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PLANT GROWTH PROMOTING RHIZOBACTERIA- IMPACTS ON CAULIFLOWER YIELD AND SOIL HEALTH

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ABSTRACT

The present investigation were conducted for two summer seasons during 2009 and 2010 in the field of Department of Soil science and Water Management, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh to study the effects of integrated nutrient management systems on growth, yield of cauliflower and soil health. The conjoint effect of plant growth promoting rhizobacteria (PGPR) at varying (50%, 75% and 100%) doses N and P fertilizers registered a significant increase in number of non-wrapper leaves, curd diameter, curd depth and curd weight of cauliflower, total microbial counts and available N and P contents of soil. The application of MK_5 isolate at 75% recommended dose of NP fertilizers not only increased the yields of cauliflower by 24% but also saved 31kg N /ha and 8 kg P/ha fertilizers over control (recommended doses of NPK). A significant increase in available N and P contents were also noted by the conjoint application of PGPR and chemical fertilizers. Hence the developed integrated nutrient module can be used for enhanced yields without destructing soil health in mid hills of North Western Himalayas.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR), are considered as efficient microbial competitors in the root zone to enhance plant growth directly and/or indirectly by reducing soil borne pathogens, enhancing efficiency of applied inputs and also helps in degrading xenobiotic compounds. In the context of increasing international concern for food and environmental quality, the use of plant growth-promoting rhizobacteria (PGPR) for reducing chemical inputs in agriculture is a potentially important issue. PGPR have been applied to various crops to enhance seed emergence, growth and crop yield, and only a few isolate have been commercialized. PGPR are also known to produce antibacterial compounds that are effective against certain plant pathogens and pests. PGPR are directly involved in increased uptake of nitrogen, synthe-sis of phytohormones, and solubilization of minerals such as phosphorus and production of siderophores that chelate iron and make it available to the plant root (Bowen and Rovira, 1999).

Cauliflower is grown throughout the year in different agro-climatic zones occupying an area of 2800 ha with annual production of 54,500 million tonnes (National Horticulture Board, nhb, 2010). The high-yielding cauliflower variety has resulted in an increase in cauliflower production but requires large amounts of chemical fertilizers which lead to health hazards and environmental pollution. In order to make cauliflower cultivation sustainable and less dependent on chemi-cal fertilizers, it is important to have effective PGPR isolate that can biologically fix nitrogen, solubilize phosphorus and induce some growth promoting substances like indole acetic acid (IAA) besides acting as biocontrol agents that can contribute to the production of cauliflower.

Therefore, the present investigations were undertaken to screen the PGPR isolates from its natural growing zones with multifarious activities, at various levels of N and P with other recommended package of practices for commercial cultivation of cauliflower.

MATERIALS AND METHODS

Soil and root samples were collected from the rhizosphere of cauliflower plants from three (Hamirpur, Bilaspur and Kangra districts) different naturally growing agro-climatic zones of Himachal Pradesh. The samples were stored at 4°C in the Soil Microbiology Laboratory, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. For isolation of PGPR, one gram of the rhizosphere soil was placed in 9mL of sterilized distilled water under aseptic conditions. The soil suspension was diluted in 10 fold series and the microbial count was determined by the standard pour plate technique (Subba Rao, 1999). After incubation of 24 - 48h, Modified Replica plate technique was used for isolation.

Thereafter, screening of the bacterial isolates for the various plant growth promoting activities like P-solubilization, siderophore formation, HCN concentration, growth on N-free medium, auxin production and antagonism against *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. were performed by adopting the standard methods as given in Table 1. The optimization of growth conditions (physical, chemical and nutritional) of selected bacterial isolates were standardized by conducting separate experiments.

Out of 30 isolates, 5 efficient isolates designated as MK_2, MK_4, MK_5, MK_7 and MK_9 were selected and characterized after successful experiments under *in vitro* and net house conditions. On the basis their multifarious plant growth promoting activities, growth and yield attributes under controlled conditions (growth chamber and net house), three isolates (MK_5, MK_7 and MK_9) were selected for field experiments along with varying (50%, 75% and 100%) doses of N and P fertilizers. The experiment was conducted during summers of 2009 and 2010 at Nauni, Solan of Himachal Pradesh. Seeds of recommended variety were treated with bacterial inoculum for 8h and untreated seeds were treated with sterilized water for same time and designated as control. Bacterial cell suspension (O.D. 1.00 at 540nm) of 72h old culture grown in nutrient broth at the rate of 10 per cent was used as inoculum for field experimentation. The seeds were sown in nursery and one month old seedlings were transplanted in the field at the spacing 60 × 45cm. The treatments combinations viz.:T1 (Control), T2 (MK5 + 50%NP), T3 (MK5 + 75%NP), T4 (MK5 + 100%NP), T5 (MK7 + 50%NP), T6 (MK7 + 75%NP), T7 (MK7 + 100%NP), T8 (MK9 + 50%NP), T9 (MK9 + 75%NP) and T10 (MK9 + 100%NP) were arranged in RBD design and replicated thrice. The sources of nitrogen and phosphorus were CAN (25% N) and SSP (16% P_2O_5) respectively. All phosphorus fertilizers were applied at the time of transplanting of seedlings and nitrogen fertilizer was applied in three split applications up to the curd formation stages. A booster dose of bacterial culture was added at every one month interval till harvesting. Weeding was done manually and the crop was irrigated when required.

Physico – chemical properties viz. pH, EC, organic matter (OM) and bulk density of soil sample was determined before start of the experiment and after the termination of the experiment and was determined as per the standard method adopted by Jackson (1973) while, available N, available P, available K was determined by the methods adopted by Subbiah and Asija (1956), Olsen et al., 1954 and Merwin and Peech (1951) respectively. The observations were recorded on different quantitative characters of cauliflower viz. (number of non-wrapper leaves, curd diameter, curd depth and curd weight and curd yield). Five plant samples at the time of harvest were also randomly collected from each plot and mixed separately to determine concentrations of N, P and K at harvest using procedure described by Jackson (1973). Statistical analysis was performed as per the design suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

On the basis of morphological, physiological and biochemical characteristics isolates were presumptively identified as *Bacillus* spp. All the five isolates were P-solubilizers, nitrogen fixers, auxin and siderophore producers. And only three (MK_5, MK_7 and MK_9) isolates were HCN producer and were also able to show antagonism towards *Rhizoctonia solani, Pythium* spp. and *Fusarium* spp. A large group of researchers gave evidences to suggest that PGPR enhance the growth and crop yield, and known to produce antibacterial compounds that are effective against certain plant pathogens and pests (Kloepper *et al*., 2004). In our studies, bacterial isolate (MK_5) showed maximum antagonistic activity against the three tested fungal pathogens and all five isolates were able to solubilize phosphate in the rhizosphere soil.

Then these isolates (MK_2, MK_4, MK_5, MK_7 and MK_9) were further characterized and the activities were also quantified. The results represented in Table 2 reveal that MK_5 had highest phosphate solubilizing efficiency (PSE *i.e.*172.21%) which was statistically at par with rest of the four isolates. In liquid medium maximum phosphate was solubilize by MK_5 (664.30 μg/ml), which was statistically at par with MK_4, MK_7 and MK_9. Growth inhibition shown by various bacterial isolates against *Fusarium spp.*, *Rhizoctonia solani* and *Pythium spp.* were also summarized in Table 2. Also the isolate MK_5 produced a significantly higher concentration of IAA (29.67 μg/mL) after 72h of incubation as compared to other isolates. All the five bacterial isolates produced a bright zone with yellowish colour around the bacterial colony on Chrome-azurol-S medium. Quantitative estimation of siderophore using Chrome-azurol-S (CAS) liquid assay revealed that bacterial isolate MK_5 produced maximum (33.02 % siderophore unit) at 72h of incubation.

Physico-chemical properties and nutrient status of soil

The physico-chemical properties of soil were recorded at the start and termination of the experiment. The physico-chemical properties of soil were recorded at the start and termination of the experiment. The data on initial soil parameters are pH (6.76), EC (0.47 dSm^{-1}), bulk density (1.04mgm^{-3}) and organic matter (1.16%). The initial available N (301.7 Kg/ha) and available K (195.3 Kg/ha) was medium, however available P (41.00 Kg/ha) was in high range. There was no significant change in basic physico-chemical properties of soil *i.e.* pH, EC, organic matter and bulk density after the termination of experiment.

However, the available nutrient contents N and P were increased by 2.56-32.00% and 6.66-40.72%, respectively over control as given in Table 3. Treatments also did no significantly influence the available K content of soil. The seed inoculation with different bacterial isolates not only improved the nutritional content particularly NPK of plants but also increased their uptake significantly over uninoculated control. This increase may be attributed to atmospheric nitrogen fixation, phosphate solubilization in the rhizosphere. Further due to enhanced uptake by increase in specific ion fluxes at the root surface in the presence of plant growth promoting rhizobacteria has also been reported by Bertrand *et al.* (2000). Apparently, synergistic effect of chemical fertilizers and PGPR could have brought significant improvement in soil available nutrients. The higher nutrient concentration with conjoint use of PGPR and chemical fertilizers may be attributed to well develop root system, significant improvement in soil physical properties, microbial and metabolic activity and higher photosynthesis rate, which might have improved absorption of nutrients by plants (Hazara *et al.,* 1987).

Plant Parameters

The application PGPR isolates at different levels of N and P significantly increased number of non wrapper leaves, curd diameter, curd weight, curd depth and curd yield over uninoculated control as given in Table 4. The improved growth and yield of cauliflower as a result of integrated use of PGPR and chemical fertilizers might be due to improved photosynthetic and metabolic activity, which led to increase in various plant metabolites responsible for cell elongation (Hatwar *et al.,* 2003). There was increase in yield by 4.78-28.56% shown by different treatments over uninoculated control but, the highest yield (357.4 q/ha) was record-ed in MK5 isolate with 100% NP fertilizers (T4 treatment) which was statistically at par with T3 (344.6 q/ha) in MK5 isolate with 75% NP fertilizers and also there was a saving of 25% N (31 kg/ha) and 25% P (8 kg/ha) fertilizers in T3 treatment.

The increased yield due to application of chemical fertilizers in conjunction with PGPR may be due to improved vegetative growth, better availability of nutrients, enhanced photosynthetic activity and improvement in soil physical properties which led to better soil physical health (Sharma, 1986). The NPK content of plant also get improved significantly. The combination of MK5 at 75% NP levels was found to be superior to rest of treatments to increase growth and yield of cauliflower, reducing the use of chemical fertilizers and in the improvement of soil health. The application of selected isolates alone and then conjoint use with N and P fertilizers resulted in a significant increase in number of non-wrapper leaves, curd diameter,

Table 1: Methods adopted to check multifarious plant growth promoting activities of the bacterial isolates.

Activity	Methods employed
Phosphate solubilizing	Pikovskaya (1948)
Nitrogen fixing ability	Jensen (1987)
Siderophore production	Schwyn and Neilands (1987)
HCN production	Bakker and Schippers (1987)
Auxin production	Gorden and Paleg (1957)
Antagonistic activity	Vincent (1947)

Table 2: Plant growth promoting activities of selected bacterial isolates

Isolates	P-solubilization %P-solubilization efficiency	Antifungal activity (% growth inhibition)* P-solubilization in liquid medium (μg/mL)	*Fusarium spp.*	*R. solani*	Indole-3-acetic acid (μg/mL)	Siderophore activity *Pythium spp.*	Zone size (mm)	% siderophore unit
MK_2	166.67	444.3	83.33	81.81	83.72	24.83	8.67	51.36
MK_4	147.22	567.67	90.38	78.40	81.39	24.67	11.33	19.40
MK_5	172.21	664.30	89.28	84.04	86.04	29.67	12.67	33.02
MK_7	151.36	640.33	85.71	77.27	81.39	25.50	13.33	19.86
MK_9	158.33	604.00	88.09	81.81	77.90	28.33	14.67	31.14
$CD_{0.05}$	76.03	131.98	5.43	7.05	10.07	3.26	2.77	15.94

Table 3: Effect of different treatments on physico-chemical properties and available nutrient contents of soil

Treatments	Soil physico-chemical characteristics pH	EC (dSm^{-1})	BD (gcm^{-3})	OM (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T1	6.78	0.43	1.57	1.09	304.6	43.39	212.2
T2	6.79	0.48	1.56	1.22	312.4	46.28	203.8
T3	6.81	0.49	1.54	1.28	345.6	52.88	213.3
T4	6.80	0.48	1.56	1.21	402.1	61.06	215.8
T5	6.75	0.39	1.55	1.12	313.7	46.39	213.1
T6	6.81	0.48	1.55	1.29	343.9	53.27	214.1
T7	6.81	0.45	1.49	1.25	395.3	59.56	205.7
T8	6.79	0.46	1.56	1.18	317.8	47.89	206.8
T9	6.80	0.43	1.50	1.22	355.0	53.05	206.8
T10	6.81	0.45	1.57	1.21	399.6	59.17	211.8
$CD_{0.05}$	NS	NS	NS	NS	16.85	2.42	NS

Table 4: Effect of different treatments on plant (growth and yield) parameters

Treatments	Plant Parameters No. of non wrapper leaves	Curd diameter (cms)	Curd weight (gms)	Curd yield /ha (q)	Curd depth (cms)	Total N content in plant (%)	Total P content in plant (%)	Total K content in plant (%)
T1	9.46	10.84	750.8	278.0	7.33	3.15	0.52	2.52
T2	9.83	13.01	820.0	303.7	7.73	3.21	0.56	2.76
T3	10.37	14.27	930.3	344.6	8.55	3.25	0.57	2.78
T4	10.97	15.35	965.0	357.4	9.85	3.25	0.56	2.58
T5	10.17	12.95	808.0	299.3	7.37	3.23	0.53	2.60
T6	10.17	13.73	881.7	326.5	8.65	3.29	0.54	3.02
T7	10.67	14.10	964.2	357.0	8.68	3.20	0.53	2.54
T8	10.03	12.50	786.7	291.3	7.63	3.20	0.54	2.65
T9	10.30	14.23	870.8	322.5	8.45	3.29	0.55	2.77
T10	10.67	14.10	944.2	349.6	8.72	3.23	0.55	2.67
$CD_{0.05}$	0.72	1.57	40.32	14.93	0.81	0.07	0.03	0.50

curd depth and curd weight of cauliflower, total microbial counts in soil and available N and P contents of soil. Taken together, the results suggested that PGPR are able to induce the production of IAA, solubilization of phosphorus and resistance to pathogens and pests, thereby improv-ing growth of plants. The use of PGPR as inoculants biofertilizer is an efficient approach to replace chemical fertilizers and pesticides for sustainable cauliflower cultivation.

Thus, the selected isolate (MK_5) with optimum doses of N and P chemical fertilizers has good prospects to be used as integrated nutrient module not only for enhanced yield but also to sustain soil health under mid hill conditions of Himachal Pradesh.

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