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STUDIES ON THE ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON THE GROWTH AND YIELD OF CHILLI (*CAPSICUM ANNUM.L.*)

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Abstract

Plant growth promoting rhizobacteria (PGPR) is one important group of microorganism which can produce growth promoting substances and increases crop growth either directly or indirectly. A pot culture experiment was carried out in the Department of Microbiology, Faculty of Agriculture, Annamalai University to study the effect of PGPR on the growth and yield of chilli. Two isolates *viz., Pseudomonas sp* and *Bacillus sp* were isolated from chilli rhizosphere soil. These isolates were studied for their production of growth promoting substances such as IAA and GA_3.then the isolates were tested for the efficacy of chilli .From the results, it was observed that the treatment 100% recommended dose of fertilizer along with *Pseudomonas sp* and *Bacillus sp* recorded maximum yield of 26.75tons of green chillies per hectare when compared with absolute control.

***Keywords*:** *Bacillus sp,* GA_3, IAA, *Pseudomonas sp,* rhizobacteria.

Introduction

Vegetables are essential components of human nutrition. Among the vegetables, chilli (*Capsicum annuum* L.) is one of the important commercial crop cultivated 96000 hectares throughout India and India ranks first in world chilli production(Khan and Raj,2006). However, the productivity is lower when compared with other countries due to several factors. One among the factors is nutrient management and chilli require good balanced nutrition for their growth and development. Owing to its high cash value and consumption rate the annual trade of chilli is approximately 17% of total spice trade in the world and is about 33% in India (Ahmed *et al*., 2000). By applying inorganic source of fertilizer only not fulfills the growth and yield of chilli and it requires additional means like bioinoculants that will enrich the soil there by enhance the crop.

Plant growth promoting rhizobacteria (PGPR) is important group of microorganisms that will influence plant growth either directly or indirectly by fixing atmospheric nitrogen or by solubilizing phosphorus or by production of growth promoting substances and by production of siderophores (Berg, 2009). PGPR have been reported to increase growth and yield of many crops rice(Ashrafuzzaman *et al.,*2009), cucumber (Malekia *et al.,*2010), maize(Sandiya *et al.,*2010), cotton(Anjum *et al*., 2007 and banana (Mia *et al.,* 2010). The use of chemical fertilizer and insecticides spoils the soil environment. Taking the above factors, the present study was focused mainly on the effect of PGPR on the growth of chilli and the experiment was carried out in Department of Microbiology, Faculty of Agriculture, Annamalai University, India.

Materials and Methods

Survey and collection of chilli rhizosphere soil samples

A survey was conducted at twelve different locations of cuddalore districts in Tamilnadu, India. Chilli rhizosphere soil samples were collected and then transported to the laboratory carefully. The soil samples were stored in refrigerator at 4 c for future studies.

Enumeration of microorganisms from chilli rhizosphere soil samples

Ten gram of chilli rhizosphere soil samples from twelve locations were taken individually and serially diluted upto 10^{-6} dilutions. From each sample take one ml of sample from 10^{-6}, $10^{-5}$and 10^{-4} dilutions and pour in steile petriplates and pour soil extract agar media, kenknights agar and rose Bengal agar media respectively to enumerate bacteria, actinomycetes and fungi respectively. The plates were kept at room temperature (37^0c) for incubation and then after incubation period enumerated the colonies using colony counter.

Table 1 : Estimation of microbial population in chilli rhizosphere soil.

S. No	Name of the location	Bacteria	Actinomycetes	Fungi
1	Annamalai nagar	12.33	7.33	8.33
2	Panruti	14.33	5.66	8.66
3	Sethiyathope	11.66	5.33	7.66
4	Kurinjipadi	9.00	6.66	10.66
5	lalpettai	10.66	3.66	9.33
6	Puthuchatiram	15.66	10.33	6.33
7	Mutulur	7.33	4.66	7.00
8	Cuddalore	9.66	8.66	9.66
9	kattumanarkoil	10.66	7.66	6.00
10	Kumaratchi	11.66	4.33	11.66
11	Anakarai	11.66	5.66	8.66
12	Bhuvanagiri	12.33	6.66	9.33
	SEd	0.412	0.302	0.289
	CD(P=0.05)	0.897	0.698	0.512

Table 2: Enumeration of *Pseudomonas sp* and *Bacillus sp* in chilli rhizosphere soil.

S. No	Location	*Pseudomonas sp*	Bacillus sp
1	Annamalainagar	7.66	6.66
2	Panruti	6.66	5.33
3	Sethiyathope	7.33	9.33
4	kurinjipadi	8.66	6.00
5	Lalpettai	8.33	7.33
6	Puthuchatiram	10.33	6.66
7	Mutulur	8.00	8.66
8	Cuddalore	8.66	7.33
9	Kattumanarkoil	8.33	6.66
10	Kumaratchi	7.33	6.66
11	Anakarai	8.66	6.00
12	Bhuvanagiri	5.33	7.66
SEd		0.454	0.512
CD(p=0.05)		0.916	1.064

Enumeration of *Pseudomonas sp and Bacillus sp* population from chilli rhizosphere soil

Pseudomonas sp and *Bacillus sp* population of twelve rhizosphere samples were enumerated using serial dilution method. The soil samples were serially diluted up to 10^{-4} dilution and from dilution 10^{-4} one ml of samples were taken separately for the isolation of *Pseudomonas sp* and *Bacillus sp* then pour kings B and nutrient agar medium respectively. After incubation, enumerate the colonies using colony counter.

Estimation of indole acetic acid(IAA) production

A quantity of 100ml broth of Kings B and Nutrient medium were prepared for *Pseudomonas* and *Bacillus* respectively. Then add L-tryptophan was added to each flask to a final concentration of 100 mg l^{-1}.One ml of culture broth containing 108 cells ml^{-1} of plant growth promoting rhizobacterial isolates were inoculated to each flask and incubated at 37^{o}Cin dark for seven days at stationary condition. After incubation, the cultures were centrifuged at 6000rpm for 5min to remove the bacterial cells. The supernatant was brought to pH 2.8 with 1 N HCl. Fifteen ml of the acidified supernatant was taken in 100ml conical flask and to it equal volume of diethylether was added and incubated in dark for 4 hrs. IAA was done at 4^{o} C in a separating funnel using diethylether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, 2 ml of methanol extract was determined using the method of Gorden and Paleg (1957). The intensity of pink color developed was read at 535 nm in a spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity in the culture filtrate was determined and expressed as g ml^{-1} of culture medium.

Table 3: Estimation of Indole acetic acid(IAA)

S.No	Name of the Isolate	IAA g ml^{-1}
1	PS-1	76.11
2	PS-2	71.49
3	PS-3	78.05
4	PS-4	92.15
5	PS-5	68.87
6	PS-6	34.97
7	BS-1	66.65
8	*BS-2*	*70.54*
9	BS-3	76.98
10	BS-4	65.75
11	Bs-5	72.32
12	BS-6	73.78
	SEd	2.122
	CD(p=0.05)	4.325

Estimation of GA_3 Production

Culture suspensions of *Pseudomonas sp* and *Bacillus sp* were taken and added in nutrient broth separetly for each organisms and incubated for five days. Then the broths were centrifuged at 10000 rpm for 20 minutes and the p^{H} was adjusted to 2.5 using 0.5% HCl. The filterates were extracted with ethyl acetate (1:3 of filterate to solvent ratio) and the extracts were used for ctetheir gibberellic acid determinations. The experiments were conducted in triplicates. In this assay gibberellic acid is converted into gibberellenic acid and estimated at 245 nm absorbance (Pandya and Desai 2014).

Table 4: Estimation of GA_3 production

S.No	Name of the Isolate	GA_3 µg ml^{-1}
1	PS-1	24.78
2	PS-2	17.45
3	PS-3	23.89
4	PS-4	31.65
5	PS-5	17.43
6	PS-6	25.22
7	BS-1	30.24
8	BS-2	26.75
9	BS-3	30.13
10	BS-4	31.21
11	BS-5	35.74
12	BS-6	24.87
SEd		1.421
CD(p=0.05)		2.912

Table 5: Effect of *Pseudomonas sp* and *Bacillus* sp on the growth and yield of chilli

S. No	Treatments	Green Chilli yield t/ha
1	Control	12.83
2	100%N P K	22.67
3	100%N P K+ *Pseudomonas sp*	23.00
4	100%N P K +*Bacillus sp*	23.12
5	100%N P K +*Pseudomonas sp* +*Bacillus sp*	26.75
	SEd	1.326
	CD(P=0.05)	2.674

Result and Discussion

Totally twelve rhizosphere soils of chilli were collected from cuddalore district, Tamilnadu (Table 1) and the results revealed that the bacterial population ranged from 7.33 to $14.33x10^{6}$ cfu g^{-1} of soil where Panruti chilli rhizosphere soil recorded maximum of 14. $33x10^{6}$ cfu g^{-1} of soil. The

actinomycepes population ranged from 3.66 to 10.33x10^5 cfu g^{-1} of soil and Puthuchaitram rhizosphere soil recorded the highest population. The fungal population ranged from 6 to 11.66 x10^4 cfu g^{-1} of soil. The table 2 revealed the population of *Pseudomonas* sp and *Bacillus sp.* The Pseudomonas population ranged from 5.33 to 10.33 x10^4 cfu g^{-1} of soil. The location Puthuchitriam recorded the highest population.

The bacillus population was ranged from 5.33 to 9.33 x10^4 cfu g^{-1} of soil and the location Sethiyathope observed maximum population. Then totally 12 isolates were taken for further studies out of which six from *Pseudomonas sp* and six from *Bacillus sp*. The *Pseudomonas sp* were designated as PS-1 to PS-6 and the *Bacillus sp* were designated as BS-1 to BS-6. These isolates were studied for their efficacy to produce IAA and GA_3. Among the isolates ,the isolate PS-4 produce the maximum amount of IAA of 92.15 µgml-1 of broth. all the isolates showed IAA production and it ranged from 34.97 to 92.18 µgml^{-1} of broth(Table 3). The similar result was observed by Berg(2009)and reported that *Pseudomonas sp produce* IAA 68.73 µgml^{-1} of broth. Among the isolates the GA3 was produced maximum by the isolate BS-5(35.75 µg/ml^{-1}) all the isolates produce GA3 and ranged from 17.43 to 35.75 µgml^{-1}.

A pot culture study was carried out to study the effect of PGPR isolates namely *Pseudomonas sp* and *Bacillus sp* on the growth and yield of chilli. The experiment was performed along with 100% recommended dose of fertilizer. The addition of co-inoculum of *Pseudomonas sp* and *Bacillus sp* along with 100% NPK recorded the highest yield of 26.75 tons of green chilli per hectare whereas 100%NPK recommended dose of fertilizer yields only 22.67 tons of green chilli per hectare. The use of PGPR inoculum namely *Pseudomonas sp* and *Bacillus sp* increases the green chilli yield up to 13%. Vessey(2003) reported that use of rhizobacteria will increase the green chilli yield upto 15% when compared with absolute control.

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