v]$
Putting(9) in RHS, $a^2-b^2+2ma - 2mb = d^2-c^2 + 2(d-c)[m + b - c]$
i.e $a^2 - d^2$ + m [2a - 2b - 2d + 2c] - b [b + 2c - 2d] + c [2d +c -2c]
Applying (10) in the second factor of LHS, (a + d) (a - d)- b [b + 2c - 2d]+ c [2d - c]
From (10), a - d = b - c and b - a = c - d
.Putting these in the above equation, b [2a - 3b + a + d]+ c [2d - c -a - d]
Applying (10) in the second factor, b [2a - 3b + a + d] - bc = 0
i.e b [3a - 3b +d - c] = 0
From (10) we get that d - c = a - b.Applying this b[4a-4b] =0
i.e a = b (11)
Analysing (4) , (5) and (11) we have z = x (12)
By construction Sides OB and OC are equal (1)
and side OD is greater than side OA (2)
Now look at figure 2.On OA, cut off F such that OD = OF.
By SAS correspondence, triangles FOB and COD are congruent.
But from (12) the sum of the interior angles of triangles AOB and COD are equal.
From this we obtain that the sum of the interior angles of triangles ABO and ABF
are equal.Consequently, we get that the sum of the interior angles of triangle ABF
is equal to two right angles (13)

Figure I (Euclidean)



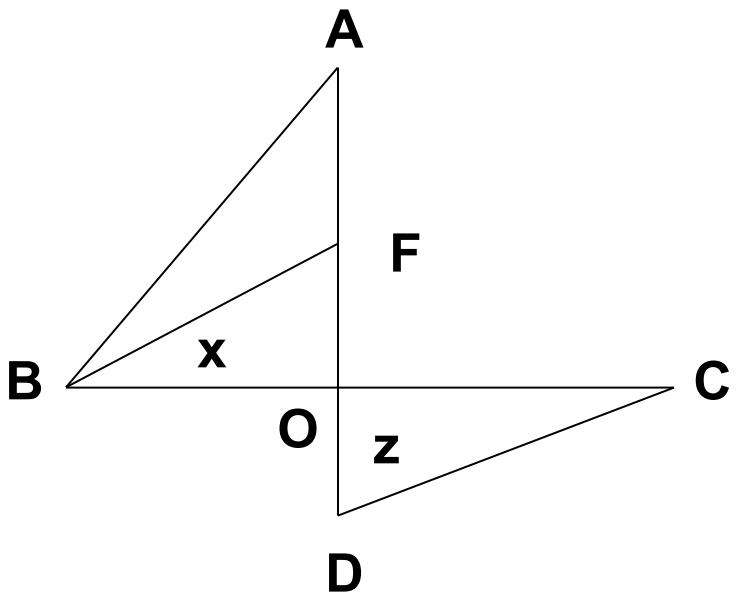


Figure II (Euclidean)

4.Discussion

Since we have derived (13) without assuming Euclid's fifth postulate which is an unsolved classical problem for more than 2300 years, beyond each and every mathematical doubt, (13) establishes the parallel postulate. [1-4] .Figure 1 can be extended to both hyperbolic and elliptic spaces.So, (13) will hold even in non- Euclidean spaces.But the mere exixtence of consistent models of non-Euclidean geometries demonstrate that Euclid V canNOT be deduced from Euclid I to IV.But our result canNOT be challenged.Questioning (13) will force us to doubt the fundamental operations of number theory and algebra.

The famous unsolved classical problems such as squaring the circle, duplicating the cube,trisection of a general angle and to draw a regular septagon are not merely difficult but IMpossible to solve.In this study, application of classical algebra explored a masterpiece result in geometry.There is something hidden treasure of mathematics.Further probes will unlock this mystery.

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04/20/2009

Comparative tracheary elements characteristics of *Canarium schweinfurthii* Engl. and *Dacroydes edulis* (G. Don) H.J. Lam growing in derived savanna and rainforest regions of Edo state, Nigeria

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ABSTRACT: Histomorphological characteristics of two woody plants belonging to the family Burseraceae Kunth growing naturally and abundantly either in the rainforest and derived savanna habitats of Edo state, Nigeria is reported. *Canarium schweinfurthii* Engl grows only in the rainforest habitats. *Dacryodes edulis* (G. Don) H.J. Lam occurs in both rainforest and derived savanna habitats. Both woody plants are well known for their sweet and edible fruits. The two taxa exhibit medium length (350-800μ_m) vessels with medium sized diameters (100-200μ_m). *Canarium schweinfurthii*, growing only in the rainforest areas has large sized (>200μ_m) vessel diameter. Vessel members of the two plants are thick-walls with simple pits arranged in rows. Fibres in the two plant species are of medium lengths (900-1600μ_m), moderately thick-walled (3-5μ_m) non pitted, non septate except in *Canarium schweinfurthii*, where septate were encountered fibre / vessel length ratio in both taxa is greater than 1 indicating that they are hypogenetically advanced and specialized. Runkel ratio of taxa is less than 1. Comparing *Dacryodes* species growing in rainforest with those in the derived savanna habitats, there is plasticity in the tracheary element dimensions (fibres and vesels) but no significant variations were encountered in their tracheary element dimensions. Higher mean maximum and minimum values in vessels (lengths and diameters) and fibre lengths were encountered in the rainforest plants, except in fibre lengths of *D. edulis* occurring in the derived savanna habitat where higher mean minimum values were obtained. [Nature and Science. 2009;7(6):90-95]. (ISSN: 1545-0740).

Keywords: Tracheary elements, *Canarium schweienfurthii*, *Dacryoyes edulis*, rainforest, derived savanna, Edo state

INTRODUCTION

Willis and Airy-Shaw (1973) reported 500 burseraceous species in the tropics of the 900 woody taxa reported in Nigeria by Keay *et a*l, (1964) and Gill (1992). Only 50 timber species are being commercially exploited. The possible reason for this low value is that no enough is known about the characteristics, qualities and the uses to which the other could be put (Gill 1992)

Relevant contributions to the tracheary element characteristics of hardwood species include Akachuku (1987), Baas *et al* (1983), Gill and Okoegwale (1990), Gill and Onuja (1982, 1984), Gill *et al*, (1985) Okoegwale and Idialu (1998).

Gill *et al* (1985), reported medium length (mean 270.64μ_m) vessels with medium sized (138.72μ_m) diameters, slightly broad tails, simple perforations transversely situated at the end walls and simple slit-like round pits arranged in rows along vessel length in *Dacryodes edulis*. They also reported long (mean 1665.35μ_m) moderately thick-walled fibres with no pits and septations in *Dacryodes* species

According to Akachuku (1987), density is largely determined by diameter and wall thickness of cells and the proportion of thick-walled tissues (vesses and fibres) and is the best singular indication of wood quality and its suitability for various purposes.

For plant species growing in both rainforest and derived savanna environment's Okoegwale and Idialu (1998), reported higher maximum and minimum values for vessel and fibre lengths of woody leguminous plants in the rainforest than in the derived savanna counterparts. These are important parameters for the determination of strength qualities and end-use of wood. It was also reported that there were significant variations in fibre wall thickness in the same plant growing in the two ecological zones which they claimed to be of relevance in comparing wood density.

The purpose of this study was to ascertain the nature of plasticity of tracheary elements (vessels and fibres) of *Canarium schweinfurthii* Engl and *Dacryodes edulis* (G. Don) H. J. Lam growing naturally and abundantly in either the rainforest or both rainforest and derived savanna habitats of Edo state, Nigeria known commonly for the their sweet and edible fruits.

It is also to assess the qualities, potentialities and phylogenetic trend of their wood and the effects of ecological variations on the tracheary elements (vessles and fibres) on *Dacryodes edulis* growing naturally in both rainforest and derived savanna habitats.

Derived savanna is a forest outlier or ecotone bordering guinea savanna, part of original forest which transformed to this type of vegetation as a result of biotic or edaphic factors resulting from population pressure. It is found at the edge of the forest.

MATERIALS AND METHODS

Wood samples of *Canarium schweinfurthii* growing naturally only in the rainforest habitat and *Dacryodes edulis* growing in both rainforest and derived savanna habitats of Edo state, Nigeria and whose ages were not ascertained were obtained. The ecological zones are located between latitude 6^0 and 7^0 5^1 and longitude 5^0 and 7^0E wood samples were collected from plants whose girths ranged from 8.0-15.0 centimeters at 1.3 meters above ground level i.e 1.3 metres diameter at breast height (d.b.h). Wood samples were air –dried for 10 days before they were made into chips. Maceration of chips was carried out using the procedure of Gill *et al* (1983) and Okoegwale and Gill (1990). Wood chips obtained, were placed in a test tube containing 10-15 m of 60% nitric acid and left overnight. It was then boiled for 5-10 minutes. The macerated materials were washed several times with distilled water. Macerated materials were not centrifuged as described by Gill *et al* (1983) and Okoegwale and Hill (1990). A diluted (1%) drop of 1.1 glycerol-safranin solution was added before placing the coverslip. Linear measurements (length and diameter, lumen diameter, wall thickness) of vessels and fibres were made on calibrated microscope. Average values were based on 100 measurements. A t-test distribution was used to analyse results

RESULTS

Table 1. Morphological characteristics of vessel and fibres of *Dacroydes edulis* (G. Don) H.J. Lam growing in derived savannah and rainforest regions of Edo state.

	SAVANNA	**RAINFOREST**
Plant tissue type	**Morphological characteristics**	**Morphological characteristics**
VESSELS		
Length	Medium, ranges from196.7-408.54μ_m, mean 368.10μ_m	Medium, ranges from 205.4-455.6μ_m. mean 380.66μ_m.
Diameter	Large-sized, ranging from 98.72-192.3μ_m ,mean 140.16μ_m	Medium-sized, ranges from 106.4-204.6μ_m. mean 166.0μ_m.
Wall thickness	Thick and ranges from 4.60-13.80μ_m mean 8.28μ_m.	Thick; ranges from 4.40-12.70, mean 6.35μ_m.
Tail length	Length, ranges from 9.6-24.9μ_m, mean 16.1μ_m	Ranges from 9.4-34.1μ_m. mean 18.6μ_m.
Perforation plate	Simple located in transverse and walls	Simple: transversely located at the end walls
Pit	Simple and arranged in rows	Simple: arranged in rows
Statistical analysis: Vessel dimensions (length, diameter, wall thickness) do not vary significantly between plants growing in the derived savanna habitats and rainforest		
FIBRE		
Length	ranges from 6.10-12.82μ_m, mean 9.02μ_m	Medium; ranges from 980.91-1260.48μ_m. mean 1152.38μ_m.
Diameter	Large-sized, ranging from 11.09-18.46μ_m ,mean 14.82μ_m	Ranges from 12.56-20.80μ_m. mean 16.0μ_m.
Lumen diameter	ranges from 6.10-12.82μ_m, mean 9.02μ_m	Ranges from 6.48-1.51μ_m. mean 9.45μ_m.
Wall thickness	Moderate; ranges from 2.13-4.67μ_m mean 3.71μ_m.8.15μ_m.	Moderate: ranges from 2.56-5.10μ_m. mean 4.65μ_m.
Pit	Absent	Absent
Septae	Absent	Absent
Fibre vessel: Length ratio	2.74	3.02
Runkle ratio	0.82	0.98
Statistical analysis: No significant variations between the plants growing in the derived savanna and rainforest		

Table 2: Morphological characteristics of vessels and fibres of *Canarium schweinfurthii* Engl growing in the rainforest region of Edo state.

DERIVED SAVANNA	
Plant Tissue Type	**Morphological Characteristics**
VESSELS	
Length	Medium, ranges from 286.0-570.4μ_m mean 400.33μ_m
Diameter	Large-sized, ranging from 256.1-311.6μ_m mean 282.40μ_m.
Wall thickness	Thick, ranging from 4.0-11.10μ_m mean 6.0μ_m.
Perforation plate	Simple, located in transverse and walls.
Pits	Simple and arranged in rows
FIBRE	
Length	Medium, ranges from 952.30-1271.18μ_m. mean 1003.23μ_m.
Diameter	ranging from 12.96-36.34μ_m. mean 26.86μ_m.
Lumen diameter	Ranging from 14.36-27.14μ_m. mean 20.47μ_m.
Wall thickness	Moderate, ranging from 3.58-8.15μ_m. mean 3.29μ_m.
Pits	Absent
Septae	Present
Fibre vessel: length ratio	2.51
Runkel ratio	0.32

Table 1. Fibre/vessel/ length ratio of C. *Schweinfurthii* and *Dacryodes edulis* growing in rainforest and derived savanna

| Plant species | | |

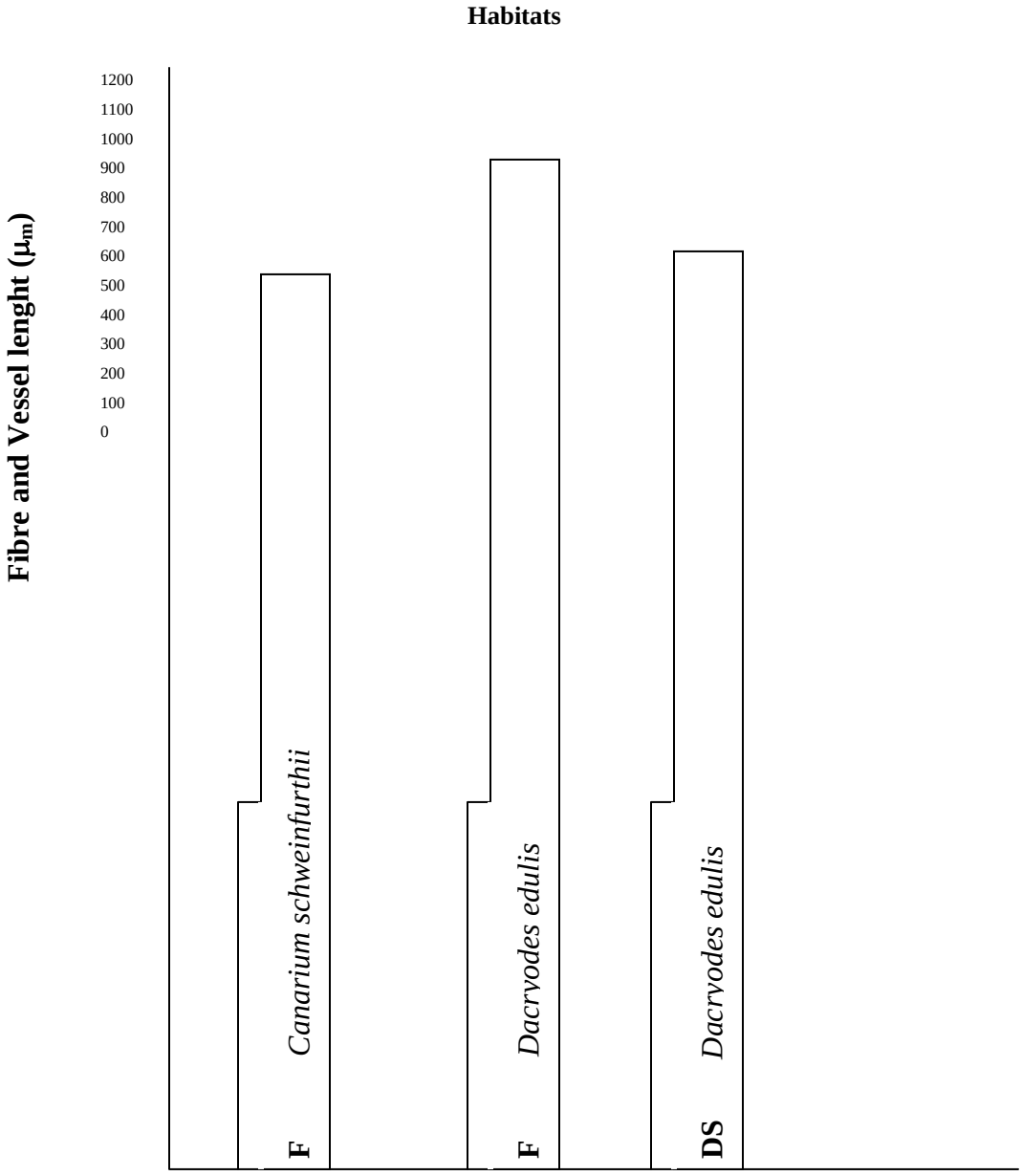

Fig 1. Fibre/vessel length ratio of *C. schweinfurthii* and *Dacryodes edulis* growing in rainforest and derived savanna

DISCUSSION

Earlier, Gill and Onuja (1984) reported medium length vessels with large diameters in *Canarium schweinfurthii* while Gill *et al* (1985) reported vessel of medium lengths with medium-size diameters in *Dacryodes edulis*

In the present investigation, vessel members of *Canarium schweinfurthii* growing only in the rainforest zone are of medium length, mean 400.33$\pm$ 187.0μ_m with large diameter of mean 282$\pm$90.5μ_m. *Dacryodes edulis* growing in the rainforest zone possess vessles of medium length, mean 380.66$\pm$ 161.58μ_m. but medium size diameters of mean 166.0$\pm$49.17μ_m. occurrence of these dimensions is in line with Gill and Onuja (1984) and Gill *et al*. (1985), who reported the same in *C. schweinfurtii* and *D. edulis* respectively.

In *D. edulis* growing in the derived savanna habitat, vessel members are of medium length, mean 368.10+ 128.9μ_m. with medium-sized diameter of mean 140.16$\pm$50.41μ_m. This is in agreement with studies carried out by Gill *et al.* (1985) on *D. edulis.*

Taxa vessels are thick-walled in both vegetation zones, with a mean vessel wall thickness of 6.0$\pm$2.0μ_m. reported in *C. schweinfurthii* growing in the rainforest zone while *D. edulis*, vessel mean wall thickness of 6.35$\pm$2.0μ_m. and 6.35$\pm$ 2.6μ_m. were recorded in rainforest and derived savanna habitats respectively.

Tailed-vessels were encountered in both vegetation zones *C. schweinfurthii* had a mean tail length of 116.0$\pm$58.22μ_m. in the rainforest habitat while *D. edulis* growing in both habitats had mean tail lengths of 18.60$\pm$7.54μ_m. and 16.10$\pm$7.1μ_m. occurring respectively in the rainforest and derived savanna areas.

The presence of tails in the plant species is in agreement with Gill and Onuja (1984) and Gill *et al.* (1985) who reported same in *C. schweinfurthii* and *D. edulis*.

Vessel members in the plant species are of simple perforation types in the two ecological zones and are transversely situated at the end walls. With simple pits occurring in them. This also agrees with Gill and Onuja (1984) and Gill et al. (1985).

However, Gill and Onuja (1984) reported simple slit-like pits arranged in rows along vessel lengths of *C. schwenfurthii* occurrence of septate fibres in *Canarium schweinfurthii* apart from been taxonomic, may be a new record for the taxon.

Fibre/vessel length ratio in the two plant species is greater than 1 and ratio approaching 10 is phylogenetically advanced and specialized and suitable for different uses.

Runkel ratio in all the taxa is below 1 indicating thus that the plants investigated are not suitable for high grade pulp as this is an important parameter in pulp industry.

Plasticity is a stronghold phenomemon in tracheary elements (vessels and fibres) of *Dacryodes edulis* growing in both rainforest and derived savanna areas, as no significant variations were encountered in these element dimensions.

However, higher mean maximum values in vessel and fibre lengths are reported in taxa growing in the rain forest than in the derived savanna habitats.

From the foregoing, woods of investigated taxa can be considered to be suitable for various uses but are however, not suitable for high grade pulp because of their relative low fibre lengths and runkel ratio.

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ISSN 1545-0740