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Original Article

Response of Turmeric to Plant Growth Promoting Rhizobacteria (PGPR) Inoculation under different levels of Nitrogen

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Abstract

A study was conducted at K.S.R College of Engineering and Technology and Gobisettipalyam of Erode dist in 2009-2010 to examine the effect of PGPR inoculation alone and in combination with three levels of mineral nitrogen fertilizer (0-56-60, 56-56-60 and 112-56-60 kg NPK/ha) on turmeric. The bacterial inoculums (50 g / kg of seed) significantly increased rhizome yield (21%), plant height (5%) rhizome weight (60%) and microbial population in soil (41 %) over their respective controls while GOT remained statistically unaffected.

Keywords: *Pseudomonas*, *Bacillus*, Plant growth promoting rhizobacteria, turmeric, rhizome, bio fertilizer.

Introduction

Nitrogen is a major limiting nutrient for crop production. It can be applied through chemical or biological means, but chemical nitrogen fertilizer is expensive (Regan *et al.*,1988). To get optimum crop yields, biological means need to be explored for acquiring nitrogen for plant growth. Plant growth promoting rhizobacteria (PGPRs) are root colonizing microorganisms which are known to fix atmospheric molecular nitrogen through symbiotic and asymbiotic or associative nitrogen fixing process. The effect of associative microorganisms in increasing crop yield and N_2-fixation has been reported by many research workers (Markus,1988; Rashid *et al.*,1999; 2000). These microorganisms not only fix atmospheric nitrogen but also produce certain plant growth promoting hormones (Frankenberger *et al.*, 1995). These bacteria belong to the genera *Azotobacter*, *Azospirillum*, *Bacillus*, *Arthrobacter*, *Enterobacter*, *Pseudomonas*, *Alcaligenes*, *Klebsiella* and *Serratia* (Dobereiner, 1992). Application of bacterial inoculants as biofertilizers has improved growth and yield of cereal crops (Dobereiner 199; Kennedy *et al.*, 1992; Rashid *et al.*, 1996). During last two decades, nitrogen fixation with non-legumes has attracted much attention of soil microbiologists. Interest in beneficial rhizobacteria associated with cereals has increased recently due to their potential use as biofertilizers (Okon *et al.*, 1994; Bashan *et al.*, 1990). Allison proposed that bacillus in addition to fixing atmospheric nitrogen, produce plant growth regulating hormones which also antagonizes against pathogens. PGPR can influence plant growth and development directly or indirectly. Direct promotion of plant growth by PGPR generally entails providing a compound to the plant that is synthesized by bacterium or facilitating the uptake of nutrient from environment (Chet *et al.*, 1994; Glick, 1998). On the other hand, indirect promotion of plant growth occurs when bacteria decrease or prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms.

There are several ways the PGPRs may directly facilitate the proliferation of their plant hosts. These may (i) fix atmospheric nitrogen and supply it to plants, (ii) synthesize siderophores which can provide iron to plants, (iii) synthesize various phytohormones, including auxins and cytokines, (iv) provide mechanisms for the solubilization of minerals such as phosphorus and (v) synthesize enzymes that can modulate plant growth and development (Brown *et al.*, 1974; Davison *et al.*, 1988; Jacobson *et al.*, 1994; Kloepper *et al.*, 1988; Lambert *et al.*, 1989 and

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Patten *et al*.,1996). A number of PGPRs contain the enzyme 1-cyclopropane, 1-carboxalate (ACC) deaminase (Glick *et al*., 1995) and this enzyme can cleave the plant ethylene precursor ACC and thereby lower the level of ethylene in a developing or stressed plant. For many plants, a burst of ethylene is required to break seed dormancy (Esashi *et al.*,1991) but following germination, a sustained high level of ethylene would inhibit root elongation (Jacobson *et al.*, 1991).

PGPRs possess traits associated with biocontrol of plant pathogens viz. (i) antibiotic synthesis, (ii) secretion of iron binding siderophores form soil and provide it to a plant and thereby depriving fungal pathogens in the vicinity of soluble iron (Dowling *et al.*, 1996; Haahtela *et al.*,1996 and Neilands *et al*., 1986), (iii) production of low molecular weight metabolites such as hydrogen cyanide, with antifungal activity (Dowling *et al.*, 1994;Loper *et al.*, 1997); (iv) production of -1,3-glucanase, protease or lipase which can lyseβenzymes including chitinase, some fungal cells (Chet *et al.*,1994), (v) out-competing phyto-pathogens for nutrients and niches on the root surface (Kloepper *et al.*, 1988; O'Sullivan *et al*.,1992) and (vi) lowering the production of pathogen(s) stress ethylene (Jacobson *et al.*,1991) in plants with the enzyme ACC- deaminase (Glick *et al*., 1995; Penrose *et al.*, 2001). Since many of the chemicals are used to control pathogens, pest, insects etc. in plants are hazardous to animals and human and can persist in natural ecosystems. So these chemicals are being replaced with environment friendly biological approaches to indirectly promote plant growth including the use of biocontrol PGPR. At present, there are fewer than 20 different biocontrol PGPR strains that are commercially available. However, this number should increase as new biocontrol strains are isolated using any one of the variety of available screening procedures (Berg *et al.*, 1996; Eden *et al.*, 1996; Gould *et al.*, 1996; Putcha *et al.*, 1997) and superior, genetically engineered, biocontrol strains are developed. A particular bacterium may promote plant growth and development by using any one, or more of these mechanisms. Moreover, a bacterium may utilize different traits at various times during life cycle of the plant.

Diazotrophs belonging to diverse bacterial genera such as *Azospirillum, Azotobacter, Acetobacter, Arthrobacter, Alcaligenes, Bacillus, Enterobacter, Herbaspirillum, Klebsiella* and *Pseudomonas* frequently colonize the important cereal crops including wheat, rice, sugarcane and maize and promote plant growth either directly or indirectly by producing certain plant growth promoting (Berge *et al.*,1991;Cavalcante *et al*., 1988a; Malik *et al*.,1994) or phyto-pathogenic substances. Javed *et al.*, (1994) selected 11 isolates of plant growth promoting rhizobacteria and reported that four of these improved the growth of maize and could be used as biofertilizers. However, results of these bacteria used as biofertilizers are variable in the field. Rashid *et al*., (1996) reported that response of wheat to diazotrophic bacteria was variable in different ecological zones of Punjab ranging from 0 to 35 percent increase in yield over control. This inconsistency in results might be due to many factors such as complex interaction among hosts, rhizobacteria, pathogens, climate and soil environment. Variability in root colonization by these bacteria is the most important factor. Hence there is a dire need to study the adaptation of diazotrophs to their host plant.

The Western Ghats (WG) is one of the world's biodiversity hotspots, which stretches from Tapti valley in the north of Gujarat to Kanyakumari in Tamil Nadu, covering a distance of 1600 km with over 100 km wide. The WG runs through different states of south-western India such as Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, Kerala and covers various types of vegetation including evergreen, tropical deciduous, scrub, montane, subtropical temperate forests and grasslands. The diversity of higher plant flora and fauna has been studied in great detail since European colonization in India. Although there has been substantial research in terms of medicinal and ecosystem values. In present study, inoculation of diazotroph bacteria (Bacillus and pseudomonas) was tested on curcuma longa to see the response of turmeric yield parameters to these beneficial bacteria for minimizing production cost.

Materials and Methods

A study was conducted at K.S.R College of technology and Gobisettipalyam, Erode District in 2009-2010. Rhizospheric soils of different agronomic parts of Kollihills in Nammakal, Tamil Nadu, India were collected for the isolation of *Pseudomonas* Spp. *Pseudomonas* isolates were isolated from the soil on nutrient agar medium or King's medium as per the standard method (Stein *et al.*, 1990). Bacillus

were isolated with the Rhizosphere isolation medium (RIM) incubated at 22^0C for 2 to 3 days (Buyer, 1995). *Bacillus* sp and *Pseudomonas* sp cultures were mixed at 1:1 ratio on the basis of number of bacterial cells named as diazotroph bacterial inoculum. Peat based inoculum was prepared and incubated for 15 days. Diazotroph inoculum was applied to seed rhizome (Madras variety also known as Perianadan) @ 50 g per kg containing 5 x 108 MPN bacterial cells/g of peat before sowing. The soil was clay loam having pH 7.9; ECe 1.9 dS/m; native N 0.041 percent; organic matter 0.57 percent and Olsen-P 7.8 mg/kg soil. Experiment had three fertilizer levels (0-56-60, 56-56-60 and 112-56-60 kg NPK/ha) alone and in combination with diazotroph inoculum with four repeats. All P_2O_5 as SSP and K_2O as SOP along with bacterial inoculum were applied at sowing as seed coating. First 1/3 N fertilizer as urea was applied with first irrigation, 1/3 at 3rd month stage and remaining 1/3 was applied at 5th month stage. Recommended insecticides were sprayed to the crop when needed. Data were recorded on Rhizome yield, stem height, stem biomass, root length, root biomass, GOT and microbial population in soil at harvest. Microbial population in soil was determined in soil samples by plate count method. Standard analytical methods were used for soil and plant analysis. Data were subjected to statistical analysis following RCBD with two factors. Duncan's multiple range test (Duncan 1955) was applied to see the significance of differences among treatment means.

Results and Discussion

The data (Table-1) indicated that diazotroph bacterial inoculation significantly increased stem height (25%) as compared to treatments without inoculation. N-fertilization also significantly influenced the stem height.

Table -1: Effect of diazotroph bacterial inoculation stem height and stem biomass

Treatments (kg NPK/ha)	Stem height (cm)			Stem biomass (gm)		
	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
0-56-60	29.00	67.40	48.20 C	15.32 c	46.35 b	30.83 B
56-56-60	32.46	96.37	64.41 B	24.60 b	52.65 b	38.62 AB
112-56-60	45.00	109.30	77.15 A	30.40 ab	54.00 a	42.20 A
Mean	35.48 B	91.00 A		23.44 B	51.00 A	

Means followed by different letters significantly differ using LSD test (P ≤0.05).

Table -2: Effect of diazotroph bacterial inoculation on root length and root biomass

Treatments (kg NPK/ha)	Root length (cm)			Root Biomass (gm)		
	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
0-56-60	13.80	17.35	15.57	7.82	15.81	11.80 B
56-56-60	15.20	21.40	18.30	10.30	18.31	14.30AB
112-56-60	16.25	24.25	20.25	12.60	20.28	16.44 A
Mean	15.08	21.00 NS		10.24	18.13 NS	

Means followed by different letters significantly differ using LSD test. (P≤0.05)

Table -3: Effect of diazotroph bacterial inoculation on no of leaves and rhizome biomass

Treatments NPK kg ha^{-1}	No of leaves			Rhizome biomass (gm)		
	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
0-56-60	3	6	4.5	21.23	89.60	55.41 C
56-56-60	5	8	6.5	54.23	208.23	131.23B
112-56-60	5	9	7.0	92.30	222.50	157.40A
Mean	4.33	7.66 NS		55.92B	173.44A	

Means followed by different letters significantly differ using LSD test (P< 0.05).

Table – 4: Effect of diazotroph bacterial inoculation on no of leaves and rhizome biomass

Treatments NPK kg ha^{-1}	GOT (%)			Microbial population		
	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
0-56-60	37.6	37.6	37.6B	80.5	117.8	88 C
56-56-60	38.3	38.0	38.2AB	108.0	161.5	135 B
112-56-60	38.6	39.0	38.8A	161.0	239.8	201 A
Mean	38.1	38.2 NS		117 B	166 A	

Means followed by different letters significantly differ using LSD test (P< 0.05).

Maximum stem biomass (54 gm) was obtained when fertilizer was applied @ 112-56-60kg NPK along with inoculum as compared to un-inoculated treatment (30.40gm). The interaction effect of inoculum and N fertilization was, however, statistically non-significant. The results are comparable to those of Boddy *et al.*,(1996). Where application of bacterial inoculants as bio fertilizers has improved growth and yield of cereal crops. Diazotroph bacterial inoculum had significant effect (41 %) on plant height (Table 1). Mineral fertilizer also significantly increased plant height. Maximum plant height (109.30 cm) was recorded from NPK applied @ 112-56-60 kg along with diazotroph bacterial inoculum which was comparable with un-inoculated treatment (45.00 cm). Interaction effect of inoculum and mineral fertilizer was also statistically significant. Nagaraja *et al.,* (2010) also reported that inoculation with PGPR strains increased plant height; root proliferation, N contents and root shoot dry weight of Maize.

The data (Table -2) further indicated that Bacillus and Pseudomonas non-significantly increased root length (33%) as compared to un-treated plants. Mineral fertilizer also had non-significant effect on root length. Maximum root length (24.25 cm) was noted in NPK treatment of 112-56-60 kg in combination with bacterial inoculum over its respective control (16.25 cm).

Interaction effect of mineral fertilizer and bacterial inoculation was also non-significant on root length. Similar results have been observed by Rashid *et al.,* (1996). It was also observed that diazotroph inoculation had non-significant effect on root biomass while mineral fertilizer significantly increased root biomass of crop. Interaction effect of inoculum and mineral fertilizer was, however, statistically non - significant. Maximum root biomass (29 %) was recorded from mineral fertilizer @ 112-56-60 kg NPK along with inoculum.

The results regarding no of leaves and rhizome biomass (Table 3) indicate non-significant effect of diazotroph bacterial inoculum and mineral fertilizer.

Interaction effect of inoculum and fertilizer was also statistically non-significant. Maximum in no of leaves (9) was recorded also from 112-56-60 kg NPK in combination with bacterial inoculation where un-inoculated with 5 leaves. Further indicated that Bacillus and Pseudomonas non-significantly increased rhizome biomass (60%) as compared to un-treated plants. Mineral fertilizer also had non-significant effect on rhizome biomass. Maximum rhizome biomass (222.50gm) was noted in NPK treatment of 112-56-60 kg in combination with bacterial inoculum over its respective control (92.30 gm).

It was also observed that diazotroph inoculation had non-significant effect on GOT while mineral fertilizer significantly increased GOT of rhizome. Interaction effect of inoculum and mineral fertilizer was, however, statistically non-significant. Maximum GOT (39.0%) was recorded from mineral fertilizer @ 112-56-60 kg NPK along with inoculum.

Data regarding microbial population in soil at harvest (Table-4) revealed that bacterial inoculation significantly increased microbial population in soil (41%) as compared with non-inoculated plants. Mineral fertilizer also had significant effect on microbial population in soil whereas interaction effect of bacterial inoculation and mineral fertilizer was non-significant in soil at the time of harvest. These results are in line with those obtained (Berge *et al.,* 1991; Cavalcante and Dobereiner, 1998).

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