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EFFECT OF SEED INOCULATION WITH GLOMUS *INTRARADICES*, PAENIBACILLUS AND *METHYLOBACTERIUM* ON GERMINATION OF MAIZE

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ABSTRACT:

In order to study the effect of seed inoculation with *Glomus intraradices*, *Paenibacillus* and *Methylobacterium* on germination in Maize, a laboratory experiment was conducted in Department of Agrl.Microbiology, Tamil Nadu Agricultural University in completely randomized design with three replicates. The experiment was conducted by treating the seed with both the culture of bacteria and the spore of *Glomus intraradices*(AM) by using lignite as the carrier substrate and gum arabica as the adhesive .The result revealed that, the highest germination percent of 97% was noticed in the treatment of the combined inoculation of AM, *Paenibacillus and Methylobacterium aminovorans.* The germination percent in the un-inoculated control was 85.56% and in the treatment with AM *(Glomus intraradices*) alone was 87.9%. There also observed a significant increase in the shoot length, root length and vigour index of the maize seedling. The highest vigour index of 4111.95 was reported in the treatment with the inoculation of AM, *Paenibacillus* and *Methylobacterium aminovorans*. This treatment also showed a percent increase of 78.64 in case of vigour index over control.

KEY WORDS: *Glomus intraradices, Paenibacillus , Methylobacterium, Gum arabica, Vigour index.*

INTRODUCTION:

Good seed germination behaviour is important for horticulture and agriculture. Uneven or poor germination and subsequently inhomogeneous seedling growth can lead to great financial losses, by e.g., reduced possibilities for mechanization, or lower prices of inhomogeneous plant batches (Ghiyasi *et al*., 2008). More recently, a real challenge faces the workers in the agricultural research field to stop using the high rates of agro-chemicals which negatively affect human health and environment. It is well known that corn crop is considered among the most important cereal crops either in Egypt or all over the world that consumes huge quantities of chemical fertilizers. Many attempts have been tried to replace a part of those harmful chemical fertilizers by biofertilizers to get yield of a good quality without loss in its quantity. *Azospirillum, Pseudomonas* and *Azotobacter s*trains could affect seed germination and seedling growth (Shaukat *et al* .,2006).Thus it has been shown that *Azospirillum* and *Pseudomonas* had the potential for agricultural exploitation and could use as natural fertilizers (Cakmakc *et al*., 2006). Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease (Lugtenberg *et al*., 2002). Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum (Raju *et al*., 1999) and pearl millet (Niranjan *et al*.,2004) and (Niranjan *et al.*, 2003). These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as α-amylase, which have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins (Bharathi *et al*., 2004).The main goal of the current trial is looking for the best seed treatment could be applied to the Maize seed to get a high yield with a good quality in addition to keep our environment clean and safe to live in.

MATERIALS AND METHODS:

The experiment was conducted at Dept. of Agricultural Micobiology , Tamil Nadu Agricultural University in completely randomized design with three replicates, during 2014 growing season at Coimbatore, Tamil Nadu. The seeds of maize COHM6 were surface sterilized with 5% sodium hypo chloride for 5 min to avoid fungal invasion, followed by washing with distilled water. The inoculum of bacteria and AM spore was coated over the seed by mixing the seed in the carrier based inoculum of these organisms that was added with an adhesive of gum arabica for better adherence of inoculum to the seed.

The culture broth of *Paenibacillus* and *Methylobacterium aminovorans* containing 10^8 cells /ml broth was added in the lignite up to 40% moisture level. The in-vitro multiplied spore of AM(*Glomus intraradices*) was added at a rate of 100 spore per 10g of lignite.

The treated seeds were allowed for germination in a Petriplate containing moist filter paper for a period of 5 days. After the germination and development of radicle, the seeds were transferred to a germination paper roll by facing radicle down wards. The plates were given with the Jensen's nutrient solution and allowed to grow for a period of 15 days.

Traits measured including, germination percentage, root length, shoot length and vigour index. Root and shoot length of individual seedling was measured to determine the vigor index with following formula: Vigor index= (mean root length +mean shoot length) × % germination (Abdul Baki and Anderson, 1973).

The statistical analysis was carried on the obtained data according, where the means compared using L.S.D. test at 0.05 level of significance.

RESULTS AND DISCUSSION:

Inoculation of Maize seed with bacteria and AM fungi significantly enhanced seed germination and seedling vigour of maize.

The germination percentage of Maize seed

The highest germination percentage of 97% was obtained when the seeds were treated with the combination of *G. intraradices, Paenibacillus* sp and *M. aminovorans* . Among the *Paenibacillus* isolates tested PB-11 showed highest germination either individually as well as in combination with AM + *M. aminovorans.* All the *Paenibacillus* isolates (PB-7, 10, 11) showed higher germination than the standard culture of *Paenibacillus polymyxa* (MTCC-9489)(Table.1).

The improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum (Raju *et al*.,1999) and pearl millet (Niranjan *et al*., 2004). The improvement in seed germination by PGPR was also found in work with wheat and sunflower(Shaukat *et al*.,2006). Seed inoculation of PPFM resulted a higher germination percent and rate of germination than un-inoculated one in sugar cane (Madhaiyan *et al*., 2005).

The shoot and root length

The treatments were showing significant difference. The highest shoot and root length was observed when the seeds were treated with the combination of *Glomus intraradices, Paenibacillus* sp and *Methylobacterium aminovorans.* Among combined application, the inoculation of *G. intraradices, M. aminovorans* with the isolate of *Paenibacillus* PB-11 showed the highest shoot (19.4 cm) and root (22.5cm) length when compared with other combination treatments of *Paenibacillus.*

All the individual inoculation and dual inoculation of AM with any one bacteria (*Methylobacterium aminovorans* or *Paenibacillus* sp) recorded less root and shoot length when compared with the

combined treatment of all the three organisms. The shoot and root length in the control reported are 12.77cm and 14.1cm per plant respectively. The combined treatment of AM and *Paenibacillus* showing more shoot and root length when compared to the combined treatment of AM and *Methylobacterium aminovorans* (Table 1& Figure 1).

Kloepper *et al* (1992) has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola (Glick *et al*., 1997) as well as wheat and potato (deFreitas and Germida ., 1992), (Frommel *et al*., 1993). *Azospirillum, Pseudomonas* and *Azotobacter* strains could effect seed germination and seedling growth(Shaukat *et al*.,2006).The IAA production ability of the isolates may significantly contribute for enhancing shoot and root length of the plants.

Vigour index

The vigour index of the maize seedling was calculated using the value of shoot length, root length and germination percentage.

All the inoculated treatments recorded 9.5 -78.6% increase in the vigour index of the seedling over un-inoculated control. The highest vigour index (4111.95) of maize was observed when the seeds were treated with the combination of *G. intraradices, Paenibacillus* sp (PB-11) and *M. aminovorans.* The un-inoculated control having the vigour index of 2301.8, and it is very less compared to the all inoculated treatments. The treatment of AM with *Paenibacillus* sp (PB-11) and *Methylobacterium aminovorans* showing a percent increase of 78.64 in case of vigour index compared to the control and also showing a percent increase of 27.9 compared to the treatment with the standard culture of *Paenibacillus polymyxa*- MTCC 9489(Table 1& Figure 2)

Many endophytic strains can promote plant growth through N_2 fixation (Ladha *et al.*, 2000), root elongation by the production of plant hormones (Kozyrovska *et al*.,1996) and increased resistance to pathogen and parasites(Chen *et al*.,1995). Total increase in shoot length, root length as well as germination lead to an increase in vigour of the plants.

CONCLUSION:

Finally, in respect to our observations in this study, we propose a beneficial role of studied *Paenibacillus* , *Methylobacterium* and the AM fungi *Glomus intraradices* application for seeds to enhance the seed properties by seed biofortification, which can improve not only seed quality ,including seedling vigour and optimizing maize hybrid yields, but also considered as suitable approach in sustainable agriculture. In this experiment, all the three inoculants are applied through seed and this lead to 100% colonization of the inoculants in the root without any loss. Further AM fungus colonizes the root cortex

and this influences the bacterial population in a positive way due to mycorrhizosphere effect. This may be the reason that the bacterial population is on the higher side in root as well as enhancement was observed when all the inoculants are applied together as consortium through the seed. This result also confirmed the endophytic potential of the selected isolates of *Paenibacillus* and *Methylobacterium*.

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Table 1. Effect of AM fungus(*Glomus intraradices*) and Bacteria (*Paenibacillus* sp and *Methylobacterium aminovorans*) on different biometrical characteristics of maize under roll towel method

Treatment	Germination percentage	Shoot length(cm/plant)	Root length(cm/plant)	Vigour Index
T1- Control	85.56 (67.67)	12.77	14.1	2301.8
T2-Glomus intraradices(GI)	87.9 (69.64)	13.13	15.3	2521.51 (9.5)
T3- GI+ Methylobacterium aminovorans	90.93 (72.48)	14.76	16.1	2821.23 (22.56)
T4- GI +Pb(MTCC-9489)	90.06 (71.62)	13.1	16.3	2651.99 (15.2)
T5- GI+ Pb(MTCC-9489) +M.a	91.93 (73.51)	16.4	18.1	3214.64 (39.66)
T6- GI +PB-7	92.96 (74.62)	15.3	16.8	2983.1 (29.5) (12.48)
T7-GI+PB-7+M.a	93.5 (75.24)	18.23	20.1	3628.58 (57.64) (12.87)
T8- GI +PB-10	92.06 (73.64)	14.1	17.3	2912.29 (26.52)(28.97)
T9- GI+PB-10+M.a	93.2 (74.89)	17.23	19.2	3420.36 (48.59)(6.39)
T10- GI +PB-11	92.9 (74.55)	16.0	17.3	3110.27 (35.12)(17.28)
T11- GI+PB-11+M.a	97.06 (80.12)	19.4	22.5	4111.95 (78.64)(27.9)
SEd	**0.35**	**0.12**	**0.11**	**144.78**
CD(0.05)	**0.74**	**0.25**	**0.22**	**300.25**

Values represent mean of three replicates ;DAS-Days After Sowing; GI-*Glomus intraradices;*The value in the parentheses indicate the Arcsine transformation for germination percentage; For Vigour index the value in the parentheses indicate the percent increase over control and standard of respective treatments; Pb-MTCC-9489- *Paenibacillus polymyxa*-MTCC-9489*;M.a-Methylobacterium aminovorans;*PB-*Paenibacillus* isolate.

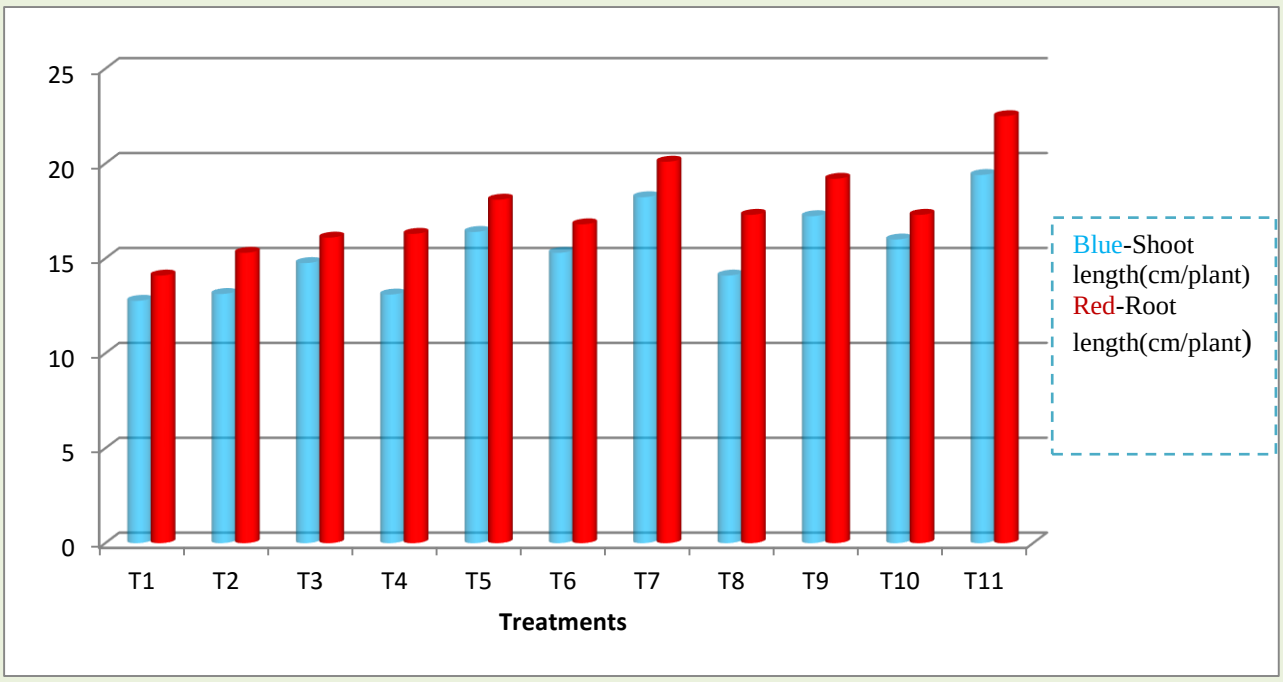

Figure 1. The influence of interaction of AM, *Paenibacillus* and *Methylobacterium* on the shoot and root length of Maize under roll towel method

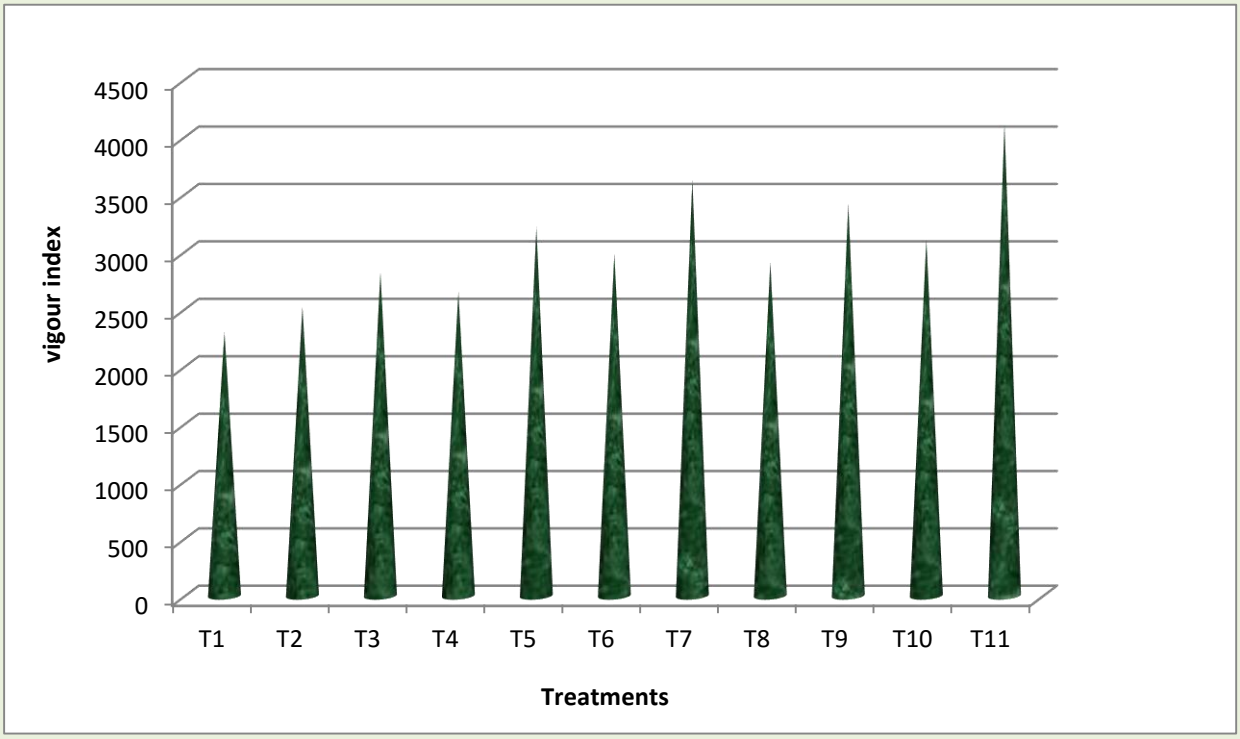

Figure 2. The influence of interaction of AM, *Paenibacillus* and *Methylobacterium* on Vigour index of Maize seedling under roll towel method