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Native rhizosphere strains of Pseudomonas and Bacillus species in plant growth promotion and wilt suppression in carnation

Mohinder Kaur, Sunita Chandel*

Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173230, Himachal Pradesh, India

Abstract

In the present work, two rhizospheric bacteria viz *Pseudomonas* sp. (10 isolates) and *Bacillus* sp.(4 isolates) were isolated from carnation. Further, the isolated microorganism were evaluated for various biological activities. Results from the study indicated that the isolates have capability of producing the phytohormones and thus have potential to be exploited for growth promoting activity in various crops including carnation as well as helpful in suppression of soil borne pathogens of devastating nature.

Key words: Microorganism, Antagonist, *Pseudomonas*, *Bacillus*, Carnation, rhizosphere

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1.0 Introduction

Many soil microorganisms are known to possess useful activities and are good producers of plant growth promoting bioactive compounds, which are of great interest in modern and eco-compatible agriculture. They are known to play key role in nutrient cycling and maintenance of soil fertility. Rhizosphere of plant is an important and versatile soil ecological environment for plants and microbial interactions that can be useful, harmful or neutral [1,2]. Their promising potential to produce secondary metabolites is studied by various workers, which can be of economic and beneficial use to plants and human beings. An accurate characterization of bacterial populations naturally associated with roots is essential for designing novel plant growth promoting and disease suppressing bio-agents that can deliver toxins beneath the surface of soil where root feeding insects and fungi cause most of the damage. Some of these products are useful in management of diseases and others for chemical reactions. Flourescent *Pseudomonas* are mainly known to stimulate the growth of several annual crops including carnation [3,4] and are producing siderophores, an iron chelating agents that may cause iron depletion in most of the plant pathogenic fungi. The ability of the microorganisms such as *Pseudomonas* and *Bacillus* spp. to synthesize various biologically active substances like phytohormones, antibiotics, metabolites are thought to participate in multifaceted process. Therefore, finding many beneficial traits in one strain is essential before suitable strain can be directly selected for rhizosphere inoculation or can be subjected to genetic engineering or other means into commercial cut flowers, which are reported to be attacked by large number pathogen, is of prime importance. Hence, it is of research interest to develop possible eco friendly technology for growth promotion and disease control by exploiting the indigenous rhizosphere strains of *Pseudomonas* and *Bacillus* species of well-known abilities for multifaceted uses and for the healthy crop production. Hence, in the present work, an attempt has been made to isolate Native rhizosphere strains of Pseudomonas and Bacillus species and to evaluate their plant growth promotion and wilt suppression in carnation.

2.0 Materials and methods

Isolation and identification of rhizosphere bacteria

Isolation of rhizobacteria and screening for antimicrobial bioactive metabolites was carried out in year 2010-11 by taking randomly rhizosphere soil samples (10g) from 15 cm depth of actively growing carnation plants from 5 selected sites viz, University campus-(UH&UF), Kandaghat –(KG) Research Station, Mahog- (MC), Oachghat-(OG) and Kotlanala-(KN) to make a composite sample for isolation of bacteria. Dilutions were made by taking the original 10g rhizosphere soil in 90 ml of sterile water to yield different dilution series ranging from 10^{-2} to 10^{-8} for each sample. Nutrient Agar, Bacillus Agar, Kings Media A and Kings Media B [5] were employed for isolation of *Bacillus* and *Pseudomonas* species. The basis of morphological and biochemical tests were considered for characterization

as listed in Bergey's manual of Systemic Bacteriology [6].

Rhizosphere bacteria for biological activity

The isolated rhizosphere bacteria were screened for antibacterial activities by well plate assay [7] against seven indicator bacteria (viz, *Bacillus subtilis, Escherchia coli, Klebsiella* sp, *Pseudomonas* sp, *Salmonella paratyphi, Salmonella typhi and Xanthomonas* sp) and eight plant pathogenic fungi(*Alternaria* sp., *Aspergillus* sp, *Fusarium* sp., *Penicillium* sp., *Phytophthora* sp., *Pythium* sp., *Sclerotium* sp., and *Trichothecium* sp.). The cell free culture supernatants of *Pseudomonas* and *Bacillus* species were prepared by centrifugation at 10,000 rpm at 4^0C for 30 min. after incubation of bacterial cultures at 30^0C and 37^0C temperatures, respectively. After addition of 100µl of 72 h old cell supernatant of bacterial strains in each well (7 mm) opposite to indicator bacteria and fungi in Nutrient Agar and Potato Dextrose Agar plates, these were incubated at 37^0C for 24-48 h and at 28^0C for 72 h, respectively for formation of clear zone around the wells. The proteolytic activity was assessed in same way using Skim Milk Agar medium whereas standard iron free succinate medium (K_2HPO_4 6.0 g; KH_2PO_4 3.0 g; $(NH_4)_2$ SO_4 1.0 g; $MgSO_4.7H_2O$ 0.2 g, Succinic acid or Citric acid 4.0 g) as developed by Meyer and Abdallah [8] was used for production of siderophores. The detection of siderophores was carried out by Chromeazurol-S (CAS) plate assay with slight modifications [9]. The bacterial inoculated plates were incubated at $30\ ^0$C for 24 h for obtaining yellow or orange halo around the wells. Siderophore production expressed in terms of mm diameter of yellow or orange halo production.

For the production of phytohormones, both *Pseudomonas* and *Bacillus* species associated with carnation plants were considered. The production of phytohormones viz, auxin, gibberellins and cytokinins were estimated. Supernatants of both bacteria obtained from Nutrient Broths through shake conditions (90 rpm) at 30^0C and 37^0C were prepared by centrifugation at 10,000rpm at 4^0C for 30 min. and used for extraction, separation and estimation of photohormones [10]. Ethyl acetate was used for extraction of auxin, purification and separation was done by chromatography in solvent system, isopropanol: ammonia: water (80:10:10) by keeping reference sample of IAA developed for 12-14 h. Quantitative measurement was carried out by colorimetric method [11]. Developed chromatographs were divided into 10 equal transverse strips between starting line and solvent front depicting Rf values 0.1 to 1.0, eluted in 1-2 ml of methanol/ ethanol for 30 min. Salper reagent (2 ml) was added into the extracts (1 ml) drop wise with continuous stirring and incubated for 60min in dark for development of pink colour. Absorbance measured at 535 mm against solvent reagent blank. Standard curve was drawn from known concentration of IAA.

The Gibberellic acid was estimated spectrophotometrically by the method as described by standard procedure as follows. To 15 ml of supernatant of bacterial species, 2 ml zinc acetate was added and

centrifuged at low speed (2000 rpm) for 15 min. Along with supernatant equal volume of HCl (30%) was added. The absorbance was read at 254 nm and standard curve was prepared with known concentration of Gibberellic acid (10-100 µg/ml). For the extraction of cytokinin, 72h old supernatant cultures of bacteria were used and extraction was carried out with ethylacetate according to Mahadevan [12] and Mandahar and Arora [13]. Separation and purification of cytokinins was performed using descending paper chromatography technique employed in dark at 25^{0}C using isopropanol: ammonia: water (10:1:1) as solvent mixture [14]. The chromatogram was removed when the solvent front reached a distance of ¾ th from the base line and solvent front depicting Rf values from 0.1 to 1.0. The control strip of same size was also obtained from the chromtogram. In addition, the bioassay of cytokinin was done by Radish cotyledons expansion test [15]. The seeds of Radish (*Raphanus sativus* L- Japanses White) were germinated in total darkness for 48 h at 28^{0}C. After removing the seed coat, smaller cotyledons were transferred to sterilize petridishes containing the test solutions and filter paper strips (elute + strips). The cotyledons were placed on petridishes and incubated at 25^{0}C under fluorescent light for 3 days. These were later blotted dried and weighed. All the treatments were replicated thrice. The bioassay response was expressed as percentage of the initial weight. A standard curve was drawn by plotting the differences against the known concentrations of the kinetin in the form of dose response of kinetin per gram fresh weight of the tissue.

Field efficacy of potential strains of rhizosphere bacteria

In field conditions, testing of potential isolates selected on the basis of their performance of biological activities related to proteolytic, siderophore, auxin, gibberiln and cytokinin production was performed. The experiment on wilt disease incidence and growth parameters of carnation was carried out with ten rhizospheric isolates of *Pseudomonas* sp. and *Bacillus* sp. The cell suspension containing 32 x 10^6 c.f.u./ml of each antagonists was prepared in Nutrient Broth medium and added (300 ml) in formalin sterilized soil in 1x1 m sized beds in randomized block design. The mass culture of wilt pathogen, *Fusarium oxysporum* f.sp. *dianthi* was prepared on maize bran medium as suggested by Dohroo and Sharma [16]. The boiled maize grains were mixed with sand in 3:1 proportion along with 2% sucrose, sterilized in autoclave at 15lbs psi for 1hr for 2 consecutive days. The sterilized medium was inoculated with one-week-old culture of pathogen and kept at 25^{0}C for 10-12 days. The medium was used for artificial inoculation of the soil by adding 200g/m^2 20 days prior to application of antagonist isolates. The rooted cuttings of carnation were purchased from Department of Floriculture and Landscaping of Dr. Y.S. Parmar University of Horticulture and Forestry, Solan-India.

Table-1: Screening of *Pseudomaonas* and *Bacillus* for production of antibacterial / antifungal activity by well plate assay method

Bacteria	Strain No.	Antibacterial activity (mm diameter)*							-
		Bacillus subtilis	***E.coli***	***Klebsiella* sp.**	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Salmonella typhi***	***Xanthomonas* sp.**	
	KG3	-	-	-	-	-	-	-	
	KN6	+24	+w	-	-	-	-	-	
	KN7	+w	+w	-	-	-	-	-	
	MC	+35	-	+w	+30	-	+w	+15	
Pseudomonas sp.	MC5	+w	-	+w	-	-	-	+w	
	MC10	-	+w	-	-	-	-	-	
	OG6	+24	+w	+w	+10	+14	-	+20	
	UF2	-	-	-	+w	-	-	-	
	UH3	+w	-	-	-	-	-	-	
	UH5	+28	+w	-	-	-	-	+21	
	KG5	+w	+w	-	-	-	-	-	
	MC1	+29	+18	+w	+w	+w	-	-	
	MC4	-	-	-	-	-	-	-	
	OG5	-	+w	-	-	-	-	-	
Bacillus sp.	OG7	-	-	-	-	-	-	-	
	UFA	-	+w	+w	-	-	-	-	
	UF1	+w	+w	-	-	-	-	-	
	UH4	+w	+w	-	-	-	-	-	
	UF8	+32	+24	+w	+28	-	-	-	
	UF9	-	-	-	-	-	-	-	
	UF10	+w	-	-	-	-	-	-	

Bacteria	Strain No.	Antifungal activity (mm diameter)**							
		Alternaria sp.	*Aspergillus* sp.	*Fusarium* sp.	*Penicillium* sp.	*Phytophthora* sp.	*Pythium* sp.	*Sclerotium* sp.	*Trichotheciu m* sp.
	KG3	-	-	-	-	+12	-	-	+w
	KN6	+22	-	-	-	+w	-	-	-
	KN7	-	+18	-	+w	-	+14	-	-
	MC	+16	+w	+12	+w	+w	+w	-	-
Pseudomonas sp.	MC5	+16	-	-	+w	+w	+w	-	-
	MC10	+w	-	+12	-	-	+w	-	-
	OG6	+w	-	+11	-	+w	+18	-	-
	UF2	-	-	-	-	+w	+w	+w	-
	UH3	-	-	+10	-	-	-	-	-
	UH5	+w	-	-	-	+16	-	-	-
	KG5	-	+18	-	-	+12	-	-	-
	MC1	+9	-	+11	-	+w	+w	-	-
	MC4	-	-	-	-	-	-	-	-
	OG5	-	-	+7	-	-	-	-	-
	OG7	-	-	-	-	-	-	-	-
Bacillus sp.	UFA	-	-	-	-	+w	-	-	-
	UF1	+14	-	-	-	+14	-	-	-
	UH4	+w	-	-	-	+14	+w	-	-
	UF8	+18	+w	+12	-	+12	-	-	-
	UF9	-	+16	-	-	-	-	-	+w
	UF10	-	+w	-	-	+16	+w	-	-

These were dipped in antagonist cell suspension for 30 min and planted in beds at a spacing of 25 x 25 cm during first week of March, 2010-11. Thereafter two soils drenching of antagonists were given at one-month interval around the root zones of the plants. All the treatments replicated thrice and data on disease incidence and growth characteristics were recorded up to the maturity of the crop and analyzed by Gomez and Gomez [17] method. All the experiments were carried out with three replications and subjected to completely randomized block and randomized block designs. Whenever needed, the data was transformed before analysis. The means were evaluated at the 5% level of significance (p=0.05) using Fisher Test.

Table-2: Evaluation of *Pseudomonas* and *Bacillus* strains for the production of proteolytic and siderophore activity by well and plate assay

Bacteria	Srain No.	Proteolytic activities* (mm diameter)	Siderophore Production** (mm diameter)
Pseudomonas sp.	KG3	+28	+18
	KN6	+21	+26
	KN7	-	+20
	MC	+12	+25
	MC5	+10	+26
	MC10	-	+20
	OG6	+24	+10
	UF2	+14	+22
	UH3	-	+20
	UH5	+w	+12
Bacillus sp.	KG5	+w	+22
	MC1	+22	+18
	MC4	-	+13
	OG5	-	+25
	OG7	-	+14
	UFA	-	+16
	UF1	+12	+28
	UH4	+w	+18
	UF8	+12	+14
	UF9	+20	+12
	UF10	+25	+22

-Indicates no activity; + Indicates activity; w Indicates weak activity; *

3.0 Results and discussion

Enumeration and identification of rhizosphere bacteria from carnation From five locations, maximum bacterial count was obtained at 102 dilutions in rhizosphere soil

samples collected from University campus (UH&UF) and minimum from Kandaghat Research Station.

On the basis of morphological and biochemical characterization, the bacteria were identified as *Pseudomonas* sp. and *Bacillus* sp. with 10 and 11 isolates, respectively. Most of *Pseudomonas* isolates showed inhibitory action against gram-positive bacteria compared to gram-negative bacteria (Table-1).

Maximum inhibitory activity (35 mm) was recorded against *B. subtilis* by *Pseudomonas* MC isolate followed by UH5 (28 mm), OG6 and KN6 (24 mm). These isolates also showed strong antagonism against *Pseudomonas aeruginosa* sp. (30 mm), *Xanthomonas* sp. (20 mm), *Salmonella typhi* (14 mm) and but weak response to *Klebsiella* sp, *S. paratyphi,* and *Escherchia coli*.

In case of *Bacillus* sp., isolate UF8 gave maximum inhibition zone (32 mm) against *B. subtilis* followed by *P. aeruginosa* (28 mm) and *E. coli* (24 mm). The isolate MC1 registered clear zone (29 mm) diameter in comparison to other test bacteria towards *B. subtilis* and *E. coli* with 18 mm diameter of clear zone formation.

It was observed that maximum zone of inhibition was exhibited by Pseudomonas isolates MC, MC5 and KN6 towards *Alternaria* sp. by giving 16-22 mm clear zone formation. No significant antifungal response was reported against *Sclerotium* sp. and *Trichothecium* sp. by the tested strains of bacteria, among the tested samples, two *Bacilli* isolates MC1 and UF8 showed moderate antifungal effect against *Fusarium* sp., *Alternaria* and *Phytophthora* sp. and weak antagonism towards *Pythium* sp. Proteolytic activity was produced maximum by *Pseudomonas* strains in the range of 24-28 mm of diameter of clear zone while that produced by *Bacillus* sp. remained in 22-25 mm range as depicted in Table-2.

Proteolytic activity expressed in terms of mm diameter of clear zone produced around the well (7mm) on skim milk agar plates at for 24h and Siderophores production expressed in terms of mm diameter of clear zone produced around the 72h old culture bit (7mm) on Chromeazurol-S-HDTMA agar plates at 30^0C for *Pseudomonas* sp. and at 37^0C for *Bacillus* species

Two strains of each bacterium KG3 and OG6 of *Pseudomonas* sp. and UF10 and MC1 of *Bacillus* sp. exhibited highly efficacious proteolytic activity. The results pertaining to siderophore production by rhizobacterial isolates revealed that all the isolates of *Pseudomonas* and *Bacillus* produced siderophore. However *Bacillus* strain UF1 gave highest production followed by *Pseudomonas* KN6 and MC5 on Chromeazusol-S-HDTMA agar plates. Proteolyte and siderophores were produced higher in KG3, OG6, UF10 and MC1 strains of both rhizospheric showing siderophore-mediated competition for iron to be the mechanism of suppression of Fusarium wilt. Both *Pseudomonas* and *Bacillus* species are reported to be a prolific producer of proteinases, which constitute a major source of enzymes used in detergent and waste utilization. There are report that some of the organisms secretes non-binding ligand called siderophore that can bind the ferric iron and make it available to the host microorganism. Involvement of these compounds in plant growth

promotion and disease suppression by these strains has been investigated [18].

Production of phytohormones

Phytohormones viz, auxins, gibberellins and cytokinins were investigated from the rhizobacteria growing in nitch of carnation. Both the bacteria also produced gibberellin like substances (Table-3). Of different *Pseudomonas* isolates, KN6 (668.30 µg/ml) showed highest production followed by *Pseudomonas* strains MC5 (405.00 µg/ml) UH3 (303.3 µg/ml), KN7 (286.70 µg/ml) MC10 (246.70 µg/ml) and UH5 (135.0 µg/ml). While two strains of *Bacillus* sp – UH4 and UFA produced maximum gibberellin content in capacity of 468.3 µg/ml and 308.3 µg/ml, respectively (Table-3).

Table-3: Potentiality of *Pseudomonas* and *Bacillus* strains for the production of gibberellins like substances at 30^{0}C and 37^{0}C for 72 h

	Strain No.	Gibberellins*(µg/ml)
Pseudomonas sp.	KG3	113.30d
	KN6	668.30a
	KN7	286.70c
	MC	80.00e
	MC5	405.00b
	MC10	246.70c
	OG6	130.00d
	UF2	25.00f
	UH3	303.30b
	UH5	135.00d
	Strain No.	Gibberellins* (µg/ml)
Bacillus sp.	KG5	205.00d
	MC1	200.00d
	MC4	221.70b
	OG5	65.00f
	OG7	205.00d
	UFA	308.30b
	UF1	251.70c
	UH4	468.30a
	UF8	205.00d
	UF9	171.70e
	UF10	271.70c

The mean values with same letters are not significantly different at $p<0.05$ for *Pseudomonas* (strains), *Bacillus* (Strains) according to Fisher's test.

* Gibberellins concentration expressed in terms of µg/ml produced by *Pseudomonas* and *Bacillus* strains in cell free culture supernatants at 30^{0}C and 37^{0}C for 72h under shake condition in nutrient broth

Table-4: *In vivo* evaluation of antagonist isolates on wilt incidence (*Fusarium oxysporum* f.sp. *dianthi*) and growth parameters of carnation

Antagonist isolates	Wilt incidence (%)	Disease control (%)	Average stem length (cm)	Average number of flowers/ plant	Average size of flower/ plant (cm)
Pseudomonas sp.					
OG6	45.33	19.05e	39.65e	2.25c	3.82d
MC	(42.33)f*	76.20a	67.67a	4.75a	7.48a
MC5	13.33	73.80b	63.50b	4.50a	7.55a
UHF	(21.44) a	61.91c	58.45c	3.50b	5.27b
KN6	14.67	50.00d	56.65d	3.25b	4.10c
Control	(22.52) a	-	24.60f	0.75d	2.25c
	21.33				
	(27.53) b				
	28.00				
	(31.90) c				
	56.00				
	(48.45) d				
Bacillus sp.					
UF4	12.00	78.05a	65.05c	4.50a	7.20a
UF1	(20.27) a	63.42c	60.67b	3.75b	6.57b
UF8	20.00	68.30b	64.72c	4.50a	6.82b
UFA	(26.56) c	46.35d	59.90b	4.00b	5.82c
UF9	17.33	14.63e	48.28c	3.00c	3.62d
Control	(24.61) b	-	28.40d	1.25d	2.35c
	29.33				
	(32.80) d				
	46.67				
	(43.11) e				
	54.67				
	(47.67) f				

Figures in parentheses are arc sine transformed values
The mean values with same letters are not significantly different at p<0.05
Averages of growth parameters is based on 5 plants per replication in each treatment

All the cell free culture supernatants of *Pseudomonas strevii* strains with raddish cotyledon bioassay showed percent significant increase in weight. Gibberellic acid (GA_3) and gibberellin like compounds were identified in culture of various

species of *Azotobacter*, *Pseudomonas and Bacillus*. Bioassay and thin layer chromatography used for characterizing plant growth substances from rhizosphere microorganism and soil revealed the presence of GA_3 like substances in range from 0.012 to 1.0 GA_3 equivalents per liter as reported by Leinhos and Birnstiel [19]. *Bradyrhizobium japonicum* strain TAL377 produced 74.64 µg/ml IAA and 261.2 µg/ml GA_3 while *Pseudomonas* strain 54RB produced 8.034 µg/ml IAA and 1766 µg/ml GA_3 giving survival efficiency of 33% and 46%, respectively due to co-inoculation with P_2O_5 in soybean [20].

The refractive index of phytohormones revealed that most of the strains produced two types of auxins like substances that occurred in Rf value range 0.3-04 and Rf 0.6-1.0.

Three MC, MC5 and UF2 strains of *Pseudomonas* sp. produced maximum auxins like substances under Rf value 0.3-0.4 and 0.6-1.0 while Rf value of 0.3 was effective for *Bacillus* sp. Ali et al [21] and Yadav et al [22] reported that auxin production of *Bacillus* spp. in broth medium supplemented with 1,000µg ml^{-1} typtophan ranges from 0.60 to 3.0 µg ml^{-1} IAA by gas chromatography and mass spectrometric analysis.

Cytokinins produced exhibited Rf value between 0.1-0.4 and 0.8-1.0 giving a peak activity at Rf 0.3 and Rf 0.8 equivalent to production of cytokinins like activity to culture supernatant in *Pseudomonas* sp. found statistically superior. Peak activity registered by *Bacillus* sp. at Rf 0.1, 0.4 and Rf 0.7-1.0. Two *Pseudomonas* isolates, UH5 (0.051 µg/ml) and MC5 (0.028 µg /ml) showed highest activity in all the Rf values.

Maximum and significant peak activity of cytokinin like substances was shown by UF1 and UF8 isolates of *Bacillus* with 0.08 µg /ml at Rf 0.1 and Rf 0.4, values, respectively. A peak activity under Rf values 0.3 and 0.8-0.9 in case of *Pseudomonas* strains UH5 and MC5 was registered. Similar findings were reported by Hussain and Hasnain [23] who reported that the strains of *Bacillus licheniformis* Am2, *B. subtilis* BC1 and *P. aeruginosa* E2 produce zeatin and zeatin riboside that enhanced the cell division, fresh weight and cotyledon size in dark as well as light grown cucumber cotyledons.

Field evaluation of effective antagonist isolates

It is evident from the data presented in Table-4 that antagonist isolates MC, MC5, UF4, UF8 of *Pseudomonas* sp. and *Bacillus* sp. recorded less wilt incidence of carnation ranging between 12.0 to 21.33 per cent with higher disease control between 61.91 to 78.0 per cent indicating the potentialities of these isolates in combating the disease while other isolates such as KN6 and UFA were found superior over control but less effective than to above isolates. These isolates further showed difference on growth parameters of carnation plants. The average spike length, average number of flowers and flower size increased in above isolates in comparison to control and less effective isolates. The average stem length was maximum in MC isolates (67.67 cm) followed by MC5 (63.50 cm) in *Pseudomonas* sp. while UF4 of *Bacillus* sp. registered 65.05 cm

and UF8 as 60.67 cm. These isolates also increased the number of flower and flower diameter per plant. The average number of flowers varied between 0.75-4.75 cm in case *Pseudomonas* sp., 1.25 -4.0 cm in *Bacillus* sp. whereas maximum average size of the flower was 7.55 cm, 7.48 cm in isolates MC and MC5 and 7.20cm in UF4 in both the antagonists, respectively.

There are reports that there may be a possible involvement of induced resistance in the biological control of soil borne pathogens such as *Sclerotinia* sp., *Verticillum* sp. and *Fusarium* sp. [24, 25]. In the present study similar antagonistic activity was reported by the strains of *Pseudomonas* and *Bacillus* species obtained from carnation rhizosphere. The average spike length, flower number and flower size was increased to 3-4 folds, thus showing the positive response of these isolates on plant growth and yield. Extra-cellular chitinase of fluorescent *Pseudomonas* exhibited antifungal activity against *Fusarium oxysporum* f.sp. *dianthi* which caused carnation wilt [26]. PGPR strains SE34 and SE49 when applied at 1 x 10^8 cfu/ml as soil drench one and two weeks after planting significantly reduced disease severity of soil borne pathogen such as *Phytopthora capsici* in vegetables [27]. The evidence of PGPR *Pseudomonas inflourescens* Pf1, *Bacillus subtilis* BSCBE4, *Pseudomonas chlororaphis* PA23 and endophytic *P. flourescens* induced defense responses by accumulation of defense enzymes against blister blight and stem blight of tea and *Phyllanthus amarus,* respectively and their probable influence on growth promotion.

The ability of soil microorganism to synthesize various metabolites can affect the soil properties, the growth and chemical composition of plants and their health that help in improving the soil fertility. *Bacillus megaterium* strain promoted growth of *Arabidopsis thaliana* and *Phaseolus vulgaris* seedlings as reported by Castro-Ortiz [28]. A gene involved in cytokinin signaling in terms of growth promotion and root development might have responded in these plants. Thus few strains in our study of both the bacteria possessing properties of plant growth promotion and antagonistic effect can be exploited in managing the wilt disease that can help to improve the overall health of the carnation.

4.0 Conclusion

In the present work an attempt has been done to isolate rhizospheric bacteria viz *Pseudomonas* sp. and *Bacillus* sp from carnation. On the basis of morphological and biochemical characterization, ten isolates of *Pseudomonas* sp. and eleven isolated of *Bacillus* sp. were isolated. Further, the isolated microorganism were evaluated for various biological activities. Results from the study indicated that the isolates have capability of producing the phytohormones and thus have potential to be exploited for growth promoting activity in various crops including carnation as well as helpful in suppression of soil borne pathogens of devastating nature.

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