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IN VITRO GROWTH CHARACTERISTICS OF MAIZE AND WHEAT ON MICROBIAL INOCULATION

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ABSTRACT

Maize and wheat seeds were inoculated with microorganisms like *Chaetomium globosum, Gliocladium roseum, Streptomyces* sp. *Pseudomonas pseudomallei* YL-1 along with control and fertilizer (NPK 17:17:17) were grown in pots for forty five days under laboratory conditions. The phenotypic traits such as shoot length, root length, leaf area were increased significantly in seedlings treated with microbes when compared to control but near equal to fertilizer-treated seedlings. Although the fresh and dry weights were more in microbial treated seedlings over control but did not show significant increase by DMRT analysis. However, based on the results obtained in this study it has been revealed that microbial inoculants could be promising substitutes for chemical fertilizers for the improvement of agricultural crops.

KEY WORDS: ***Seed inoculation, microbes, leaf area, shoot length, maize, wheat***

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INTRODUCTION

Environmental and health concerns about the extended use of pesticides in agriculture necessitate the finding of alternative methods to control pathogens and better crop production. Plant beneficial microorganisms are of interest for application in agriculture as biofertilizers or as pesticides as well as for phytoremediation applications[1]. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Many rhizobacteria[2], actinobacteria specifically belonged to the genus Streptomyces[3,] Diazotrophic bacteria[4] were used to improve crop fertility by enhancing the amount of nitrogen available to the plant and modifying physiological changes in the host plants. Maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) is an important crop for meeting increasing global demands for food, and livestock. In the present study, we attempted to evaluate the influence of microbes which were earlier isolated from different plants on growth characteristics of maize and wheat under laboratory conditions.

MATERIALS AND METHODS

(i) Plant species and sampling

Seeds of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) were surface sterilized with ethanol (70%) for 2 m, Sodium hypochlorite solution (0.75% Chlorine) for 10 m followed by sterile distilled water six times to avoid surface contaminants[5].

(ii) Inoculum

Fungi such as *Chaetomium globosum* Kunze ex Fr., *Gliocladium roseum, an actinobacterial Streptomyces* sp. and bacterial strain *Pseudomonas pseudomallei* YL1were obtained from culture collections of Department of Applied Botany, Kuvempu University which were isolated from some medicinally important host plants in our previous study. The starter cultures of these bio agents were grown on Petri plates containing PDA medium for five days. After five days, discs of size 5 mm of these fungi were inoculated into 500 ml conical flasks containing 200 ml of Potato Dextrose Broth (Dextrose–20 g; Potato–100 g; and distilled Water–1000 ml) and incubated at 30±2º C under constant shaking conditions (100 rpm) in the dark for 10 days. Fungal mycelium was harvested and washed several times with sterile water. Surface sterilized seeds (50 g) of maize were mixed with a mechanically homogenized culture of *C. globosum, G. roseum* and *Streptomyces* sp. in sterile water[5]. One chemical fertilizer N: P: K (17:17:17) was applied at the rate of 60 mg Kg^{-1} of soil, in order to compare the efficacy of microbes towards plant growth promotion[5].

(iii) Seed bacterization

Surface sterilized seeds were dried over night under sterile airflow. Cells of *P. pseudomallei* was grown on King's B medium (proteose peptone – 20 g; Glycerol -15 ml; potassium hydrogen phosphate- 1.5 g; magnesium sulphate (hydrated) -1.5 g; agar-20 g; and distilled Water-1 L) for 48 h at 30±2º C. The inoculum was scrapped with a sterile glass rod and taken in 1% carboxy methyl cellulose (CMC) Sodium salt solution. The sterilized seeds were mixed with the bacterial suspension in the CMC and dried overnight in sterile polythene sheets under sterile stream of laminar airflow (Dileep et al. 1998). In total, there were six treatments maintained in this study were 1) *C. globosum*, 2) *G. roseum,* 3) *Streptomyces* sp. 4) *P. peudomallei* strain YL-1, 5) Fertilizer (N:P:K:17:17:17), and an 6) un-inoculated control. All these treatments were maintained separately in three replications.

(iv) Soil preparation and growth conditions

Soil sample was prepared by adding a mixture of Soil: Sand: Farmyard manure (3:1:1). This mixture was disinfected with 1% formalin for 48 h and left for five days to aerate to eliminate excess formalin[5]. Treated and untreated seeds were sown in pots (9 inch height by 10 inch diameter) containing soil prepared by adding mixture of Soil: Sand: Farmyard manure. The pots were kept under the normal growth conditions approximately 75% relative humidity, 22º C to 26ºC, 12 h day light. The emerged plants were irrigated on every second day with equal amount water to pots containing control plants and pots with treatments.

(v) Measurements of growth parameters

Seed germination percentage was calculated as number of seeds germinated/ total number of seeds sown x 100. The plantlets were harvested on 7^{th}, 15^{th}, 30^{th} and 45^{th} days after sowing. Leaf area was calculated as leaf area (cm^2) = K x length x breadth, where K = Kemp's constant. The harvested plantlet shoot length (cm) was measured from the above ground level to growing tip (stem apex) and root length (cm) was measured from below ground level to root tip. Fresh weight of measured plantlets were recorded immediately after harvesting and dry weights were recorded until constant weight obtained after drying in a hot air oven at 55º C for two days.

Confirmation studies of root colonization

Roots of microbial treated plants of age 45 days were washed thoroughly in running tap water, cut into 1 cm pieces and treated overnight with 10% KOH solution at room temperature. Then root pieces were washed 3-5 times with sterilized distilled water and treated with 1% trypan blue in lactophenol[6]. The stained root samples were mounted on slides and observed microscopically. The per cent infection was calculated as follows:

$$\text{Per cent colonization} = \frac{\text{No. of root segments colonized}}{\text{Total no. of segments observed}} \times 100$$

(vi) Statistical analysis

Difference among effects of different treatments on plants growth was calculated by Duncan's Multiple Range Test (DMRT) at 0.05%[5,6].

RESULTS

After seven days of seed inoculation, maximum seed germination was found in seeds treated with *G. roseum* (89 %) and NPK (92%) in maize and *C. globosum* (87 %) in wheat (Fig 1) (Table 1, 2). After 45 days of sowing, maize shoot length was significantly increased over control seedlings by NPK treatment (129.2 %), *G. roseum* (125.0 %) and *C. globosum* (124.3 %). Wheat shoot length was increased significantly by *Streptomyces* sp., (158.9 %), NPK (141.0 %), *C. globosum* (136.4 %) over the control. Similarly the maize root length was increased significantly in the seedlings treated with NPK (170.0 %), *G. roseum* (166.4 %), *C. globosum* (159.4 %), and *P. pseudomallei (*158.0 %)*, Streptomyces* sp. (158.0 %), over the control seedlings. Wheat root length increased significantly by NPK (158.5 %), *G. roseum* (119.2 %), *P. psedomallei* (116.4 %), *Streptomyces* sp. (115.7 %) and C*. globosum* (113.5 %) over the control seedlings. Although fresh and dry weights were more in microbial treated seedlings over control, they did not show any significant when subjected to DMRT. Leaf area was maximum in seedlings treated with NPK (134.4 %), in maize and *C. globosum* (175. 9 %) in wheat seedlings over the control seedlings (Fig 2). The maximum root colonization was found in roots treated with *G. roseum* (81.0 %) in maize and *C. globosum* (74.0 %) in wheat seedlings.

Table1

Growth parameters of maize seedlings by microbial seed inoculation after 7th, 15th, 30th, 45th days after sowing

	7th day						15th day					
	control	NPK	P. pdl	C. gbs	S. sp	G. rsm	control	NPK	P. pdl	C. gbs	S. sp	G. rsm
Shoot length (cm)	8.6^a	11.0^a	10.9^a	12.6^b	10.9^a	11.5^b	16.0^a	27.0^b	24.2^b	28.0^b	24.0^b	25.0^b
Root length (cm)	8.0^a	8.2^a	8.4^a	9.5^a	8.3^a	8.4^a	8.3^a	13.0^b	11.6^a	13.7^b	11.5^a	11.7^a
Fresh weight (g)	0.90^a	0.99^a	1.01^a	1.1^a	0.86^a	1.05^a	1.4^a	1.64^a	1.66^a	2.0^a	1.14^a	1.85^a
Dry weight (g)	0.11^a	0.21^a	0.22^a	0.30^a	0.27^a	0.27^a	0.16^a	0.23^a	0.23^a	0.28^a	0.28^a	0.28^a
Leaf area (cm)	7.4^a	9.3^a	12.6^b	9.5^a	8.7^a	12.1^b	20.9^a	27.5^b	29.0^b	31.0^b	27.5^b	29.0^b
Root colonization (%)	-	-	51	64	51	61	-	-	61	69	63	68
	30th day						45th day					
	control	NPK	P. pdl	C. gbs	S. sp	G. rsm	control	NPK	P. pdl	C. gbs	S. sp	G. rsm
Shoot length (cm)	25.0^a	49.5^c	38.0^b	49.0^c	39.0^b	45.0^b	41.0^a	53.0^b	41.7^a	51.0^b	41.5^a	51.5^b
Root length (cm)	12.0^a	23.5^b	20.9^b	23.5^b	18.0^a	23.0^b	17.0^a	29.0^b	27.0^b	27.1^b	27.0^b	28.3^b
Fresh weight (g)	2.11^a	2.62^a	2.47^a	3.07^a	1.98^a	2.91^a	2.54^a	3.03^a	2.96^a	3.17^a	2.38^a	3.08^a
Dry weight (g)	0.20^a	0.35^a	0.39^a	0.29^a	0.29^a	0.29^a	0.61^a	0.42^a	0.49^a	0.48^a	0.52^a	0.42^a
Root colonization (%)	-	-	61	76	66	67	-	-	61	79	65	81

Note: Data based on average of three replications; Different letters following means in rows indicate a significant difference among treatments based on DMRT (P≤ 0.05), P. pdl =P. pseudomallei, C. gbs =C. globosum, S. sp = Streptomyces sp., G. rsm = G. roseum

Table2

Growth parameters of wheat seedlings by microbial seed inoculation after 7th, 15th, 30th, 45th days after sowing

	7th day						15th day					
	control	NPK	P. pdl	C. gbs	S. sp	G. rsm	control	NPK	P. pdl	C. gbs	S. sp	G. rsm
Shoot length (cm)	11.0^a	31.4^c	21.8^b	32.3^c	29.9^c	19.8^b	19.7^a	40.8^c	27.2^a	37.3^c	42.3^c	31.6^b
Root length (cm)	5.4^a	13.0^b	8.6^a	11.0^b	8.4^a	17.1^a	6.3^a	15.9^b	9.8^a	11.4^a	18.1^b	11.0^a
Fresh weight (g)	0.2^a	0.41^a	0.31^a	0.49^a	0.38^a	0.21^a	0.21^a	0.7^a	0.39^a	0.6^a	0.79^a	0.53^a
Dry weight (g)	0.01^a	0.07^a	0.04^a	0.09^a	0.07^a	0.04^a	0.05^a	0.31^a	0.09^a	0.1^a	0.33^a	0.12^a
Leaf area (cm)	6.8^a	11.7^a	12.0^a	17.1^b	18.3^b	11.1^a	9.1^a	19.0^b	17.3^b	20.9^c	21.1^c	14.9^b
Root colonization (%)	-	-	51	51	66	68	-	-	69	71	76	81
	30th day						45th day					
	control	NPK	P. pdl	C. gbs	S. sp	G. rsm	control	NPK	P. pdl	C. gbs	S. sp	G. rsm
Shoot length (cm)	28.1^a	52.0^c	39.3^b	48.8^c	57.4^c	38.7^b	39.0^a	55.0^b	44.0^a	53.2^b	62.0^b	45.0^a
Root length (cm)	10.9^a	20.9^b	17.6^b	18.0^b	24.3^b	13.8^a	14.0^a	22.2^b	16.3^a	15.9^a	16.2^a	16.7^a
Fresh weight (g)	0.41^a	1.2^a	0.7^a	1.1^a	1.29^a	0.7^a	0.6^a	.82^a	0.94^a	1.3^a	1.0^a	0.68^a
Dry weight (g)	0.13^a	0.21^a	0.09^a	0.36^a	0.32^a	0.09^a	0.08^a	0.28^a	0.09^a	0.3^a	0.32^a	0.30^a
Root colonization (%)	-	-	59	68	77	51	-	-	66	74	69	51

Note:Data based on average of three replications; Different letters following means in rows indicate a significant difference among treatments based on DMRT (P≤ 0.05), P. pdl =P. pseudomallei, C. gbs =C. globosum, S. sp = Streptomyces sp., G. rsm = G. roseum

Figure 1
Germination (%) of maize and wheat seeds inoculated with microbes and NPK

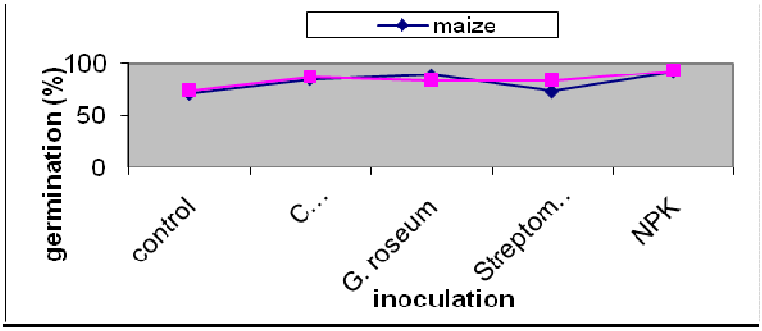
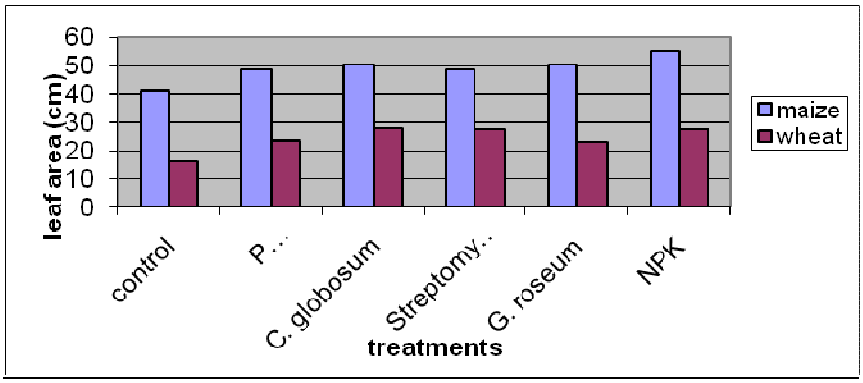


Figure 2
Leaf area (cm) of maize and wheat seedlings control and inoculated wih microbes and NPK.



DISCUSSION

Microbial isolates used to evaluate growth characteristics of maize in this study have shown to be antagonistic against various phytopathogens of rice *in vitro* (Shankar et al. 2006). Plant host stimulation by these symbionts have been already documented in jowar, rice, ground nut and finger millet and consisted of larger number and more number of tillers, leaf elongation, leaf area, thickness, stem length and altered root architecture[7]. Inoculation of *Aureobasidium pullulans* in wheat and chilli seedlings exhibited maximum growth against NPK treated seedlings in our previous study[8]. Diazotrophs such as Azospirillum, Azotobacter, Bacillus and Pseudomonas frequently colonize the important cereal crops including wheat, rice and maize and promote plant growth by producing certain PGPR[9]. Many studies and reviews have reported plant growth promotion, increased yield, and solubilization of P (phosphorus) or K (potassium), uptake of N (nitrogen), etc. through inoculation with PGPR[10, 11]. Several studies were reported the significance of siderophores produced by certain genera of PGPR in plant growth promotion[12]. Siderophores are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore-producing PGPR in rhizosphere increases the rate of Fe3+ supply to plants and therefore enhances the plant growth and productivity of crop. There is a significant increase on growth parameters, such as ear dry weight comparison to the control in spring wheat by inoculation of *Azospirillum brasilense*[13]. Several workers have been reported that in vitro studies have documented satisfactory results in the use of *Streptomyces* against some root pathogens and enhancing growth of the crops and vegetables[14] .The growth promotion ability of these microbes in the present study indicated that microbial mutualists could be ecofriendly alternative tools for chemical fertilizers for sustainable agriculture.

CONCLUSION

Microbes are an integral part of the soil and contribute to soil and plant health. Microorganisms have the ability to fix atmospheric nitrogen, solubilize and mobilize phosphorus, produce antibiotics and disease suppressing molecules. Current and future progress in our understanding of PGPR diversity, colonization ability, mechanisms of action, formulation, and application could facilitate their development as reliable components in the management of sustainable agricultural systems.

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