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Effect of arbuscular Mycorrhizal (AM) fungi (*Glomus fasciculatum* L.) for the improvement of growth and yield of maize (*Zea mays* L.)

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ABSTRACT

Maize is an important cereal crop of India, stands 3rd in area and production after rice and wheat. Currently, it is cultivated over an area of 8.49 m ha with a production of 21.28 million tonnes. Maize is one of the most important cereal crops in the world agriculture economy both as food for man and feed for animals. It is a miracle crop, having high yield potential, wider adaptability and it is grown throughout the world. Maize is called "Queen of Cereals" because of its productive potential compared to any other cereal crop. In this present study, four AM Fungal spores isolated from five different locations in Cuddalore. The isolated fungal spores are Glomus fasciculatum, Glomus mossae, Gigaspora margarita and Acaulospora laevis. Among the fungal spores, efficient strain selected from Phoaphatase activity, Glomus fasciculatum used for growth of Zea mays by produced the acid phosphatase activity (24.80 µg/ 24 h. 10 g $^{-1}$ of root) and alkaline phosphatase activity (23.00 µg/ 24 h. 10 g $^{-1}$ of root) on 90 DAS.

Key words: AM fungi, *Glomus fasciculatum*, Phosphatase and Maize.

INTRODUCTION

Maize (*Zea mays* L.) is commonly cultivated in temperate regions where prolonged periods of suboptimal temperature and short-term cold spells often occur in spring during the crucial stages of early vegetative development.Maize is grown in almost all the states of India. The crop occupies an area of 6.11 million hectares with a production of 9.12 million tonnes during the period of 2012 - 2013 in India. In Tamil Nadu, it is cultivated over an area of 29,300 hectares with a production of 46,500 tonnes during the period of 2012 - 2013. Maize grain contains about 10% protein, 4% oil, 70% carbohydrates, 23% crude fibre, 10.4% albuminoides, 1.4% ash. A maize grain has significant quantities of vitamin - A, nicotinic acid, Riboflavin and vitamin - E. Maize crop is utilized in many ways like other grain crops. Over 85% of maize produced in the country is consumed as human food [1, 2, 3].

Arbuscular mycorrhizal (AM) fungi are worldwide distributed soil fungi, forming symbiosis with most plant families. Their importance in natural and semi natural ecosystems is commonly accepted and materialized by improved plant productivity and diversity as well as increased plant resistance against biotic and abiotic stresses [4]. Arbuscular Mycorrhiza (AM) fungi are highly beneficial mycorrhizae which are found in association with every taxonomic group of plants and the list of species not infected is probably far shorter than the infected ones. These fungal associations are beneficial to crop plants in many ways, including enhancing the nutrient availability especially phosphorus, enhancing water uptake, inducing resistant against diseases and increasing the yield [5].

MATERIALS AND METHODS

2.1. Details of Locations

The survey was conducted at five different locations in coastal areas of Cuddalore district in Tamil Nadu, India comprising, Killai, Pudhuchattiram, Kaayalpattu, Samiyarpettai and Poondiyankuppam.

2.2. AM fungal colonization in Maize roots

The percentage mycorrhizal colonization of the roots was determined by the method of Phillips and Hayman [6]. The roots were washed gently in tap water. The washed roots were cut into one cm length and then immersed in 10 per cent KOH solution for clearing the host cytoplasm and nuclei for stain penetration. Then, it was autoclaved at 15 lbs/sq. inch pressure for about 20 min. Then, the root bits were taken out and washed with tap water for about three times or until no brown colour appeared in the rinsed water. The roots were acidified with two per cent hydrochloric acid for proper staining. The acid was poured off and root bits were stained with 0.05 per cent tryphan blue in lactophenol solution and boiled for 10 min. These root bits were examined under compound microscope. Root segments in each replication were used to determine AM fungal colonization per cent.

$$\text{Per cent root colonization} = \frac{\text{Number of root bits with infection}}{\text{Total number of root bits examined}} \times 100$$

2.3. SURVEY FOR THE OCCURRENCE OF AM FUNGAL SPORES

AM fungal spore population in the maize rhizosphere soil samples were estimated by wet sieving and decanting method of Gerdemann and Nicolson [7]. One hundred gram of rhizosphere soil sample was taken and mixed thoroughly in one litre of tap water and allowed to settle down the heavier particles for few seconds. The suspension was decanted through a coarse soil - sieve (500 - 800 µm sieve) to remove large pieces of organic matter. The liquid which passed through the sieve was collected separately and stirred to resuspend all particles. The suspension was decanted through a sieve fine enough to retain desired spores (38 - 250 µm sieve). The material retained on the sieve was washed with a stream of water to ensure that all colloidal materials were passed through the sieve. The small amount of remaining debris were transferred to a shallow layer of water in a petridish and examined under a Stereo zoom microscope. The spore numbers from each soil sample were counted and expressed per 100 g of soil.

2.4. Characterization of AM fungal spores

The AM fungal spores obtained from wet sieving and decanting method were cleaned from soil particles by sucrose density gradient centrifugation method and washed with distilled water [8] and the number of spores present in each soil was counted with Stereo zoom microscope (45 X). During counting, identical spores were separated into groups, mounted and identified. Based on the taxonomic key of Gerdemann and Trappe [9], the spores of *Glomus fasciculatum, Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis* were identified.

2.5. Screening for Phosphatase activity of maize roots

The phosphatase activity was measured in maize roots as described by Morton [10].

2.5.1. Enzyme extract

About 10 g of root samples from each treatment were ground thoroughly with acid washed sand in a pre-chilled pestle and mortar in grinding medium containing 20 ml of 0.2 M acetate buffer (pH 4.5) for acid phosphatase or 0.2 M borate buffer (pH 8.5) for alkaline phosphatase activity. The homogenate was passed through four layers of cheese cloth and the filtrate was centrifuged at 3000 rpm for five min and the supernatants were used as enzyme source.

2.5.2. Estimation of acid phosphatase

About 1.0 g of p-nitrophenyl phosphate was dissolved in 100 ml distilled water as substrate. One ml of substrate was pipetted out into a test tube and two ml of enzyme extract and five ml of 0.2 M acetate buffer (pH 4.5) were added. This was incubated for 24 hrs and one drop of 10 per cent Trichloro acetic acid (TCA) was added and centrifuged at 5000 rpm. From this one ml of clear supernatant was taken in a test tube added with, one ml of folin ciocalteau reagent and 2 ml of 20 per cent sodium carbonate and boiled for one minute (at 100°C). Then the test tube was cooled and the volume was made upto 10 ml with distilled water. The colour intensity was read in UV spectrophotometer (Elico, India) at 725 nm (red filter). Standard curve using P-nitrophenol was drawn and using this, the activity was estimated.

2.5.3. Estimation of alkaline phosphatase

Alkaline phosphatase activity was measured by adopting the procedure described for acid phosphatase by replacing borate buffer (0.2 M; pH 8.5) instead of acetate buffer.

2.6. Effect of *Glomus fasciculatum* on the growth of Maize (CO-1)

The pot culture experiment was conducted to study the effect of *Glomus fasciculatum* on the growth and yield of Maize var CO-1. The study was conducted at Department of Microbiology, Annamalai University, Annamalai Nagar. The soil used in the pot culture experiment was clay loamy in nature. The experiment was arranged in Randomized Block Design (RBD) with three replications. For sowing in inoculated pots, blackgram seeds were soaked with trehalose at 15 mM, polyvinyl pyrollidone (PVP) at 2% and glycerol for 30 min in different formulations (20 ml/kg of seeds), (Spacing, 15cm × 10cm: 3 seedlings/hill and 12 seedlings/pot). Gap filling was done after 10 DAS. The crop was given hand weeding on 30th DAS and well protected against pests and diseases. A water level of 5cm depth was maintained through the crop period. Five representative samples of plant hills in each pot were pegmarked for periodical observation.

2.7. Treatment schedule

Design-RBD Replications-3

T_1 – Uninoculated control
T_2 – 100% RDF
T_3 – 25% RDF + *Glomus fasciculatum*
T_4 – 50% RDF + *Glomus fasciculatum*
T_5 - 100% RDF + *Glomus fasciculatum*
(The growth and yield parameters will be analyzed after harvesting)

2.8. Biometric observations

2.8.1. Plant height

Plant height was measured from the ground level to the tip of the primary branches were recorded on 30 DAS, 60 DAS and at harvest. The mean value was worked out and expressed in cm.

2.8.2. Dry matter production

Randomly, five plants from each plot were uprooted as destructive sampling and the roots were washed thoroughly at 30 DAS, 60 DAS and at harvest. The plants were initially sun-dried followed by oven drying at 80ºC±5ºC for 72 hours till a constant weight was attained. The weight of over dried plant samples was recorded and the dry matter production (DMP) was computed to kg ha^{-1}.

2.8.3. Number of grains per cob

Grain number per cob was obtained by manual counting, number of grains after separation of grains from cob.

2.8.4. Number of grains per row

The number of grains per row was counted by manually at randomly selected three rows per cob. Then average number of grains of selected row was taken as number of grains per row.

RESULTS AND DISCUSSION

The results of root colonization percentage and AM fungal spore population obtained are presented in Table - 1. The maize root colonization per cent and AM fungal spore population were in the range from 29.50 % to 61.32 % and 49.00 to 98.00 respectively. The sample collected from Pudhuchattiram recorded the highest root colonization percentage and spores (61.32% and 98.00). The sample collected from Poondiyankuppam recorded the least root colonization percentage and spores (29.50 % and 49.00). The isolated spores were characterized according to Gerdmen and Trappe [11] and the four AM fungal species *viz., Glomus fasciculatum, Glomus mossae, Gigaspora margarita* and *Acaulospora laevis* were found to be present in all the soil types. The characteristics of Arbuscular mycorrhizal fungal spores are given in Table – 2.

The AM fungal spore diversity was studied from the maize rhizosphere soils. The spores of different species *Glomus fasciculatum, Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis* were accounted separately (Fig-1). Among the four isolates, *Glomus fasciculatum* (58 per 100 g^{-1} soil) recorded the highest AM fungal spore numbers at Pudhuchattiram. The AM fungi *Acaulospora laevis* recorded the least AM fungal spore population at Poondiyankuppam (5 per 100 g^{-1} soil).

Table – 1: Distribution of AM fungi in Maize rhizosphere soils of Cuddalore District

S. No.	Location	Root colonization (%)	AM fungal spores numbers (100 g^{-1} of soil)
1	Kaayalpattu	42.43	78
2	Killai	52.64	79
3	Samiyarpettai	34.95	59
4	Pudhuchattiram	61.32	98
5	Poondiyankuppam	29.50	49

Table-2: Morphological Characterization of Arbuscular mycorrhizal fungal spores of maize rhizosphere soils of Cuddalore district

S. No	Character	*Glomus fasciculatum*	*Glomus mosseae*	*Gigaspora margarita*	*Acaulospora laevis*
1.	Size of spore	100 – 120 µm	120 µm	200 – 300 µm	400 µm
2.	Spore shape	Globose hypogeous	Globose	Ectocarpic	Globose
3.	Colour of the spore	Yellow to reddish brown	Yellow to brown	White when young and slightly yellowish at maturity	Outer wall – brown Inner wall – Hyaline Ellipsoid
4.	Sporocarp	Present	Present	Absent	Present
5.	Thickness of spore wall	4 – 14 µm	3 – 4 µm	> 20µm	4 – 8 µm
6.	Subtending hyphae	Absent	Cylindric flared	Bulbous (30 – 50 µm)	Not observable

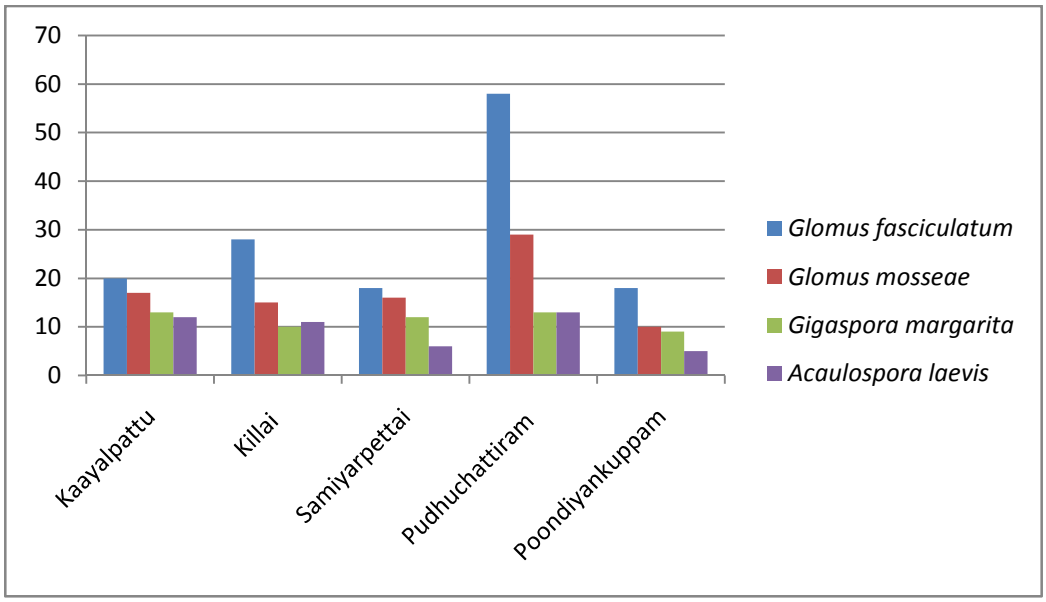

Fig-1: Distribution of different AM Fungal species in Rhizosphere soils of maize Isolated from coastal soils of Cuddalore District

Phosphatase activity were considered as the parameters to study the effectiveness of AM fungi *viz.*, *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis* were estimated on 30 DAS, 60 DAS and 90 DAS. Based on the results, the efficient AM fungus was selected. The results were presented in Table - 3 and Table - 4. The values in the Table – 3 and Table – 4 represents the acid and alkaline phosphatase activity of maize (PKM-1) rhizosphere soil samples was influenced by four AM fungal species *viz.*, *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis*. The results closely revealed that similar to root colonization and spore numbers. The *Glomus fasciculatum* recorded maximum acid phosphatase activity (24.80 µg/24 hrs 10 g^{-1} of root) and alkaline phosphatase activity (23.00 µg/24 hrs 10 g^{-1} of root) on 90 DAS. The minimum acid and alkaline phosphatase activities (9.03 µg/24 hrs 10 g^{-1} of root and 11.90 µg/ 24 hrs 10 g^{-1} of root) were recorded in the *Acaulospora laevis* treatment.

The effect of *Glomus fasciculatum* on improvement of growth parameters in Maize Co - 1 (*Zea mays* L.) was determined and the findings of the present research was given below. The effect of *Glomus fasciculatum* on plant height of Maize Co – 1 (*Zea mays* L.) was measured in the present research. The observations recorded on plant height at harvest are presented in Table – 5. Maximum plant height was recorded during the harvest and highest plant height (177.53 cm) was recorded in the treatment T_5 (75% RDF + *Glomus fasciculatum*). The

treatment T_2 was on par with the treatment T_5 (Control - 100% RDF) (Plant height – 176.80 cm). Lowest plant height (166.31 cm) was observed in the treatment T_1 (Control).

Table – 3: Screening of AM fungi for their efficiency to mobilize the phosphorus based on acid phosphatase activity in the rhizosphere soils of maize from Cuddalore district

AM Fungi	Acid Phosphatase activity (µg/ 24 hrs. 10 g $^{-1}$ of root)		
	30 DAS	60 DAS	90 DAS
Glomus fasciculatum	11.23	18.20	24.80
Glomus mosseae	7.25	10.15	19.00
Gigaspora margarita	6.30	8.10	12.60
Acaulospora laevis	3.70	4.44	9.03
S.Ed	0.38	0.97	1.32
CD(p=0.05)	0.76	1.94	2.64

Table – 4: Screening of AM fungi for their efficiency to mobilize the phosphorus based on Alkaline phosphatase activity in the rhizosphere soils of maize from Cuddalore district

AM Fungi	Alkaline Phosphatase activity (µg/ 24 hrs. 10 g $^{-1}$ of root)		
	30 DAS	60 DAS	90 DAS
Glomus fasciculatum	12.58	19.85	23.00
Glomus mosseae	10.44	17.37	21.33
Gigaspora margarita	8.73	12.75	17.30
Acaulospora laevis	4.20	8.30	11.90
S.Ed	0.69	0.99	1.33
CD(p=0.05)	1.38	1.98	2.66

Maqsood *et al.* [12] reported a significant increase in maize plant height by inoculation of different bacterial strains in combination with *Glomus fasciculatum*. Similar results that plant height increases with *Glomus fasciculatum* and *Pseudomonas fluorescens* were reported by Ayub *et al*. [13] reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens [14, 15].

The effect of *Glomus fasciculatum* on Dry matter production was investigated. The observations recorded at harvest are presented in Table – 5. Maximum dry matter production was recorded during the harvest and more dry matter production (260.34 t ha^{-1}) was recorded in the treatment T_5 (75% RDF + *Glomus fasciculatum*). The treatment T_5 was on par with the treatment T_2 (100% RDF) (Dry matter production – 259.88 t ha^{-1}). Less dry matter production (196.23 t ha^{-1}) was observed in the treatment T_1 (Control). Kumar *et al*. (1998) [16] reported that *Glomus fasciculatum* along with rock phosphate fertilization significantly increased dry matter yield of maize plants.

The effect of *Glomus fasciculatum* on Number of grains per cob and Number of grains per row in Maize Co – 1 (*Zea mays* L.) was tested and the results were presented in Table – 5. Maximum number of grains per cob (480.67) and number of grains per row (31.33) was noticed in the treatment T_7 (75% RDF + *Glomus fasciculatum*). The treatment T_7 was on par with the treatment T_2 (100% RDF) (Number of grains per cob - 480.05 and Number of grains per row - 30.93). Minimum Number of grains per cob (432.80) and Number of grains per row (15.10) was observed in the treatment T_1 (Control).

These results are in line with the findings of Khan *et al*. [17] reported significant effect of bacteria and AM fungal application applications on number of cobs per plant. Kumar *et al*. [18] who reported significant increase in number of plants per meter row length by inoculation of *Azotobacter chroococcum.* The results of the present research are also in agreement with those of [19, 20, 21, 22, 23] who reported that number of grains rows per cob, grain weight per cob and 1000 grain weight was increased with application of fertilizers in combination with bioinoculants. The findings of Capuno *et al.* [24] are almost similar to the present study who found that biofertilizers increased the 1000 grain weight of sorghum and maize.

Table - 5: Effect of *Glomus fasciculatum* on the growth parameters of maize

Treatments	Plant height (cm)	Dry matter production (t ha^{-1})	Number of grains cob^{-1}	Number of grains row^{-1}
T_1 - Control	166.31	196.23	432.80	15.10
T_2 – 100% RDF	176.80	259.88	480.05	30.93
T_3 – 25% RDF + *G. fasciculatum*	170.23	205.75	449.47	20.15
T_4 - 50% RDF + *G. fasciculatum*	173.56	231.56	468.00	25.95
T_5 - 75% RDF + *G. fasciculatum*	177.53	260.34	480.67	31.33

CONCLUSION

From this study we concluded that the AM Fungi had promote the growth of *Zea mays* (Maize). The external hyphae of AM fungi used for uptake and transport of nutrients *viz.*, nitrogen, phosphorus and potassium has been well demonstrated. AM Fungi have great potential to enhance the nutrient uptake, particularly more efficient in phosphorus uptake and plant growth. It also produce the Alkaline Phosphatase and Acid phoasphatase to solubilize the Phosphorus in soil. This study revealed that the highest phosphatase activity was recorded in the application of AM Fungal spore *Glomus fasciculatum* which facilitated the growth of *Zea mays* L. than other AM Fungal spores.

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