Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 672-676
Received: 13-03-2017
Accepted: 14-04-2017

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Effect of conjoint application of nutrient sources and PGPR on soil microbiological properties of cauliflower

Pardeep Kumar, Jagriti Thakur and Mohit

Abstract
The present investigation entitled "Studies on conjoint application of nutrient sources and PGPR on microbiological properties of cauliflower (*Brassica oleracea* var. *botrytis* L.) cv. Pusa Snowball K-1 was carried out at the Experimental Farm of Department of Soil Science and Water Management, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, (HP) during 2014-15 and 2015-16. The experiment was laid out in a randomized complete block design with three replications comprising seven treatments viz. T_1(100% NPK + FYM), T_2(100% NPK + FYM+ PGPR), T_3(100% NPK+ 50% FYM and 50% VC on N equivalence basis + PGPR), T_4 (75% NPK+50% FYM and 50% VC on N equivalence basis), T_5 (75% NPK+50% FYM and 50% VC on N equivalence basis+ PGPR), T_6 (50% NPK+ 50% FYM and 50% VC on N equivalence basis) and T_7 (50% NPK+ 50% FYM and 50% VC on N equivalence basis + PGPR). The seedlings were transplanted at a spacing of 60 x 45 cm in 3 x 2 m size plots. Conjoint use of fertilizers, manures and PGPR significantly influenced soil microbial properties of cauliflower crop. Treatment T_3 resulted in significantly maximum microbiological properties which was found statiscally at par with T_5.

Keywords: INM, PGPR, VC, FYM, cauliflower, microbiological properties

Introduction
Cauliflower (*Brassica oleracea* var. *botrytis* L.) a member of cruciferae is a commercial crop of table purpose vegetable and as a seed crop of Himachal Pradesh. The crop is native of Southern Europe in the Mediterranean region and was introduced in India from England in 1822 (Chatterjee and Swarup, 1972) [4]. Its white tender curds are used as vegetables, curries, soups and pickles. The popularity of the crop has been constantly increasing and at global level. Area under cauliflower production in India is 4,14,000 hectare with a production of 78,97,000 metric tons (Department of Agriculture, GOI, 2016). In Himachal Pradesh, it is grown throughout the year in different agro-climatic zones which brings remunerative returns to the small and marginal hill farmers. Among the different cultivars, snowball group is major contributor to give lucrative returns to the farmers of the state either from seed crop and/ or off season table purpose crop. Current trends in agriculture are focused on reduction in the use of chemical pesticides and inorganic fertilizers, compelling the search for alternatives that enhance environmental quality. For boosting crop production, nutrient balance in the soil is the key component. Agriculturist have been focusing their attention towards the efficient and judicious utilization of available resources to increase the total productivity and profitability per unit area to meet out the food and other demands of ever increasing population. At this juncture of time there is a need of eco-friendly, low cost renewable non-bulky source(s) of nutrients and biological antagonists to enhance the crop productivity and to sustain soil health.

Microbial inoculants/ biofertilizers have emerged as a promising component of integrated plant nutrient management because of its manifold role in soil plant system. From the microbial point of view, soil is nutritional desert. Soil adjacent to roots (the rhizosphere) is relatively nutrient rich, because, depending on plant species, age and environmental conditions, as much as 40% of the photosynthates moving into roots are lost to the soil in the form of soluble exudates, mucilage, shed cells or cell wall material (Lynch and Whipps, 1991) [12]. Therefore, root zone harbour great microbial activity.

Soil contains an array of microorganisms, some of them are beneficial like nitrogen fixers, phosphate solubilizers, plant growth promoting rhizobacteria and some are harmful to plant growth like cyanide producing DRMO (Deleterious rhizosphere microorganisms) and other disease causing microorganisms. Populations of bacteria in the rhizosphere are enormous, ranging from 10^6-10^{12} cfu/g soil (Alexander, 1976) [1]. These rhizosphere residents are considered as passive by standards of the root environment, which affect plant health, development and environmental adaptations, both beneficially and detrimentally (Hornby, 1990) [8].

Effective strategies for the selection of efficient strain(s) of beneficial soil microbes are need of the hour. Selection of efficient beneficial strains/ isolates based on host plant specificity or adaptation to a particular soil, climatic conditions or pathogens is vital for achieving consistent and reproducible results under field conditions (Bowen and Rovira, 1999) [3].

Rhizobacteria (PGPR) encompasses all bacteria that inhabit plant roots and exert a positive effect by various mechanisms i.e. direct influence (increased solubilization and uptake of nutrients or production of plant growth hormones) and indirect effect (suppression of pathogen by producing siderophore or antibiosis). During the last two decades the PGPR have received prominent attention because of their multifarious activities to improve plant growth, primary production and disease control (Panwar *et al.,* 2004) [14]. An attempt on the use of PGPR have been made under field/ controlled conditions to supply nutrient elements, growth promoting substances and to control soil borne disease of cabbage and cauliflower (Mariano *et al*., 2002) [14]. PGPR are of great interest in sustainable crop production and sustainance of soil health. However, the extent of benefits from PGPR depends on their number and efficiency, which is governed by a large number of soil and environmental factors. It has also been observed that PGPR isolated from native rhizosphere are more effective in growth enhancement and crop protection than other strains because of better adaptability of bacterial strains.

Materials and Methods

The details of experimental site, materials and methodology employed during the course of experiment have been presented.

Experimental location

The experimental site is situated at the experimental farm of the department of Soil Science and Water Management, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP). It is located at 30º 51′ N latitude and 76º 11′ E longitude and an elevation of 1175 m above mean sea level having average slope of 7-8 per cent.

Climate

The study area Nauni falls in sub-temperate, sub-humid agro-climatic zone of Himachal Pradesh (Zone-2). The area receives an annual rainfall of 1100 mm and about 75 per cent of it, is received during the monsoon period (mid-June-mid September). Winter rains are meager and received during the months of January and February. Winter showers during the month of January and February are very common.

Experimental details

The experiment was arranged in 'Randomised Block Design' wherein following treatments were tried in triplicate.

Treatments

Treatment code Treatment detail

T_1 100% NPK + FYM

T_2 100% NPK + FYM+ PGPR

T_3 100% NPK+ 50% FYM and 50% VC on N equivalence basis + PGPR

T_4 75% NPK+50% FYM and 50% VC on N equivalence basis

T_5 75% NPK+50% FYM and 50% VC on N equivalence basis+ PGPR

T_6 50% NPK+ 50% FYM and 50% VC on N equivalence basis

T_7 50% NPK+ 50% FYM and 50% VC on N equivalence basis + PGPR

Observations recorded

Total culturable microbial count (bacteria, fungi and actinomycetes count) in soil

The serial dilution and plating techniques suggested by Rao (1999) [23] was employed for isolation and identification of viable bacteia, actinomycetes and fungi count. Media were prepared for desired micro flora. The autoclaved and cooled (45 ºC) medium was poured into sterile plates and allowed to solidify. One gram of sieved (2 mm) soil was added to 9 ml sterile water blank and shaked for 15-20 minutes. Serial dilutions of 10^{-2} ,10^{-3}, 10^{-4},10^{-5}, 10^{-6} and 10^{-7} were prepared and 0.1 ml of aliquots of various dilutions were added, over cooled and solidified medium in petriplates. Pour plate method was employed. The plates were rotated for uniform distribution of bacterial cells and fungal spores in the aliquot under the media and allowed to solidify. After the media solidified, the plates were inverted and incubated at 28 ºC for 3-4 days. The appearance of colonies on the surface of medium in the plates were observed. Population count of bacteria, fungi and actinomycetes were noted using dilution plate technique by employing nutrient agar (NA), potato dextrose agar medium (PDA) and kenknight's media respectively. The population were expressed as colony forming units (cfu/g soil).

The composition of these media are as below:

Nutrient agar (NA)

Beef extract	3g
Peptone	5g
NaCl	5g
Agar Agar	20g
Distilled Water	1000ml

Potato dextrose agar media (PDA)

Potatoes	250g
Dextrose	20g
Agar-Agar	20g
Distilled Water	1000ml

Kenknight media

K_2HPO_4	1g
$NaNO_3$	0.1g
KCL	0.1g
$MgSO_4.7H_2O$	0.1g
Glucose	1.0g
Agar-Agar	20g
Distilled Water	1000ml

Microbial biomass Carbon (Soil Fumigation-Extraction Method)

Microbial biomass Carbon was determined by soil fumigation- extraction method detailed by Vance *et al* (1987) [25]. In this method, 10 g of soil was fumigated with 20 ml of $CHCL_3$ in vacuum desiccators for 24 hours in dark and other 10 g of same soil sample was refrigerated, then both the samples (fumigated and unfumigated) were extracted with 0.5 M K_2SO_4 for half an hour and then the extract was treated with H_2SO_4 and orthophosphoric acid and heated on hot plate at 120 ºC for 30 minutes. After that diluted to 250 ml with distilled water and 2-3 drops of ferroin indicator was added and titrated against 0.005 N FAS (Ferrous Ammonium Sulphate) and calculated by

$$\text{MB-C } (\mu g/g \text{ soil}) = \frac{OC_F - OC_{UF}}{K}$$

Where,
O_{CF} = Total amount of EC (Extractable C) in fumigated soil
OC_{UF} = Total amount of EC (Extractable C) in unfumigated soil
K = Factor (0.45)

Soil microbial activity (CO_2 evolution method)

Soil microbial activity was determined by the CO_2 evolution method described by Parmer and Schmidt (1964) [15]. In this method, 100 g of soil was taken in one liter of flask, then water was added in order to maintain 30-35% moisture. A test tube containing 15 ml of 1N NaOH was suspended in flask. The flask was incubated at 30±2 ºC with appropriate control and then withdraws the test tube at different time interval (24,48,72 and 96 h). To this 1ml of 50% $BaCl_2$ was added and this was titrated against 1 N HCl with phenolphthalein used as indicator. The results were expressed as mg CO_2/g soil.

CO_2 Evolution (mg CO_2/g soil) = (B-V) NE

Where;
B = Volume of HCl used for blank
V = Volume of HCl used for sample
N = Normality of acid
E = 22 (Equivalent weight of CO_2)

Results and discussion

Total Culturable Microbial Count (cfu/g)

Bacterial count

The bacterial population was significantly enhanced by the application of combined use of organic, inorganic fertilizers and PGPR after harvest of crop during both the years of experimentation (Table 1). The pooled data pertaining to soil bacterial count revealed that soil receiving 100% NPK +50% FYM and VC on N equivalence basis + PGPR (T_3) recorded maximum microbial count (201.83 × 105 cfu g^{-1} soil) which was statistically at par with T_5 (200.38× 10^5 cfu g^{-1}soil) while minimum (154.68 × 10^5 cfu g^{-1}soil) bacterial count was noted in T_6.

Fungi count

During both the years (2015-2016), the fungal population was significantly enhanced by the combined application of organic, inorganic fertilizers and PGPR after harvest of crop (Table 1).The pooled data pertaining to soil fungi revealed that the soil receiving 100% NPK +50% FYM and VC on N equivalence basis + PGPR (T_3) recorded maximum fungal count (4.40 × 10^5 cfu g^{-1} soil) which was statistically at par with T_5 (4.38 × 105cfu g^{-1} soil) while minimum (2.69 × 10^5 cfu g^{-1} soil) microbial count was noted in T_6.

Actinomycetes count

The perusal of data in Table 1 revealed that actinomycetes population was significantly enhanced by the conjoint application of organic, inorganic fertilizers and PGPR. The pooled data pertaining to soil actinomycetes count revealed that the soil receiving 100% NPK +50% FYM and VC on N equivalence basis + PGPR (T_3) recorded maximum actinomycetes count (3.48 × 10^5cfu g^{-1} soil) which was statistically at par with T_5 (3.45 × 10^5 cfu g^{-1} soil) while minimum (2.73 × 10^5 cfu g^{-1}soil) actinomycetes count was noted in T_6.

Table1: Effect of different nutrient sources and PGPR on total culturable microbial count of cauliflower

Treatments	Total culturable microbial count(x 10^5cfu g^{-1})								
	Bacteria			Fungi			Actinomycetes		
	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled
T_1	184.23	185.73	184.98	3.75	3.91	3.83	3.17	3.23	3.2
T_2	198.3	199.43	198.87	4.32	4.35	4.34	3.41	3.44	3.43
T_3	200.27	203.4	201.83	4.35	4.44	4.4	3.43	3.52	3.48
T_4	171.7	174.07	172.88	3.36	3.38	3.37	2.94	3.05	2.99
T_5	199.3	201.47	200.38	4.37	4.39	4.38	3.41	3.49	3.45
T_6	153.3	156.07	154.68	2.63	2.75	2.69	2.69	2.76	2.73
T_7	165.9	167.47	166.68	2.79	2.85	2.82	2.79	2.88	2.84
Mean	181.86	183.95		3.65	3.72		3.12	3.2	
CD (0.05)	2.59	2.58		0.04	0.03		0.03	0.02	
T	2.59			0.04			0.03		
Y	1.38			0.02			0.02		
T× Y	NS			0.05			NS		

The maximum microbial count *viz.* bacterial, funal and actinomycetes was observed in treatment T_3 which was found statiscally at par with T_5. The soil harbour a dynamic population of microorganisms, their abundance in rhizosphere gives an indication of their possible role in decomposition of organic matter, fixation of atmospheric nitrogen, phosphate solubilization, transformations of nutrient elements, etc. The application of chemical fertilizers along with PGPR inoculants registered a significant increase in total microbial population over uninoculated control. These results are in conformation with those of Patil and Varade (1998) [16], Selvi *et al.* (2004) [20] and Qureshi *et al.* (2005) [17]. They have reported that 100 per cent N alone and control treatment had minimum microbial population; however, the balanced fertilization i.e., 100 per cent NPK + FYM and / or biofertilizer enhanced the bacterial count in the soil.

Zhang *et al.* (2006) [26] reported that different environmental parameters, content of soil organic carbon, total nitrogen and altitude could affect the diversity of soil flora, including nitrogen fixing bacteria. The increased microbial population may be due to the fact that organic manure provided necessary food and micro environment for their quicker multiplication and growth (Kumari and Kumari, 2002) [11]. They further observed that VC application increased population of phosphate solubilizing bacteria and free living organism too. Soil enzymatic activities increased as the soil microbes degrade organic matter through the production of diverse extracellular enzymes, after the application of

vermicompost to soils (Tejada and Gonzalez, 2008) [24]. This may be attributed to the VC containing higher amount of growth promoting substances, vitamins and enzymes which in turn increased the microbial population and root biomass production. FYM is one of the suitable medium in which microbial inoculants grow to a reasonably higher number with long shelf life (Sharma, 2002) [21]. Organic manures, are materials with high organic carbon which might have increased porosity, drainage, and water holding capacity (Edwards and Burrows, 1988) [7] which have enhanced the congenial conditions to harbour more microbes. Similar improvement in biological properties of soil with organic nutrition has already been reported by Dubey and Agrawal (1999) [6] and Saini *et al.* (2005) [18] also.

Microbial biomass carbon (mg MB-C/ 100g soil)

It is evident from the data presented in Table 2 that microbial biomass carbon was significantly increased by conjoint application of bacterial inoculant with chemical fertilizers during both the years of study. Pooled data revealed that maximum microbial biomass was recorded under treatment T_3 (112.55 mg MB-C/100 g soil) which was statistically at par with T_5 (110.65 mg MB-C/100g soil) whereas, the minimum microbial biomass-C was recorded with treatment T_6 (61.50 mg MB-C/100g soil).

The microbial biomass carbon ranged from 53.47 mg MB-C/100 g soil to 113.53 mg MB-C/100 g soil under field conditions. These findings are in accordance with those of Anderson and Domsch (1978) [2] who reported the range of microbial biomass carbon in agricultural soil from 15 to 240 mg MB-C/100 g soil. These results are further supported by the findings of Saini *et al.* (2005) [18] and Sparling (1985) [22] who also reported significant influence of integrated nutrient management on microbial biomass carbon.

Table 2: Effect of different nutrient sources and PGPR on microbial biomass carbon of cauliflower

Treatments	Microbial biomass carbon (mg MB-C/ 100g soil)		
	2014-15	2015-16	Pooled
T_1	92.63	94.27	93.45
T_2	108.37	111.37	109.87
T_3	111.57	113.53	112.55
T_4	84.8	86.05	85.43
T_5	109.77	111.53	110.65
T_6	53.47	69.53	61.5
T_7	73.9	75	74.45
Mean	90.64	94.47	
CD $_{(0.05)}$	2.27	1.98	
T	2.13		
Y	1.14		
T×Y	3.01		

Microbial activity (CO_2 evolution/g soil)

The rate of CO_2 evolution in treatment comprising different levels of NPK + organic manures + PGPR increased up to 48 h and then followed a sudden decrease and remained in decreasing trend with increase in incubation period (Figure 1). However, the rate of CO_2 evolution was the maximum under treatment T_3 and minimum was recorded with T_6 after 24 hrs of incubation period.

This might be ascribed to increase in microbial population by conjoint application of bacterial inoculation with chemical fertilizers. The results are further in conformation with those of Schinner *et al.* (1980) [19], Islam and Weil (2002) [9] and Kaushal *et al.* (2013) [10].

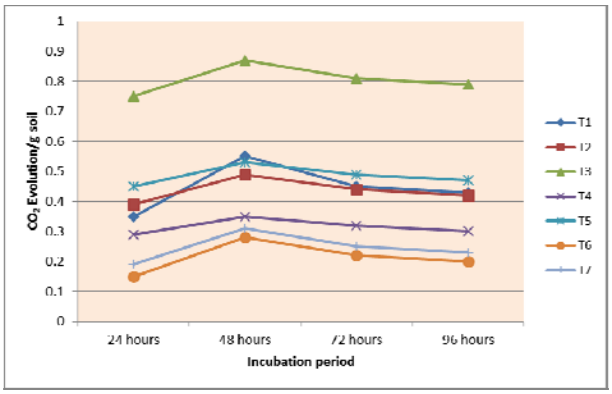

Fig 1: Effect of different nutrient sources and PGPR on microbial activity of cauliflower

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