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Biocontrol of Fusarium Wilt Disease of Cucumber (*Cucumis sativus* L.) in Greenhouse and Field

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Abstract The bacteria present around the rhizosphere, suppress diseases and promotes plant growth are known as plant growth promoting rhizobacteria (PGPR). PGPR are ecofriendly, stimulate the plant growth factors and reduce the incidence of crop diseases as well as supply the nutrients for growth of plants. 13 PGPR strains, 9 were isolated from cucurbit rhizosphere from fields, three from MTCC assessed for positive rapid screening bioassay were used for the seed priming and further tests. The potting mixture was sterilized for 1h at 121^0C at 15-18 psi for two consecutive days before use. Both the soil samples were checked for their micronutrient content, conductivity, water holding capacity and also important macronutrients. The soil samples were loamy with good water holding capacity, with adequate macronutrients (NPK) and micronutrients. In the field tests and greenhouse, the treated seeds of *Cucumis sativus* were compared for their vine length, node number of first female flower, fruits number/ plant,fruit weight in grams, diameter of the fruit in centimeter, total yield / 6 fruits in gram and growth conditions to the control. In 13 PGPR strains tested only *Bacillus amyloliquefaciens* MIC6 and *Pseudomonas aeruginosa* MTCC2581 showed maximum germination and yield manages *Fusarium* wilt both in greenhouse as well as in field conditions.

Keywords: PGPR, soil, growth, yield

Introduction

In sustainable agriculture soil borne pathogens can be controlled by biological agents like plant growth promoting rhizobacteria (PGPR) as they colonize around host root system and create competition for space as well as nutrition. PGPR are ecofriendly, stimulate the plant growth factors and reduce incidence of crop diseases, as well as supply the nutrition for growth of the plant. To minimize the use of chemicals, synthetic fertilizers and pesticides, sustainable agriculture has been in practice for the past three decades as an

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ecofriendly concept. In modern agriculture PGPR is being used as biofertilizer as well as for biological control of certain seed and soil-borne plant pathogens.

Pseudomonas cepacia, *P. fluorescens*, *Bacillus polymyxa* and *B. subtilis* were used for the biocontrol of *Fusarium* wilt of melon. Among these *P. fluorescens* has shown highest reduction in *Fusarium* wilt incidence and good antagonistic activity (Hamed *et al.*, 2009). Seventy-eight PGPR isolates were tested *in-vitro* in greenhouse against *F. oxysporum*, which causes root and crown rot of sorghum. *Bacillus* sp. KBE2-5, *B. stearothermophilus* KBE5-7, *B. cereus* KBE5-1 and *B. cereus* NAE5-5 suppressed the disease through root colonization. *F. oxysporum* was managed by PGPR (Idris *et al.*, 2007). Bafti *et al.* (2005) reported that *Streptomyces olivaceus* strain 115 antagonised *F. oxysporum* f. sp. *melonis* causing root rot and *Fusarium* wilt of cucurbits in Kerman Province, Iran. Soil treated with *S. olivaceus* in a greenhouse reduced wilt compared to control.

In case of pumpkins, insufficient NPK availability results in weak vegetative growth, poor fruit setting, low fruit quality and decreased nutritional quality (Oloyede, 2012). The external supply of bacterial inoculants as bio-fertilizers and bio-control agents increased the nutritional capacity of the soil and improved the plant growth and yield. PGPR replenished the micronutrients to the barren land and also antagonised the plant pathogen by producing various types of antimicrobials (Glick, 2012). The aim of the present study was to know the better biocontrol agents which increase the micronutrients of the soil and infer *Fusarium* disease resistance to the cucumber plants.

Materials and methods

Microorganisms used

Nine PGPR isolates (*Sarratia* sp. MIC1, *Pseudomonas aeruginosa* MIC2, *Bacillus cereus* MIC3, *B. subtilis* MIC4, *B. cereus* MIC5, *B. amyloliquefaciens* MIC6, *B. cereus* MIC7, *B. licheniformis* MIC9 and *B. subtilis* MIC10) were isolated from the rhizosphere of healthy Cucurbitaceae field soils were collected from the different districts in Karnataka (Mysore, Mandya, Hassan and Haveri). The collected soils were maintained in the culture collection of the Department of Studies in Microbiology, Manasagangothri, Mysore. Three standard PGPR strains were obtained from Microbial Type Culture Collection (MTCC) Chandigarh, India and these included *Pseudomonas aeruginosa* MTCC2581, *Bacillus coagulans* MTCC3543 and *B. subtilis* MTCC2763; these were used as positive controls. Similarly, bavistin and Mefenoxam + Chlorothalonil also used as a positive control and water as

negative control. *Fusarium* spp. were isolated from Cucurbitaceae growing field soil, and grown on potato dextrose agar (PDA), incubated at 25^0 C for 5-6 days.

In-vivo evaluation of PGPR

All PGPR strains were grown in nutrient broth for 48 h and centrifuged at 8000 rpm for 5 min to obtain a pellet, that was then washed with sterile distilled water. PGPR suspensions (O.D.-10^8 cfu/ml) and test fungicides along with adhesive carboxymethyl cellulose (CMC) were used to treat surface sterilized cucurbit seeds for 6 h on a rotary shaker at 150 rpm. Seeds soaked in distilled water amended with CMC (0.1g/10 ml) served as control (Ramamoorthy *et al.,* 2002). The seeds were then drained overnight before subjecting to a germination test by paper towel method (ISTA, 2005). Seedling vigour was calculated as per Abdul-Baki and Anderson (1973). Each rhizobacterial isolate was maintained in triplicate and all experiments were replicated three times.

Greenhouse and field tests

For conducting greenhouse experiments, field soil sample was collected, sieved and blended with peat and cow dung 2:1:1 (v/v/v). The potting mixture was sterilized for 1 h at 121^0C at 15-18 psi for two consecutive days before use. The tests were performed in fields at the Department of Studies in Biotechnology, University of Mysore, Mysore with required plots dimensions of 6×6×4.5 meter (with 2 hills and 20 seeds per lane). Cucurbit seeds were soaked with the PGPR isolates used for the test along with positive control (bavistin) and negative control (sterile water). The seeds were inoculated with PGPR inoculum adjusted to optical density of 10^{-8}/ml. Natural manure was supplied twice (seedling and flowering stages) and urea was provided as nitrogen source (Zhao *et al.,* 2011).

Chlorophyll

Total chlorophyll content was determined following the method of Ashraf and Iram (2005). Fresh leaves were cut into 0.05 g segments and extracted overnight with 80% acetone at -10^0 C. The extract was centrifuged at 14,000 g for 5 min and the absorbance of the supernatant was read at 645 and 663nm using a spectrophotometer (Thermo Biomate 3S™). Total chlorophyll was calculated using the following formula:

Total chlorophyll (µg/ml) = [20.2 (A_{645}) + 8.02 (A_{663}) × VW] /1000
Where, A= Absorbance at the given wavelength
V= Final volume of the chlorophyll in 80% acetone
W= Weight of the fresh cucumber leaves

Growth characters of cucumber

The growth characters of vein length, first female flower opening day, node number of first female flower, days until first fruit harvest, number of fruits, fruit weight, fruit diameter and fruit length were determined as reported by Tiwari *et al.* (2009).

Statistical analysis

All data from laboratory and greenhouse experiments were analyzed separately for each experiment and were subjected to arcsine transformation and analysis of variance (ANOVA) (SPSS, version 16). Significant effects of treatments were determined by the F values ($P \leq 0.05$). Treatment means were separated using Turkey's HSD test.

Results

Soil

The field and greenhouse soil samples were loamy with pH range of 6.2-6.5. The macro and micronutrient content of greenhouse and field soil samples are listed in Table 1. The NPK requirement varies with the basic nutrient content of soil. On the basis of nutrient requirement for cucurbits additional nutritional supplements were provided. In the greenhouse tests, most of the treated plants showed increased vigour compared to the control plants (Table 2). No differences in the germination percent were observed in greenhouse plants. However, *P. aeruginosa* MTCC2851, *P. aeruginosa* MIC2, *B. amyloliquefaciens* MIC6 and *B. licheniformis* MIC9 treated plants showed higher vigour. The plants in the greenhouse grew higher than the control. In case of field percent germination was minimum, except *P. aeruginosa* MIC2, *B. subtilis* MIC4 and *P. aeruginosa* MTCC2581 treated plants gave maximum yield compared to untreated plants in the field (Table 3).

In-vivo evaluation

The 13 treated seed samples, primed samples show increase in germination but had higher vigour index as compared to control. *B. amyloliquefaciens* MIC6 and *P. aeruginosa* MTCC2581 treated cucumber plants showed higher germination and vigour index (Fig.1). Carbendazim and Mefenoxam + Chlorothalonil treatment resulted in maximum germination and vigour.

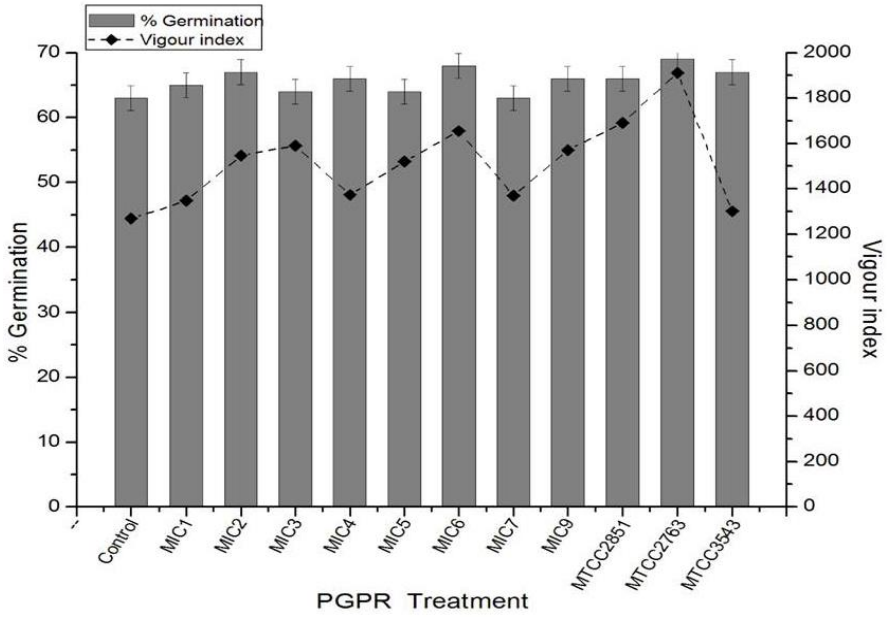

Figure 1. Effect of PGPR on seed germination and seedling vigour of the cucurbit after 8 days of germination.

Growth characters and yield

In the field studies primed cucurbit seeds showed good growth compared to the control. Among the treatments the vein length was maximum with *Pseudomonas aeruginosa* MIC2 and *Bacillus cereus* MIC7 treated plants. The first female flower appeared in 41 days and 46 days in *B. cereus* MIC5 and *P. aeruginosa* MIC2 primed seeds respectively and these were ahead of those appearing in control. Fruit weight, length and total yield were more in case of *B. subtilis* MIC10 followed by *B. cereus* MIC7 treated as compared to the control (Table 4).

Table 1. Physical and chemical properties of experimental field and greenhouse soil.

	RH	P^H	EC	N	K	P	Ca	Mg	S	Na	Cl	Cu	Zn	Mn	Fe	B	Mo
	%		$dS\ m^{-1}$	%	%	%	%	%	%	%	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
FS	7-8	6.2	1.7	5.2	3.5	0.35	0.12	0.12	0.6	0.36	0.82	6.2	34	51	158	38	0.5
GH	4-5	6.5	2.2	4.83	2.3	0.23	0.19	0.19	0.4	0.32	0.73	4.96	22	28	156	35	0.6

Note : FS-Field soil, GH - Greenhouse soil. EC – Electric conductivity

Table 2. Greenhouse treatment of Cucurbits with the plant growth promoting rhizobacteria against the *Fusarium oxysporum* f. sp. *cucumerianum*

Isolates	MRL (cm)	MSL (cm)	Germination (%)	Vigour index	Fresh weight (g/seedling)	Dry weight (g/ seedling)
MIC1	10.18 ± 0.50^{ef}	10.00 ± 0.20^{de}	65 ± 1.15^{abc}	1324 ± 28.34^{de}	0.394 ± 0.059^{c}	0.052 ± 0.034
MIC2	12.16 ± 0.76^{cd}	14.18 ± 0.38^{a}	63 ± 0.57^{abc}	1676 ± 28.68^{b}	0.381 ± 0.067^{c}	0.034 ± 0.008
MIC3	14.11 ± 0.24^{b}	10.14 ± 0.25^{cde}	60 ± 1.15^{c}	1470 ± 14.83^{cd}	0.538 ± 0.064^{bc}	0.052 ± 0.004
MIC4	11.16 ± 0.78^{de}	9.12 ± 0.09^{def}	67 ± 1.00^{ab}	1358 ± 59.43^{de}	0.838 ± 0.112^{ab}	0.063 ± 0.011
MIC5	13.18 ± 0.24^{bc}	10.10 ± 1.70^{de}	64 ± 4.50^{abc}	1491 ± 20.43^{cd}	0.387 ± 0.063^{c}	0.027 ± 0.007
MIC6	12.17 ± 0.42^{cd}	11.02 ± 0.20^{bcd}	67 ± 1.15^{ab}	1561 ± 66.02^{bc}	0.640 ± 0.180^{abc}	0.038 ± 0.015
MIC7	11.02 ± 0.20^{de}	9.31 ± 0.61^{def}	60 ± 0.57^{c}	1233 ± 15.81^{e}	0.797 ± 0.180^{ab}	0.050 ± 0.021
MIC9	13.88 ± 0.92^{bc}	11.21 ± 0.41^{bcd}	61 ± 1.52^{c}	1546 ± 29.87^{bc}	0.790 ± 0.110^{ab}	0.054 ± 0.021
MIC10	11.14 ± 0.24^{de}	12.24 ± 0.72^{abc}	63 ± 1.00^{bc}	1472 ± 52.48^{cd}	0.780 ± 0.110^{ab}	0.052 ± 0.005
MTCC2581	16.16 ± 0.90^{a}	12.41 ± 0.91^{ab}	68 ± 1.52^{a}	1951 ± 66.99^{a}	0.872 ± 0.052^{a}	0.061 ± 0.002
MTCC2763	10.96 ± 0.82^{de}	8.61 ± 0.81^{ef}	62 ± 2.51^{bc}	1220 ± 126.96^{e}	0.660 ± 0.080^{abc}	0.046 ± 0.010
MTCC3543	11.02 ± 0.20^{de}	9.11 ± 0.81^{def}	63 ± 1.00^{bc}	1267 ± 33.46^{e}	0.430 ± 0.080^{c}	0.021 ± 0.006
Negative control	9.19 ± 0.22^{f}	7.56 ± 0.68^{f}	61 ± 0.57^{c}	1033 ± 63.88^{f}	0.340 ± 0.130^{c}	0.015 ± 0.006
Positive control	10.47 ± 0.73^{def}	9.50 ± 0.53^{def}	64 ± 0.62^{abc}	1284 ± 71.57^{e}	0.790 ± 0.140^{ab}	0.172 ± 0.206

MSL = mean shoot length, MRL = mean root length, Vigour index = (MSL+MRL)*% of germination. Values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$. Note*: Sarratia* sp. MIC1, *Pseudomonas aeruginosa* MIC2, *B. cereus* MIC3, *B. subtilis* MIC4, *B. cereus* MIC5, *B. amyloliquefaciens* MIC6, *B. cereus* MIC7, *B. licheniformis* MIC9, *B. subtilis* MIC10, *P. aeruginosa* MTCC2581, *B. coagulans* MTCC3543 and *B. subtilis* MTCC2763

Table 3. Field tests with primed Cucurbits with the plant growth promoting rhizobacteria against the *Fusarium oxysporum* f. sp. *cucumerianum*

Isolates	MRL (cm)	MSL (cm)	Germination (%)	Vigour index	Fresh weight (g/s)	Dry weight (g/s)	Chlorophyll (mg/g)
MIC1	4.96 ± 0.14^{g}	10.67 ± 0.35^{defg}	66 ± 2.00^{b}	1031 ± 26.79^{ef}	5.75 ± 0.007^{def}	0.80 ± 0.051^{cd}	7.80 ± 1.20^{abc}
MIC2	10.32 ± 0.23^{bc}	9.34 ± 0.06^{fgh}	72 ± 2.00^{ab}	1415 ± 36.21^{bc}	8.17 ± 0.210^{b}	0.95 ± 0.039^{abc}	9.84 ± 0.58^{a}
MIC3	11.65 ± 1.22^{ab}	9.21 ± 0.09^{gh}	65 ± 3.51^{b}	1364 ± 140.56^{bcd}	4.62 ± 0.747^{fg}	0.68 ± 0.020^{cde}	7.94 ± 0.60^{ab}
MIC4	10.10 ± 0.40^{bc}	14.12 ± 1.07^{a}	63 ± 2.08^{b}	1532 ± 70.95^{b}	7.19 ± 0.299^{bcd}	0.89 ± 0.096^{bc}	7.99 ± 0.39^{ab}
MIC5	7.21 ± 0.81^{e}	11.38 ± 0.04^{bcde}	70 ± 3.0^{b}	1302 ± 98.37^{bcd}	6.41 ± 0.550^{cde}	0.73 ± 0.046^{cde}	7.43 ± 1.12^{abc}
MIC6	7.48 ± 0.54^{e}	11.25 ± 0.80^{cde}	63 ± 3.05^{b}	1184 ± 63.94^{cde}	7.07 ± 0.205^{bcde}	0.87 ± 0.022^{bc}	8.57 ± 0.45^{ab}
MIC7	4.96 ± 0.16^{g}	6.35 ± 0.47^{i}	65 ± 1.52^{ab}	739 ± 37.17^{g}	2.39 ± 0.415^{h}	0.38 ± 0.096^{e}	6.36 ± 1.23^{bc}
MIC9	5.46 ± 0.49^{fg}	11.12 ± 0.79^{cdef}	71 ± 3.21^{ab}	1182 ± 54.21^{cde}	7.38 ± 0.474^{bc}	0.90 ± 0.007^{abc}	8.66 ± 0.63^{ab}
MIC10	6.37 ± 1.14^{efg}	11.62 ± 0.71^{bcd}	63 ± 3.51^{b}	1147 ± 159.37^{def}	7.04 ± 0.220^{bcde}	0.77 ± 0.770^{cde}	7.39 ± 0.59^{abc}
MTCC2581	9.23 ± 0.11^{cd}	11.56 ± 1.22^{bcd}	71 ± 3.05^{ab}	1489 ± 94.46^{b}	11.26 ± 0.715^{a}	1.23 ± 0.249^{ab}	8.40 ± 1.47^{ab}
MTCC2763	7.80 ± 0.40^{de}	9.67 ± 0.35^{efgh}	73 ± 10.58^{ab}	1272 ± 154.82^{bcde}	4.67 ± 0.712^{fg}	0.61 ± 0.186^{cde}	8.57 ± 0.79^{ab}
MTCC3543	6.86 ± 0.39^{ef}	13.12 ± 0.31^{ab}	68 ± 2.64^{b}	1358 ± 63.65^{bcd}	5.68 ± 0.040^{ef}	0.64 ± 0.134^{cde}	7.98 ± 0.70^{ab}
Negative control	6.08 ± 0.13^{efg}	8.33 ± 0.42^{h}	63 ± 1.52^{b}	913 ± 56.72^{fg}	3.87 ± 0.410^{g}	0.45 ± 0.660^{de}	5.33 ± 0.59^{c}
Positive control	13.10 ± 0.30^{a}	12.56 ± 0.38^{abc}	82 ± 0.57^{a}	2112 ± 20.80^{a}	10.28 ± 0.788^{a}	1.31 ± 0.338^{a}	9.85 ± 0.73^{a}

MSL = mean shoot length, MRL = mean root length, Vigour index = (MSL+MRL)*% of germination. Values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Note*: Sarratia* sp. MIC1, *P. aeruginosa* MIC2, *B. cereus* MIC3, *B. subtilis* MIC4, *B. cereus* MIC5, *B. amyloliquefaciens* MIC6, *B. cereus* MIC7, *B. licheniformis* MIC9, *B. subtilis* MIC10, *P. aeruginosa* MTCC2581, *B. coagulans* MTCC3543 and *B. subtilis* MTCC2763

Table 4. Determination of different quantitative characters in treated Cucumber seeds.

	Vine length in cm	Days to 1st female flower open	Node no. of first female flower	Days to first fruit harvest	Fruits No. /Plant	Fruit weight in g	Fruit length in cm	Diameter of fruit in cm	Total yield /6 fruits in g
MIC1	53.34 ± 0.53^{d}	50.00±1.15^{b}	5.57±0.29^{bcd}	57.66±0.66^{bcd}	6.00±0.57^{defg}	187.09±6.43^{f}	15.93±0.50^{b}	4.23±0.05^{f}	1219.96±14.00^{d}
MIC2	60.73 ±0.37^{b}	46.33±0.88^{d}	5.26±0.63^{cd}	60.00±0.57^{abc}	7.66±0.88^{abc}	246.00±6.08^{cd}	18.23±1.91^{ab}	5.39±0.08^{a}	1447.96±32.34^{bc}
MIC3	55.28 ±0.47^{cd}	49.66±0.88^{bc}	5.58±0.09^{bcd}	63.00±0.57^{a}	5.66±0.66^{fg}	176.30±5.99^{f}	17.23±0.96^{b}	4.98±0.07^{bcde}	1155.96±29.98^{a}
MIC4	42.43 ± 0.39^{g}	47.33±0.57^{bcd}	7.32±0.06^{a}	56.66±0.88^{d}	8.33±0.88^{abcd}	211.86±5.78^{e}	16.23±0.64^{b}	5.03±0.03^{abcd}	1285.20±31.45^{ef}
MIC5	56.91 ± 0.37^{c}	41.63±0.83^{e}	6.11±0.06^{abc}	57.33±0.88^{bcd}	5.00±0.57^{efg}	250.60±3.09^{bcd}	17.59±0.82^{b}	5.20±0.05^{abc}	1480.63±11.22^{d}
MIC6	48.62 ± 0.59^{e}	56.00 ± 0.57^{a}	6.26±0.40^{abc}	50.66±0.88^{e}	5.66±0.88^{cdef}	243.56±5.43^{cd}	18.11±1.06^{ab}	4.87±0.06^{cde}	1428.20±13.56^{g}
MIC7	65.06 ± 0.50^{a}	50.33±0.88^{b}	6.58±0.22^{ab}	57.00±0.57^{cd}	6.00±0.57^{efg}	273.13±6.32^{b}	19.46±0.58^{ab}	4.90±0.03^{bcde}	1713.40±27.71^{e}
MIC9	37.55 ± 0.67^{h}	55.00±0.57^{a}	5.21±0.21^{cd}	59.33±0.33^{bcd}	8.00±0.57^{bcde}	231.30±4.10^{de}	18.15±0.59^{ab}	4.91±0.03^{bcde}	1478.93±43.21^{cd}
MIC10	50.39 ± 0.48^{e}	55.33±0.33^{a}	4.45±0.16^{d}	57.66±0.33^{bcd}	6.33±0.88^{efg}	311.06±1.80^{a}	22.35±1.74^{a}	4.89±0.04^{cde}	1849.93±32.45^{cd}
MTCC2581	53.64 ± 0.48^{d}	48.66±2.1^{cd}	7.24±0.12^{a}	51.66±0.33^{e}	8.33±0.88^{bcde}	231.10±4.21^{de}	19.35±0.47^{ab}	5.27±0.02^{ab}	1462.53±18.76^{fg}
MTCC2763	46.14±0.25^{f}	49.65±0.33^{bcd}	6.23±0.05^{abc}	58.66±0.54^{bcd}	6.66±0.66^{efg}	250.06±0.86^{cd}	16.16±0.60^{b}	4.95±0.08^{bcde}	1556.70±28.53^{cd}
MTCC3543	43.04±0.43^{g}	50.00±3.1^{d}	6.34±0.08^{abc}	58.00±0.57^{bcd}	9.00±0.57^{a}	244.66±0.99^{cd}	15.89±0.34^{b}	4.77±0.10^{de}	1484.16±9.44^{cd}
Negative Control	33.81± 0.11^{i}	52.00±4.2b^{cd}	6.15±0.08^{abc}	57.66±0.88^{bcd}	4.00±0.57^{g}	175.63±1.78^{f}	16.90±0.39^{b}	4.62±013^{e}	1064.90±16.03^{cd}
Positive Control	59.81 ±0.66^{b}	49.00±0.33^{de}	7.04±0.04^{a}	60.33±0.33^{ab}	9.00±0.57^{ab}	265.36±0.91^{bc}	19.25±0.56^{ab}	5.11±0.08^{abcd}	1586.73±5.74^{b}

Values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$.
Note*: Sarratia* sp. MIC1, *P. aeruginosa* MIC2, *B. cereus* MIC3, *B. subtilis* MIC4, *B. cereus* MIC5, *B. amyloliquefaciens* MIC6, *B.cereus* MIC7, *B. licheniformis* MIC9, *B. subtilis* MIC10, *P. aeruginosa* MTCC2581, *B. coagulans* MTCC3543 and *B. subtilis* MTCC2763

Discussion

The micronutrients analysed from the field soil and greenhouse soil showed maximum amount of all nutrients present. Seventeen essential elements are required for optimal growth and development, of crop plants. These minerals, when required in relatively high amounts are considered as macronutrients and in trace amounts they are micronutrients. If any element is lacking in the soil or not adequately balanced with other nutrients, growth suppression or even complete inhibition is evident. Micronutrients often act as cofactors in enzyme systems and participate in redox reactions, in addition they have several other vital functions in plants and their deficiency can decrease the yield (Farooq *et al.,* 2012). Inorganic and organic forms of micronutrients have the potential to increase their concentrations in grain. The most effective way of fertilization could be via soil, through foliar and by adding fertilizers to the irrigation water. Care should be taken not to over fertilize crops with micronutrients because of consequent toxicity, losses in quality and quantity in grain yield (Rengela *et al.,* 1999).

The soil samples are loamy with good water holding capacity, with macronutrient NPK content of 5.2-0.35% in case of field soil and 4.83-0.23% in case of greenhouse soil. The micronutrients ranged from 0.9-0.1% in both field and greenhouse soils. Similar results were obtained in case of field and greenhouse by adding compost manure to soil, which also increases the water holding capacity of soil, executes microbial activity and by improves soil structure and ultimately enhances the yield of the plant (Nair and Ngouajio, 2010). The effect of CCF (cell-free culture filtrate) on, radial growth and microsclerotial production of *Macrophomina phaseolina* varied with the concentration significantly reduced fungal biomass at all the three concentrations. At the concentration of 50% all three pseudomonad strains reduced radial growth (Das *et al.,* 2012).

Farooq *et al.* (2012) observed that micronutrients were essential components for plant growth and human health. Priming or coating of seeds with micronutrients improves crop yield and grain nutrient content. Soil and foliar applications are most prevalent methods of micronutrient addition but the cost involved and difficulty in obtaining high quality micronutrients were constraints. Being an easy and cost effective method of micronutrient application, seed treatment is proposed as an attractive option for poor farmers in developing countries. Microbes promote plant nutrients and reduce the need for chemical fertilizers as well as increase N, P and K uptake by plants. The phosphate availability in soil can be increased by mineralization of organic or inorganic phosphate. In case of pumpkin insufficient NPK availability results in weak vegetative growth, poor fruit setting, lower fruit quality and decreased nutritional quality (Oloyede, 2012). The most effective fertilization could be through soil, foliar and by adding fertilizers to the irrigation water. Care should be taken not to over

fertilize crops with micronutrients because of consequent toxicity, losses in quality and quantity of grain yield (Rengela *et al.,* 1999). Efficiency of bacterial inoculants increases the soil nutritional condition, of bacterial inoculation has much stimulatory effect in nutrition deficient soil (Egamberdiyeva, 2007). In our study the soil samples field samples show N-5.2, P-3.5, K-0.35 (NPK) provides with the micronutrient required to the growth of the cucurbit plants and increases the yield.

The 436 rhizobacterial isolates were screened against *Phytophthora* blight of pepper (*Phytophthora capsici*) by root-dip method, selected for radical and seedling assay for field tests. These bacterial suspensions of five strains were saturated with *Phytophthora* blight infected pepper plants by artificial pathogen inoculation, reduced the disease in both tests. Before transplanting in the field with natural inoculation managed the pepper plants against *P. capsici.* Increased pepper fruit yield and strain-treated roots had less infection rates by *P. capsici* (Sang *et al.*, 2008).

In the seed samples tested only *B. amyloliquefaciens* MIC6 and *P. aeruginosa* MTCC2581 showed maximum germination and vigour which managed the *Fusarium* wilt both in greenhouse as well as in field in our tests. *Verticillium* wilt is the destructive soil-borne disease in cotton, managed by the use of bio-organic fertilizer (BIO) both in nursery and field. The pathogenic fungi *Alternaria alternate, Coniochaeta velutina* and *Chaetothyriales* sp. were observed in the control were as *Humicola* sp., *Metarhizium anisopliae* and *Chaetomium* sp., were considered to be beneficial fungi found in BIO treatment. In nursery the BIO treatment reduced the wilt causing *V. dahliae* population considerably in rhizosphere (Lang *et al.,* 2012). Economic losses were high due to the extensive spread of wilt caused by *Fusarium* in melon (*Cucumis melo* L.). By using different BIO and different antagonistic microbes wilt was controlled in both pot and field. The treated plants increased the yield both in nursery and in pot soil compared to control. BIO reduced the concentration of colony-forming units (cfu) of pathogen infected plant. The beneficial microbes in the rhizosphere increased were by BIO application (Zhao *et al.,* 2011).

In two different soil types the stimulatory effect of plant growth promoting rhizobacteria was tested shows maximum germination (72),vigour index (1415), fresh weight (8.17g/s), dry weight (0.95g/s) and chlorophyll (9.85 mg/g) content of the cucurbits compared to the positive control. The bacterial strains *P. alcaligenes, B. polymyxa* and *Mycobacterium phlei* MbP18 were examined in two types of soils in Germany. In the pot experiments with calcarcous calcisol soil better plant growth, nitrogen (N), phosphorus (P) and potassium (K) and in loamy sand soil bacterial inoculants stimulated only root growth and NPK uptake by roots (Egamberdiyeva, 2007).

In the world wide Phytophthora blight is the most severe disease caused by *Phytophthora capsici,* which has no single method to control.

PGPR strains were applied as soil drench 1 and 2 weeks after planting and *P. capsici* was applied to squash roots at 3 weeks after planting. PGPR strains lowered the disease severity in greenhouse compared to treated control. PGPR strains applied as 2-, 3, and 4- strain mixtures reduced disease severity. Treatment with T4 + SE56 demonstrated significantly lower levels of disease, indicating either an additive or synergistic effect on disease reduction by mixing PGPR strains. Applied as seed treatment, only PGPR strain1PC-11 reduced Phytophthora blight disease in all trials (Zhang *et al.,* 2010).

The PGPR, *Enterobacter cloacae, Pseudomonas putida* and *Stenotrophomonas maltophilia* used as biofertilizers helped in reducing the costs. The strains a produced IAA from the tryptophan and antifungal metabolites against soil borne pathogens. Among these *E. cloacae* fixed the nitrogen whereas phosphate was solubilised by *P. putida.* The PGPR isolates, *Sphingobacterium canadense* and *Burkholderia phytofirmans* inoculated to two corn line varieties, under greenhouse condition and in the sterilised sand varieties as 39M27 and 39D82. The sterilized sand varieties showed increase in the total dry weight of root and shoot both in green house as well as in the field. The CR7 and CR1 mixtures gave the best results with 39M27 and 39D82 in the sand (Mehnaz *et al.,* 2010). Plant growth promoting effects of *Pseudomonas* sp., and its combined effects with different nutrient uptake (NPK) and yield of wheat (*Triticum aestivum* L.) in both greenhouse and field conditions were tested at the Research farm of agriculture and natural resources research center of Khorasan, Iran. The *Pseudomonas* sp. was checked for the production of Phosphorous and IAA. It increased the plant yield and growth. The *Pseudomonas* sp., treated seeds increased the yield both under greenhouse and field conditions to the extent of 96-80% (Zabihi *et al.,* 2011).

The seed samples tested only *B. amyloliquefaciens* MIC6 and *P. aeruginosa* MTCC2581 showed maximum germination vigour, manages *Fusarium* wilt both in greenhouse as well as in field. Biological fertilizers like *Pseudomonas* sp., and *Pantoea* sp., were increased yield, length, shoot fresh weight and dry weight (Isfahani and Besharati, 2012). *Pseudomonas florescence* treated plants shows increased germination, vigour, plant height, weight and yield in case of *Sclerospora graminicola* infected pearlmillet (Raj *et al.,* 2004).

In our treatment the vein length was maximum with *P. aeruginosa* MIC2 and *B. cereus* MIC7 treated plants. The first female flower appeared in 41 days and 46 days in *B. cereus* MIC5 and *P. aeruginosa* MIC2 primed seeds, respectively, and these were ahead of those appearing in control. Fruit weight, length and total yield were more in case of *B. subtilis* MIC10 followed by *B. cereus* MIC7 treated as compared to the control. *Trichoderma viride* and *P. florescence* shows maximum germination and yield in case of dry root rot (*Macrophomina phaseolina*) infected to

chickpea (Manjunatha *et al.,* 2013). In both summar and rabi seasons mixture of *Trichoderma, Azotobacter* and farm yard manure gives maximum level of vein length, fruit number and yield compared to the controls in cucumber (Anjanappa *et al.,* 2012). The PGPR isolates *Enterobacter cloacae*, *P. putida*, *Sphingobacterium*, *Azospirillum zeae*, *Burkholderia phytofirmans* and *Stenotrophomonas maltophilia* treated to maize, *S. canadense* and *P. putida* shows higher root and shoot length against Fusarium infection (Mehnaz *et al.,* 2010).

In 13 PGPR strains tested against Fusarium wilt of cucumber two of the strains were give positive results with maximum germination and vein length. Both the strains gave maximum yield in case of the field and greenhouse conditions.

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