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Performance of *Glomus* sp. and maize grown under different levels of fertilizer treatment

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ABSTRACT
The effect of different N , P and K fertilizer on the maize grown under glass house in inoculated and uninoculated condition was analyzed for growth and colonization pattern of arbuscular mycorrhizal fungi (Glomus sp.) . At the final stage of growth mycorrhizal inoculation showed better growth as compared to fertilizer treatment. Enhancement in root growth could be observed in AM inoculation and phosphorus and Nitrogen supplementation. The fertilizer application in combination of phosphorus and potassium exhibited better dry biomass yield in inoculated plants. The fertilizer treatment reduced the AM colonization in maize roots. Similarly data on the number of vesicles in roots and spore number in soil showed poor performance with fertilizer treatment. Data analysed statistically and presented in this paper.

Key words : Mycorrhiza, Maize, Fertilizer, Glomus, Tea

INTRODUCTION

Among the biological processes involved in the rhizoplane, the unique role of symbiotic bacteria and the mycorrhizal fungi which ensure fixation and mobilization, and availability of nitrogen and phosphorus of plants have been well recognized (Freitas *et.al*., 2004; Singh *et al*., 2004). AM fungi are cosmopolitan in distribution and found in association with plants in agricultural fields, aquatic environments, forests, along sea coasts, sand dunes and captive plantations (Trappe and Fogel , 1977 Cornquist , 1981; Trappe , 1989 ; Rahangdale and Gupta , 1999, 2001; Ranjan *et al*., 2000 ; Mosse , 1973, Hall, 1984, Miller *et al*., 1986, Kabir and Koide, 2000, Vosatka *et al.*,1999; Jentscheke *et al.*, 1999; Beena *et al*., 2000) . Arbuscular mycorrhizal fungi are of immense value for being a transporter and carrier of various minerals like P , Zn, Mn, Mg , Cu, and Al etc. from soil to the host plants to enhance growth and productivity in plants (Maiti and Variar , 2000; Ammani *et al.* 1985; Secilia and Bagyaraj , 1994).

A plethora of research findings on many agricultural, horticultural and ornamental crop plants recommended mycorrhizal inoculation for improved growth and yield as well as for effective utilization of the soil phosphates (Gautum and Mahmood , 2002; Rani and Bhaduria, 2001; Mulani *et al.*,2002 ; Nelson and Achar, 2001).. The latter aspects of the AM inoculation will effect saving in amounts of the usable phosphate fertilizer. Therefore, mass inoculation of crops with AM fungi requires a suitable technology (Bagyaraj ,1989; Sreenivas and Bagyaraj ,1988) Present work has been done on arbuscular mycorrhizal fungi occurring in the tea gardens. The present study attains more importance due to the study sites that Bhuyanpirh tea plantations of Orissa, India that never surveyed before has been taken into consideration.

MATERIALS AND METHODS

Source of AM fungi

The AM fungi used in this study was isolated from from the tea (*Camelia sinensis* L.) plantations of the Bhuyanpirh tea estate of M/S Orissa Tea Plantation Limited situated in Tarmakanta about 48 Km away from Keonjhar city of Orissa, India. The pure culture of AM fungi that was identified as *Glomus* sp., was maintained and multiplied in pot culture with maize as host plants.

Experimental set up

Maize Seedlings were raised in the earthen pots having 5-Kg capacity containing soil sand mixture (1:1) as growing media irrigated with fresh water and cultivated under glass house conditions at 32 ±2 ºC and 80 ± 5 % relative humidity. Total of 40 uniform and 15 days old seedlings of maize were distributed in to four treatments groups i. e. 10 seedlings of each group were sub irrigated according to following experimental protocol.

- Experimental group I: only soil (Black cotton soil +sand + compost , 2:1:1) + maize plants + daily watering
- Experimental group II: soil inoculated with AM fungi + maize plants +daily watering
- Experimental group III. soil inoculated with AM fungi + 15 day-old- maize seedlings treated with following sub treatments for 60 days of growth period. Six subgroups were taken into consideration under this experimental set. In three subgroups, urea (for N_2), muriate of potash (for Potassium), single super phosphate (for phosphorus) were given individually in separate pots at the rate of 5g, 3g and 1g respectively. In the fourth and fifth subgroups, phosphorus was added along with the urea or potash at the same rate. Uninoculated control was set along with above-mentioned treatments of fertilizer.

In this experiment the maize plants were uprooted and measured for the growth parameters at an interval of 30 days up to 75 days of growth period. Final observations were taken on fresh and dry biomass, plant height (shoot and root length), leaf number, and mycorrhization in terms of % colonization, spore count and vesicle number in the treated and the untreated control plants .

Analysis of growth parameters and AM colonization

Simple biological norms were taken into consideration for the determination of growth parameters including shoot height, root length, leaf no., leaf area, wet and dry biomass of shoot, root, and leaf, shoot: root ratio. AM infection and colonization in the roots of maize grown under different treatments were analyzed following the root clearing & staining technique, and slide method (Phillips and Hayman, 1970; Kormanic and McGraw, 1982). Total number of vesicles / cm root was also calculated during this experiment. Rhizosphere soil of maize grown under different treatments was treated for the AM spore isolation and total number of spore present in 100/g of soil were counted simply with the help of stereo zoom microscope (Gerdemann and Nicolson,1963).

The response of host plants towards its mycorrhization by the indigenous fungi was evaluated by measuring the growth parameter such as fresh and dry biomass , plant height, leaf number and mycorrhization in terms of % colonization, spore count and vesicle number. Data obtained at the final stage of 75 days of growth have presented in this paper .

RESULTS AND DISCUSSION

The maximum length of stem (71.87cm) was noted in the plants inoculated without fertilizer treatment after 60 days of sowing (Table 1). In all the inoculated plants, shoot length, root length, fresh and dry biomass and leaf number increased as compared to the uninoculated plants (C1). Fertilizer supplementation alone and /or in

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Table 1 : Effect of AM fungi (isolated from tea plantation) on growth (measured after 75 days of experiment) of maize(host plant) grown under different treatments (shoot length, root length, leaf number)

	SL	LN	RL	SFW	RFW	LFW	SDW	RDW	LDW
C1	137.78 ± 7.80	14.66 ± 0.33	22.62 ± 0.86	30.35 ±1.38	5.05 ±0.69	14.1 ±0.79	3.01 ±0.22	4.38 ±0.69	1.27 ±0.16
C2	95.07 ± 7.27	11.83 ± 0.65	20.41 ± 0.95	26.25 ±1.49	2.03 ±0.28	11.94 ±0.84	2.42 ±0.21	1.76 ±0.28	1.55 ±0.20
R1	60.00 ± 3.69	11.00 ± 1.00	16.10 ± 0.44	12.13 ±1.01	2.36 ±0.23	10.37 ±0.30	1.09 ±0.17	2.04 ±0.23	1.06 ±0.18
R2	51.20 ± 0.00	11.00 ± 0.00	10.90 ± 0.00	11.80 ±0.00	1.34 ±0.00	7.76 ±0.00	1.10 ±0.00	1.16 ±0.00	0.62 ±0.00
R3	73.53 ± 1.45	13.00 ± 0.00	16.83 ± 0.81	9.87 ±0.74	2.41 ±0.31	7.95 ±0.89	0.25 ±0.19	2.09 ±0.31	0.54 ±0.12
R4	52.66 ± 4.58	10.66 ± 0.67	14.06 ± 2.94	31.39 ±2.07	1.69 ±0.18	9.14 ±0.48	2.14 ±0.13	1.46 ±0.18	0.98 ±0.15
R5	56.86 ± 9.24	13.00 ± 0.00	14.23 ± 3.29	25.42 ±3.60	1.98 ±0.43	20.25 ±3.19	1.98 ±0.49	1.71 ±0.43	3.62 ±1.32
R6	76.20 ± 5.00	11.33 ± 0.67	12.35 ± 0.25	16.71 ±1.61	0.87 ±0.07	7.06 ±0.66	1.07 ±0.03	0.75 ±0.07	0.45 ±0.06
R7	47.86 ± 0.94	13.00 ± 1.00	18.33 ± 0.64	20.88 ±1.25	1.88 ±0.54	11.82 ±0.46	1.73 ±0.38	1.63 ±0.54	1.19 ±0.16
R8	59.56 ± 5.20	12.66 ± 0.33	16.23 ± 2.17	24.51 ±2.18	1.31 ±0.60	13.53 ±2.23	2.38 ±0.16	1.13 ±0.60	1.84 ±0.94
R9	95.86 ± 2.06	12.33 ± 0.33	20.26 ± 0.94	22.72 ±5.30	2.78 ±0.30	13.7 ±1.01	2.14 ±0.54	2.41 ±0.30	1.42 ±0.20
R10	68.13 ± 8.01	12.66 ± 0.33	16.46 ± 1.27	25.25 ±2.18	1.53 ±0.34	13.1 ±1.48	2.22 ±0.12	1.32 ±0.34	1.30 ±0.40

C1= uninoculated untreated
C2= inoculated untreated
R1= P +AM(Single super phosphate)
R2= N+AM (Urea)
R3= K +AM (Muriate of Postash)
R4= N+P+AM
R5= K+P+AM
R6= P(AM -) without AM
R7= N(AM -) without AM

SL =shoot length (cm)
LN = number of leaf
RL =root length (cm)
SFW shoot fresh weight (g)
RFW = root fresh weight (g)
LFW = leaf fresh weight (g)
SDW = shoot dry weight (g)
RDW = root dry weight (g)
LDW = leaf dry weight (g)

combination did not show any significant differences . However, both fresh and dry weight of leaf increased in treatments that received the potassium and phosphorus along with mycorrhizal inoculation(R1 and R3).

At the final stage of growth, the mycorrhizal inoculation showed better growth and development as compared to only fertilizer treatment .Under phosphorus supplementation, shoot length of the plants was enhanced in the uninoculated control (R8). Where as AM inoculation promoted better root growth, root weight and leaf weight (R1) . In contrast, Nitrogen induced root growth and also contributed to biomass production (R2) . Fertilization with phosphorus gave higher biomass yield in the AM inoculated plants (R1) where as nitrogen and Potassium induced better plant growth with consequent increase in the biomass yield in uninoculated plants (R7 and R8). With Phosphorus and Nitrogen combination (R9) , higher biomass yield was observed in the uninoculated plants. K was also responsible for enhancement in dry

Table 2 : Status of mycorrhization in maize roots inoculated under different treatments

Code	% colonization	no. vesicles	no. spores/100g soil
C1	0	0.00	0.00
C2	84.00 ±7.21	214.67 ±15.30	173.33 ±11.66
R1	10.00 ±10.00	3.67 ±3.67	50..00 ±0.00
R2	23.33 ±14.53	3.33 ±2.03	82.50 ±12.50
R3	20.00 ±10.00	8.00 ±2.08	90..00 ±27.83
R4	16.67 ±12.02	4.67 ±3.71	62.50 ±17.50
R5	16.67 ±6.67	6.67 ±2.33	56.66 ±10.13
R6	0.00	0.00	0.00
R7	0.00	0.00	0.00
R8	0.00	0.00	0.00
R9	0.00	0.00	0.00
R10	0.00	0.00	0.00

biomass yield in the inoculated plants in combination with phosphorus (R5).

The maximum % of colonization occurred when plants were inoculated with AM fungi as compared to other treatments, which showed poor colonization in their roots (Table 2). The highest % colonization was observed in the inoculated plants not treated with fertilizer (C2) followed by nitrogen, potassium and phosphorus treated plants. Phosphorus along with nitrogen and potassium applied together showed similar response. Similarly, data on the number of vesicles and spore per 100 g soil showed poor performance with fertilizer treatment. The maximum number of spores (173.33 /100 g soil) and vesicle number (214.67/root) were obtained in the inoculated but

Table 3 : ANOVA for shoot length,leaf number and leaf area at different stages of growth of maize under different treatments

shoot length	Treatment (between time)	17420	3	5806	30.27***
	Residual (within treatment)	84	4	144	
	Total	25860	47		
leaf number	Treatment (between time)	266.9	3	88.95	121.7***
	Residual (within treatment)	32.16	44	0.731	
	Total	299	47		
leaf area	Treatment (between time)	45000	2	2500	0.2767**
	Residual (within treatment)	117600	29	4057	
	Total	162600	31		

* = Significant at 5 % level, ** = Significant at 1 % level, *** = Significant at 0.01% level,
NS = non significant

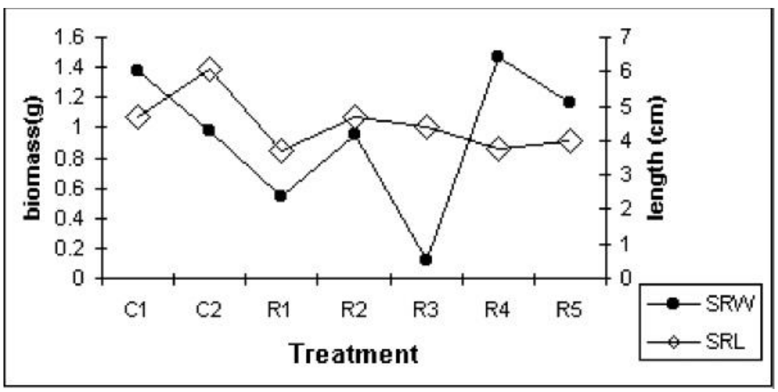

Fig. 1 Shoot and root ratio of maize grown with fertiliser treatments (with AM)

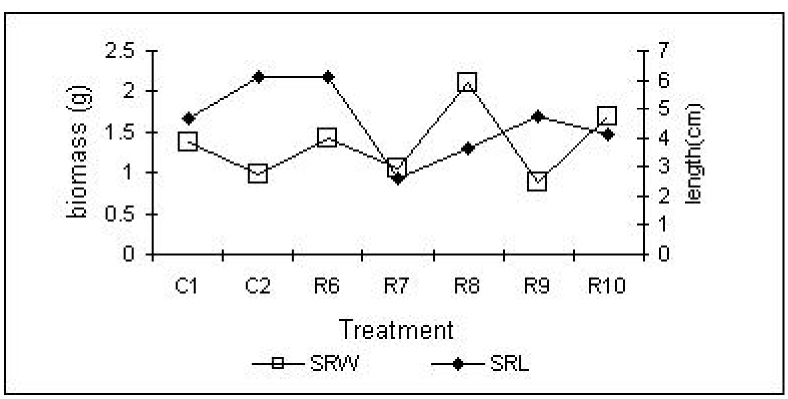

Fig. 2 : Shoot root ratio of maize grown with fertiliser treatment (With out AM)

Abbreiviations for fig.1 and fig. 2

C1=uninoculated garden soil, C2=inoculated garden soil ,

R1=P+AM,R2=N+AM, R3 = K+AM, R4=N+P+AM,R5=K+P+AM

R6=P,R7=N, R8 = K , R9=N+P, R10=K+P,

untreated plants (C2). In the present study all the three variable i.e. root length, dry biomass and % colonization were found to be interrelated (Table-3).

Shoot root ratio with regards to length was higher in C2 followed by Phosphorus application in alone and combination with N (R6 and R4) under mycorrhizal conditions (Fig.1). Biomass with regards to yield, shoot root ratio was found to be higher in R8 followed by R10 i. e. plants treated with K alone and in combination with Phosphorus under nonmycorrhizal conditions(Fig.2).The pH of different compositions ranged from 6.13 to 6.53 . The pH of soil treated with fertilizer N P K both alone in uninoculated condition ranged 6.23 to 6.52 and in inoculated condition it was ranged in6.13 to 6.37. The pH of soil of this experiment did not vary much.

The biomass yield in different parts of maize plants showed the highest % of root biomass (72%) in plants supplemented with potassium (R8) (Fig. 3) . The maximum shoot biomass (47%) was obtained in plants treated with nitrogen and phosphorus both under inoculated conditions (R4). The highest leaf biomass was recorded at 50% in plants treated with phosphorus (R1) and potassium (R3) under mycorrhization. In uninoculated and fertilizer treated plants , the highest shoot biomass (47%) was recorded in phosphorus treated (R6) plants where as potassium (R8) treatment gave the highest leaf biomass under uninoculated conditions.

A critical analysis of variance for shoot length, at different stages of growth of maize exhibited the significant variance at 0.1% level (Table –3). Analysis of variance for leaf number and leaf area of different stages of maize growth under this treatment revealed that the variation in the periodical effect of fertilizer component was significant at 1% level only. Data recorded for root length and root dry biomass and % colonization was subjected to statistical analysis (Table 4). It was observed that root length and root dry biomass was not correlated with % colonization in maize plants of different soil compositions.. However, data analyzed for correlation between colonization and number of spores and /or vesicles seemed to be correlated. Similarly, vesicle number and spores were found to be significantly correlated.

In the present study only inoculated plants showed better growth performance as compared to fertilizer treatments. Fertilizer not only affected plant growth but also the performance of AM fungi. This findings also support the reports that high phosphorus concentration in soils inhibited mycorrhizal infection (Menge *et al.,* 1978; Jasper *et al.,* 1989) . This might be due to soil conditions or absorption capacity of plants and /or AM fungi.

The adverse effect of nitrogen and potassium on the mycorrhizal colonization of maize was observed during this study which confirmed the reports of Hayman (1975) who reported that N fertilizer had a large negative effect on the mycorrhizal population. Alexander and Firnley (1983) reported reduction in mycorrhizal colonization following an application of 300 kg N per ha. Menge *et al.* , (1978) noted that daily fertilization of citrus with more than 100 ppm N as a mixture of

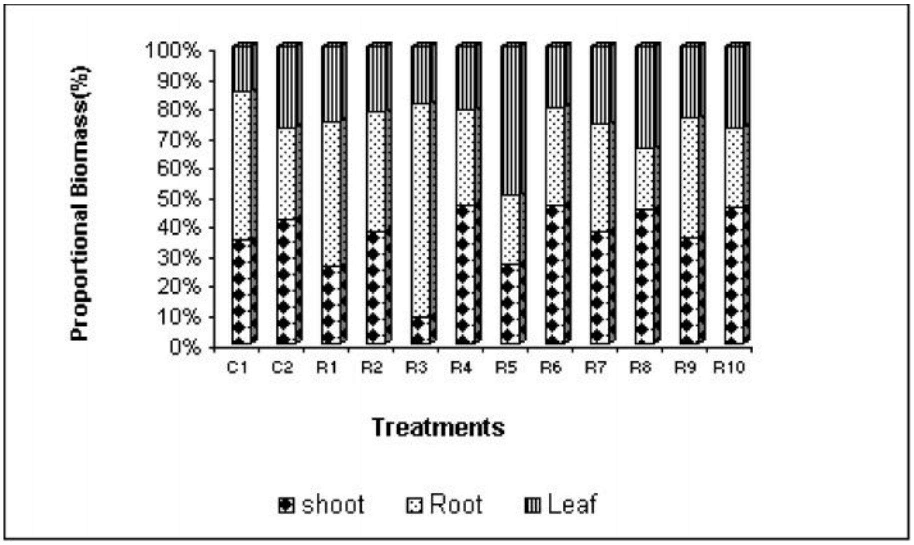

Fig 3 : Proportional distribution of dry biomass in maize grown with mycorrhizal and other treatments
C1=uninoculated garden soil, C2=inoculated garden soil ,
R1=P+AM,R2=N+AM, R3 = K+AM, R4=N+P+AM,R5=K+P+AM

Table 4 : Correlation coefficient of % colonization and vesicles number of maize grown under different treatments

I variable	II variable	R squared
% colonization	root length	0.7436^{NS}
% colonization	root dry biomass	0.7961^{NS}
% colonization	spores number	0.9751***
% colonization	vesicle number	0.988***
vesicle number	spores number	0.9438**

* = Significant at 5 % level, ** = Significant at 1 % level, *** = Significant at 0.01% level,
NS = non significant

nitrate and ammonium retarded mycorrhizal development. Davis *et al*. (1993) reported that nitrate salts were more inhibitory to AM fungi development.

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