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Impact of integrated nutrient management systems on cauliflower (*Brassica oleracea* var *botrytis)* yield and soil nutrient status

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ABSTRACT

Field experiment was conducted in two consecutive summer seasons (2009 and 2010) to evaluate effect of integrated nutrient management system (INMS) on yield of cauliflower (*Brassica oleracea* var *botrytis* L.) and soil fertility. Studies employed integrated use of PGPR strains (*Bacillus* spp.*)* and NP fertilizer doses. Three isolates (MK_5, MK_7 and MK_9) were selected for studies along with varying doses of N and P fertilizers. Experimental set up was laid down in a randomized block design (RBD) with three replicates. Results revealed that application of MK_5 isolates combined with recommended dose of 75% NP fertilizers significantly increased the cauliflower yield by 27.29% as compared to control (recommended doses of NPK). Soil status was assessed after harvest and concentration of nutrients increased in soil with the combined use of fertilizers and PGPR.

Key words : Cauliflower, P-solubilization, Siderophore, Soil fertility, Sustainable, Xenobiotics

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is a fresh market and seed crop. Its white tender curds are used as vegetables, curries, soups and pickles. It is grown throughout the year in different agro-climatic zones occupying an area of 2800 ha with annual production of 2800 ha with annual production of 54 500 million tonnes as per the data recorded by national horticultural board, 2010. The high-yielding cauliflower varieties has resulted in an increase in cauliflower production but requires large amounts of chemical fertilizers which lead to health hazards and environmental pollution. Organic sources and recycling do not on their own suffice to meet increased demands for food on a fixed land area. On the other hand, because of possible environmental concerns and economic constraints, crop nutrient requirements often cannot be met solely through mineral fertilizers. INMS refers to integration of inorganic and biological components to increase crop productivity and maintenance of soil fertility for future use. This is all done without any deleterious effect on the physico-chemical and biological properties of the soil on a long term basis (Gruhn *et al.* 2000). The conjoint application of PGPR and chemical fertilizers significantly increased yield and weight in wheat over uninoculated control (Akhtar *et al.* 2009). Integrated application having judicious combination of mineral fertilizer with organic and biological sources of nutrients are not only complimentary but also synergistic as organic inputs have beneficial effects (Roy *et al.* 2006). So, INMS was applied to make cauliflower cultivation sustainable and to improve soil productivity and fertility. The combined effect of plant growth promoting rhizobacteria (PGPR) and rates of nitrogen and phosphorus fertilizers at varying doses was observed on growth, yield, concentration of nutrients and quality of cauliflower. Therefore studies were done to enhance growth and yield of cauliflower by employing INMS.

MATERIALS AND METHODS

Experimental studies were conducted in two consecutive summer seasons (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. Soil and root samples were collected from the rhizosphere of cauliflower plants from three different naturally growing agro-climatic zones of Himachal Pradesh and were stored at 4°C in the Laboratory. One gram of the rhizosphere soil was placed in 9 ml of sterilized distilled water under aseptic conditions. For isolation of endophytic rhizobacteria from roots, sample was surface sterilized by 0.2% mercuric chloride ($HgCl_2$) for two min followed by repeated washing in sterilized distilled water. The serially diluted suspension of soil and roots were separately spread on pre-poured nutrient agar medium. After incubation of 24-48 hr, the isolated colonies that developed on enriched medium (master plate) were replica plated onto the selective media:

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Nitrogen free medium for nitrogen fixing activity, Pikovskaya medium for phosphate solubilizing ability. The microbial count was expressed as colony forming units (cfu) per gram of soil.

Standard methods were adopted for screening of the bacterial isolates for the various plant growth promoting activities like P-solubilization, siderophore formation, HCN concentration, auxin production and antagonism against *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. P-solubilization in liquid PVK medium containing TCP (tri-calcium phosphate) was also estimated. The culture supernatant was used for determination of the soluble phosphorus was estimated spectrophotometrically as described by Bray and Kurtz (1945).

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 of multiple plant growth promoting activities under controlled conditions (growth chamber and net house)**,** three isolates (MK_5, MK_7 and MK_9) of *Bacillus* spp. were selected for field experiments along with varying doses of N and P fertilizers.

Seeds of cauliflower of recommended varieties (Pusa Snowball K-1) by the university were surface sterilized in 0.2% mercuric chloride. These were treated with bacterial inoculum for 8 hours and untreated seeds were treated with sterilized water for same time and designated as control. Bacterial cell suspension with OD 1.00 at 540 nm (Sachdev *et al.* 2009) of 72 hr 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 cm × 45 cm. Trials were conducted during the months of March to June in 2009 and 2010 under temperate and dry conditions. The treatment combinations, viz. T1 (Uninoculated 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. N and P were taken into account as cauliflower growing soils of the state are deficient in nitrogen and also have problems of phosphorus fixation. The sources of nitrogen and phosphorus were calcium ammonium nitrate (CAN, provides 25% N) and single super phosphate (SSP, provides 16% P_2O_5) respectively. Recommended doses in uninoculated control was CAN@500 kg/ha, SSP@475 kg/ha and muriate of potash (MOP) @120 kg/ha. All P fertilizers were applied at the time of transplanting of seedlings and N fertilizer was applied in three split applications up to the curd formation stages. The bacterial culture was applied as seed dipping and as liquid culture. A booster dose in liquid form having same optical density was applied at the rate of 10 ml/pot after one month of sowing. Physico-chemical properties, viz. pH, EC, organic carbon (OC) and bulk density of soil sample were determined as per the standard method adopted by Jackson (1973) before start and after the termination of the experiment. Available N, P and K were 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 (leaves and curd) 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. The data recorded for various parameters under net house and field experiments were subjected to statistical analysis using completely randomized design (CRD) and randomized block design (RBD), respectively as described by Gomez and Gomez (1984). The level of significance was tested for different variables at 5% ($CD_{0.05}$).

RESULTS AND DISCUSSION

On the basis of morphological, physiological and biochemical characteristics, it is clear that the isolates belong to genus *Bacillus* as per criteria of Bergey's Manual of Systematic Bacteriology. All the five isolates were P-

Table 1 Plant growth promoting activities of selected bacterial isolates

Isolates	P-solubilization		Antifungal activity (% growth inhibition)*			Indole-3-acetic acid	Siderophore activity	
	% P-solubilization efficiency	P-solubilization liquid in medium (µg/ml)	*Fusarium* spp.	*R. solani*	*Pythium* spp.	(µg/ml)	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 2 Effect of different treatments on physico-chemical properties and available nutrient contents of soil

Treatment	Soil physico-chemical characteristics						
	pH	EC (dS/m)	BD (g/cm)	OC	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T1	6.78	0.43	1.57	0.63	304.6	43.39	212.2
T2	6.79	0.48	1.56	0.71	312.4	46.28	203.8
T3	6.81	0.49	1.54	0.74	345.6	52.88	213.3
T4	6.80	0.48	1.56	0.70	402.1	61.06	215.8
T5	6.75	0.39	1.55	0.65	313.7	46.39	213.1
T6	6.81	0.48	1.55	0.75	343.9	53.27	214.1
T7	6.81	0.45	1.49	0.73	395.3	59.56	205.7
T8	6.79	0.46	1.56	0.68	317.8	47.89	206.8
T9	6.80	0.43	1.50	0.71	355.0	53.05	206.8
T10	6.81	0.45	1.57	0.70	399.6	59.17	211.8
$CD_{0.05}$	NS	NS	NS	NS	16.85	2.42	NS

solubilizers, nitrogen fixers, auxin and siderophore producers. 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 1 reveals 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 is summarized in Table 1. MK_5 isolate also produced a significantly higher concentration of IAA (29.67 μg/ml) after 72 hour 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 72 hour of incubation (Schwyn and Neilands 1987).

Physico-chemical properties and nutrient status of soil

The data on initial soil parameters were pH (6.76), EC (0.47 dS/m), bulk density (1.04 mg/m^3) and organic carbon (0.67). 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 carbon 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 2. Treatments also did not 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. Similar increase in available N in soil due to addition of microbial inoculum was observed by Parmar and Sharma (2001) in cauliflower crop. This increase may be attributed to atmospheric nitrogen fixation, phosphate solubilization in the rhizosphere (Richardson 2001). 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

Table 3 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 (tonnes)	Curd depth (cm)	Total N content in plant (kg/ha)	Total P content in plant (kg/ha)	Total K content in plant (kg/ha)
T1	9.46	10.84	750.8	27.8	7.33	70.44	17.67	65.00
T2	9.83	13.01	820.0	30.37	7.73	74.41	18.39	66.38
T3	10.37	14.27	930.3	34.46	8.55	77.39	20.72	72.00
T4	10.97	15.35	965.0	35.74	9.85	85.94	26.22	79.50
T5	10.17	12.95	808.0	29.93	7.37	75.44	18.00	65.83
T6	10.17	13.73	881.7	32.65	8.65	71.28	21.05	67.67
T7	10.67	14.10	964.2	35.70	8.68	85.50	22.42	81.67
T8	10.03	12.50	786.7	29.13	7.63	70.58	19.08	68.50
T9	10.30	14.23	870.8	32.25	8.45	73.00	21.20	70.33
T10	10.67	14.10	944.2	34.96	8.72	90.86	21.61	86.00
$CD_{0.05}$	0.72	1.57	40.32	14.93	0.81	11.14	2.63	4.38

absorption of nutrients by plants (Hazara *et al.* 1987).

Plant parameters

Experimental results revealed that conjoint application of 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 3. Studies conducted for 2 years with the application of biofertilizers originating from a mixture of isolates of *Bacillus* spp. showed increase in plant growth and productivity (Adesemoye *et al*. 2008). 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 (35.74 tonnes/ha) was recorded in MK_5 isolate with 100% NP fertilizers (T4 treatment) which was statistically at par with T3 (34.46 tonnes/ha) in MK_5 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 application of selected isolates along with N and P fertilizers resulted in a significant increase in number of non-wrapper leaves, curd diameter, curd depth and curd weight of cauliflower, total microbial counts in soil and available N and P contents of soil.

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