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Impact of Plant Growth Promoting Bacterial Root Endophytes on Growth and Nutrient Status of Brown Sarson (*Brassica rapa* L.)

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ABSTRACT

Root samples of brown sarson (Brassica rapa L.) were collected from 22 villages of three districts of Kashmir valley viz. Anantnag, Srinagar and Baramulla. All the root samples collected from various locations harbored bacteria capable of growth on TSA media. A total of 81 morphologically dissimilar isolates were selected and characterized on the basis of Gram's staining, cell and colony morphology. The study revealed that Gram negative bacteria formed dominant group. Similarly colonies with circular forms, entire margins and convex elevation were most dominated among all the isolates.Based on overall performance of isolates for each plant growth promoting attributein vitro, 12 isolates were selected for pot house studies.The inoculation of selected isolates revealed that isolate SB51 resulted in highest fall in pH (6.12) in comparison to control (6.85) and highest fall in EC was observed upon inoculation with SB51 (0.11 dSm^{-1}) against control (0.17 dSm^{-1}).The NPK content increased significantly in all the inoculated plants in comparison to uninoculated control (except SB26 for P), with highest N and K content observed in plants inoculated with SB51. The inoculation of bacterial root endophytes significantly increased leaf pigment status of brown sarson in comparison to uninoculated control. In inoculated plants chlorophyll 'a', 'b', total chlorophyll and carotenoid content ranged from 1.54-2.06, 0.83-1.11, 2.37-3.17 and 0.39-0.55 mg g^{-1} fresh weight in comparison to 1.48, 0.76, 2.24 and 0.337 mg g^{-1} fresh weight in uninoculated control.

Key words: *Endophytes, brown sarson, nutrients, leaf pigments*

INTRODUCTION

Rapeseed and mustard are the major oilseed crops, traditionally grown everywhere in the country due to their high adaptability in conventional farming systems[44]. Among the seven edible oilseed cultivated in India, rapeseed-mustard (*Brassica* spp.) contributes 28 percent in the total production of oilseeds. In India, it is the second most important edible oilseed after groundnut sharing 27 percent in the India's oilseed economy[42].

The ever-increasing population of the world has already touched the mark of 7.3 billion. To feed this burgeoning population, farmers heavily rely on the use of chemical fertilizers especially inorganic nitrogen and pesticides.

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Application of these synthetic products has many repercussions, in the form of ground and surface water contamination due to leaching and denitrification, which is detrimental for human and animal health. Secondly, manufacturing of industrial nitrogen fertilizer uses non-renewable resources like natural gas and coal and causes production of greenhouse gases viz. CO_2 and NO_2 thereby contributes to the global warming[4]. Therefore, it is high time to opt for alternative fertilizers and pesticides (bioinoculants) which can be used in sustainable agricultural practices without affecting the environment. In recent years, interest in endophytic microorganisms has increased, as they seem to play a key role in promoting better plant environment and are promising because of their potential use in sustainable agriculture etc. This comprehension may represent the basis for the utilization of endophytic population as inoculants in organic agriculture. Consequently, the development of more adapted microorganisms may be favored, thus resulting in genotypic selection. The understanding of the mutual relationship between host plant and biochemical diversity pattern of indigenous microbial population seemed to be a requirement for evaluating the impact of microbial inocula, which could affect a pre-existing balance among indigenous populations and may prove useful in the assessment of the fate of released strains and their impact on resident microbial communities. Therefore, in the present investigation isolates from brown sarson plants of diverse localities in Kashmir were isolated and compared, their impact on growth and nutrient status of brown sarson was evaluated *in vivo*.

MATERIALS AND METHODS

Collection of root samples

A survey was conducted to collect representative root samples of apparently healthy brown sarson (*Brassica rapa*L.) plants from three districts of Kashmir valley *viz.* Anantnag, Srinagar and Baramulla. The samples were randomly collected from two blocks of each selected district. Three villages were chosen per block. In the Anantnag district the villages chosen were Akura, Bona Nambal from block Dachnipora, Hutmara, Panzmulla and Rakh Chandipora from block Khoverpora. In the Srinagar District the villages chosen were Rawalpora, Rangreth, Khunmoah from South Srinagar and Zakura, Gulab Bagh, Ahmad Nagar, Dhara, Tailbal and Batapora from North Srinagar. Three sites were chosen from each village to collect root samples. The sampling was done at peak flowering stage of the crop.

Isolation of root endophytic bacteria

The samples were collected in polythene bags and immediately shifted to laboratory for further studies. The fresh healthy root samples from each site were cut and surface sterilized by 1% (w/v) active chloride (added as a sodium hypochlorite [NaOCl] solution)[49]. The roots were then crushed in a sterilized petri plate and a loopful of root sap was streaked on TSA plates. Simultaneously, from each batch, uncrushed root sample were kept on TSA medium plates as a control to ensure proper surface sterilization of root samples. The plates were incubated at 28 ± 2^0C and growth was observed daily for 2-3 days. Well established endophytic bacterial colonies were picked and restreaked on TSA medium for purification. The isolates were maintained on TSA slants at 4^0C in a refrigerator till further studies.

Morphological characterization of isolated endophytic bacteria:

The colony morphology was studied on plates after streaking a loopful of isolated colony and colony color, colony size, colony texture and gum production were observed. The bacterial isolates were Gram stained. A smear was prepared from isolated colonies and stained with Gram's stain. Slides were observed under Geytnor microscope at 100X. Cell shape, size, Gram's reactions were observed and these were photographed.

Plant growth prompting ability *in vitro*

All the endophytic bacterial isolates were given a particular score for each beneficial trait it possessed (IAA production, HCN production, siderophore production, phosphate solubilization activity, ammonia production and antifungal behaviour) and isolates having higher cumulative score were used to study

their impact on growth of brown sarson under *in vivo* conditions besides their impact on soil physio-chemical properties.

Identification of promising isolates based on morphological, biochemical and physiological characteristics.

Bacterial isolates were grown at 28±2°C for 24 h on LB medium slants/plates. The bacterial cultures were examined for various morphological, biochemical and physiological characteristics as per procedures described in Bergey's Manual of Determinative Bacteriology[15]. The inter-relationship of the microorganisms in each section of Bergey's Manual is based on characteristics such as morphology, staining reactions, nutrition, cultural characteristics and biochemical test results for specific metabolic end products.

In vivo studies

The plant growth promoting efficiency of selected endophytic bacterial isolates was assessed under pot culture conditions using brown sarsonas a test host. Seeds ofbrown sarsonwere surface sterilized by 0.2% mercuric chloride. They were sown in the pots (on 19th October, 2014) of 4 kg capacity containing 2 kg silty clay loam soil (unsterilized) and were inoculated with the respective individual isolates (control was inoculated with only broth). All the pots were inoculated with 3 ml of inoculum of bacterial isolates after every 15 days till the harvesting of crop. Control was also maintained by inoculating with broth only devoid of bacterial culture. Pots were irrigated when needed. After maturity of the crop (24th May 2015), plants were uprooted and observations on various yield attributes were recorded *viz.* no. of primary branches, no. of secondary branches, no. of siliqua, no. of seeds per siliqua, oil content and yield per plant. The yield per plant was simply calculated by collecting the seeds per plant and weighing them. Oil content was determined by following the solvent extraction technique[1], 3 g of brown sarson seeds were crushed in 3g of Na_2SO4 and the resultant powder containing oil was taken in test tubes then 20 mL of hexane was poured in the test tubes as mobile phase. Elute containing oil was stored in a vile and hexane was evaporated in hot water bath. The remaining oil was weighed and its percentage was calculated using formullae: Oil percentage = oil content/seed weight × 100.

Impact of bacterial inoculation on soil physical properties

Soil pH and Electrical conductivity

The pH of all the treated soils was determined in 1:2.5 suspension with glass electrode pH meter[19]. After determining pH, soil suspensions were kept overnight in undisturbed condition and electrical conductivity was measured by electrical conductivity meter[19].

Nutrient analysis of plant samples

Collection of plant samples

The plant samples of brown sarson were taken from each pot at physiological maturity (180 days after sowing) and were decontaminated using 2 per cent teepol solution and 0.1N HCl and washed by double distilled water in a series. Samples were air dried on filter papers and then oven dried at 60 ±5°C for 24 hours[5]. The samples were first crushed in stainless steel blender then passed through 2 mm mesh and stored in polythene bags for subsequent chemical analysis. The nutrient analysis was done as given in Table 1.

Table 1: Methods for analysis of plant samples

S. No.	Nutrient	Method
1.	N	Colorimetric method[25]
2.	P	Vanadomolybdate color reaction method[23]
3.	K	Photometric method[18]
4.	Zn, Cu, Fe and Mn	Atomic absorption spectrophotometric estimation
5.	Ca and Mg	Versenate titration method[19]
6.	S	Turbidometric method[6]

Effect of inoculation on leaf pigment content of brown sarson plants

The pigment contents (chlorophyll a, chlorophyll b and carotenoid) in fresh leaves were determined as per Arnon method[3]. The measurement of chlorophyll a, chlorophyll b and carotenoid contents was made spectrophotometrically at 662, 644 and 440.5 nm, respectively. The pigment contents in the extract were calculated by following the formula of Wettstein[50].

Statistical Analysis

The experiment was arranged in randomized block design and analysis was performed using SPSS software. The mean values were compared at $p \leq 0.05$.

RESULT AND DISCUSSION

Morphological and plant growth promoting traits

The bacterial isolates were diverse in their colony characteristics viz. color, texture, secretions, forms, margins, elevations etc. Colony secretions varied from gummy to non-gummy, colony forms varied from circular to irregular, colony margins varied from entire, serrate to lobate, colony elevation varied from flat, raised, convex to umbonate with different colors- light yellow, white, brown, orange, faint white, sharp white, waxy white, deep orange etc. In agreement with our findings bacterial endophytic colonies from sweet potato roots were of similar morphology, round shaped, and color white and pale to bright yellow[22]. Similarly, there was a large variation in colony morphology of different isolates from soybean, differences were observed in colony—color, shape, and size[17]. In present study Gram negative bacteria predominated i.e. 51 out of 81 isolates (62.96%), circular forms (58.02%), entire margins (60.49%), convex elevation (38.27%) and rod shape (67.90%) predominated among all the isolates, similar to our findings, Liu *et al*[27]., reported the existence of thin flat, faint yellow, opaque, round with smooth edge colonies among endophytic bacteria. Gupta *et al*[12]., reported the similar findings on colony shape, color, margins, elevation and gram staining. Lopez *et al*[28]., reported all the bacterial root endophytes from cactus to be Gram negative except one. Similar findings were shared by Mbai *et al*[30]. Plants select plant growth promoting endophytic bacteria that are competitively fit to occupy compatible niches without causing pathological stress on them. However, when screening bacteria for plant growth promoting (PGP) agents, it is better to select bacteria for achieving the most promising isolates having suitable colonization and PGP traits. In most researches, it has been seen that following incubation, bacterial flora are taken at random from petri dishes for further study. However, this type of selection may remove some superior bacteria in terms of PGP traits. Therefore, it is essential to study all the isolated bacteria in an economic way and select the best bacteria in terms of PGP traits. In the present study all the isolates were studied for their potential to enhance plant growth and twelve most promising isolates were selected for *in vivo* studies.

During this study it was observed that 44 isolates (54.32% of all isolates) produced IAA with the average production of 8.15 µg/mL. The highest IAA production (19.54 µg/mL) was observed in isolate SB28 and lowest (2.2 µg/mL) in SB70, which is in accordance with Verma *et al*[48]., and Khamna *et al*[21]., who reported that 56% isolated endophytic bacteria produced IAA, the production varied from 1 to 23 µg/mL. In present study, among the 81 isolates only 28 isolates were able to produce ammonia in peptone water with average production of 31.76 (µg/mL). Nimnoi *et al*[32]., isolated 10 ammonia producing actinobacteria from healthy shoots and roots of *Aquilaria crassna*

(eaglewood) and amount of ammonia ranged between 2 to 60 mg Ml^{-1}. Similarly, out of 36 selected endophytic bacterial isolates from five mangroves and two salt-marsh plant species, 61.1 percent of bacteria were ammonia producers and 69.4 percent were acetoin producers[10]. In present study 22 endophytic bacterial root endophytes associated with brown sarson roots produced chitinase enzyme with average production of 15.83 units/mL. The presence of chitinase activity in 22 out of 72 bacterial root endophytes. This result of chitinase production of bacterial endophytes induced in a colloidal chitin containing environment as previously reported[29] is in accordance with our findings. The study revealed that 31 out of 81 isolates released free phosphate from tri-calcium phosphate with average release of 95.88 mg/L. Similarly Lopez *et al*[28]., reported five P solubilizers out of 14 root bacteria from cactus while Forchetti *et al*[9]., reported five P solubilizers out of eight root endophytes from sunflower. Sgroy *et al*[41]., reported no P solubilizer among the 29 endophytic isolates from roots of *Prosopis strombulifera*. Screening of 81 bacterial root endophytes for siderophore production revealed that 22 isolate had siderophore producing ability with average siderophore production of 12.81 (% siderophore unit) and average siderophore zone of 9.95 mm. Shobha and Kumudhini[43] also reported various bacterial isolates as efficient are efficient siderophore producer and observed that *Bacillus* isolate JUMB7 produces 10% siderophores, which is similar to our findings. Pal and Gokarn[35], reported *Klebsiella* sp. producing 3.22% and 11.99% siderophore units, which falls within our range. In the present study 15 endophytic isolates produced HCN with highest absorbance (at 625 nm) observed in isolate SB51 (0.217) and lowest in isolate SB46 (0.017). Bacterial endophytes are capable of producing HCN has widely been reported by many researchers[47] isolated eight endophytic bacterial isolates from *Amaranthus hybridus*, *Solanum lycopersicum* and *Cucurbita maxima*capable of HCN production.

The isolated bacterial root endophytes were screened for their antifungal activity against various soil borne pathogens viz. *Pythium aphanidermatum*, *Dematophora necatrix*, *Fusarium oxysporum* and *Fusarium solani*. The study revealed that 25 isolates inhibited the growth of *Dematophora necatrix* with average inhibition of 28.17 percent. Similarly only 21 bacterial root endophytes showed the antifungal behavior against *P. aphanidermatum* with average inhibition of 32.55 percent. Only 17 bacterial endophytes showed antifungal activity against *Fusarium oxysporum* with average inhibition of 33.15 percent. In the same way, only 18 bacterial root endophytes showed antifungal activity against *Fusarium solani* with average inhibition of 33.25 percent. It has been reported that the proportion of endophytes able to suppress disease symptoms has been found to be high in comparison to that observed for rhizosphere bacteria[37]. Ziedan[51] reported that out of 25 bacterial isolates obtained from inner tissue of peanut plant roots (90 days old) only three isolates of *Bacillus subtilis* and one of *P. fluorescens* showed ability to suppress *A. niger* and *F. oxysporum*. Further, *B. subtilis* was best antagonizing isolates followed by *P. flourescens*. Microbial production of extracellular metabolites like HCN reportedly contribute biocontrol nature of root pathogens[13]. It has recently been reported that the metabolites like, HCN, ammonia, chitinase, siderophore etc. have a prominent role in biocontrol activity of bacterial endophytes[20]. The broad spectrum inhibition of phytopathogens by isolates, SB13 and SB14 found in present study could be as a result of HCN toxicity brought about in fugal pathogen

niches by the bacterial isolates or may be due to the production of chitinase or siderophore.

Bacterial identification on morphological, biochemical & physiological basis

Twelve isolates having most outstanding attributes were identified up to genus level by their morphological, physiological and biochemical characters as per procedures described in Bergey's Manual of Determinative Bacteriology. Morphological characters used were colony morphology, pigment production, Gram staining, bacterial morphology, bacterial arrangement and endospore production. Study of physiological and biochemical characters included indole production, citrate utilization test, Voges-Proskauer reaction, methyl red test, oxidase test, acid production, H_2S production and hydrolysis of cellulose and starch. The studies revealed that all the selected isolates were rods, excepting SB73 which was minute cocci in shape. Only five isolates were Gram positive while rest were Gram negative. All the Gram positive isolates were spore formers with spore position central except in SB64 wherein spores were terminal in position. Only one among all the 12 isolates was indole producing (SB28) thus, 11 out of 12 were devoid of indole production. Only two isolates (SB51, SB26) were methyl red positive while none of the isolate was found to be positive with respect to Voges-Proskauer reaction, however citrate utilization ability was found in SB13, SB58, SB26, SB46 and SB55. Only isolate SB46 was negative to oxidase activity while rest were positive, however all the isolates were positive to catalase, H_2S production was observed in SB64, cellulose hydrolyzing ability was observed in none of the isolate, while starch hydrolyzing ability was observed in SB51, SB64 and SB28. Acid production was observed in three isolates namely SB14, SB58 and SB43 isolates (Table 2). Li *et al*[24]., also reported the similar findings with respect to citrate utilization test, Voges-Proskauer reaction and methyl red test in bacterial root endophytes of typha. Similarly, Sun *et al*[45]., with respect to methyl red test H_2S production and hydrolysis of cellulose in bacteria root endophytes of rice.

After analyzing the above mentioned attributes it was concluded that isolate SB13, SB14, SB64, SB43 and SB46 belong to genus *Bacillus*, isolates SB26, SB51, SB58, SB28, SB55 and SB67 belong to genus *Pseudomonas* and isolate SB73 belongs to genus *Micrococcus*. Similar to our findings many researchers have reported the predominance of *Bacillus* sp. and *Pseudomonas* sp.in plant root tissues[47]. A total of 87 culturable endophytic bacterial isolates were obtained from adult plant leaves, various parts of the berry (e.g., crown, pulp, peduncle and seed), stems, and roots of seedlings of coffee (*Coffea arabica*) plants collected from Colombia (*n* = 67), Hawaii (*n* = 17), and Mexico (*n* = 3). Both gram positive and gram negative bacteria were isolated, with a greater percentage (68%) being gram negative. The highest number of bacteria among the coffee berry tissues sampled was isolated from the seed, and includes *Bacillus*, *Burkholderia*, *Clavibacter*, *Curtobacterium*, *Escherichia*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas*[46]. Which is similar to our findings. Panchal and Ingle isolated five different bacterial species from roots of safed musli. *Rhodoferax, Pelomonas, Uliginosibacterium, Pseudomonas, Aeromonas, Rhizobium, Sulfurospirillum*, *Ilyobacter*, *Bacteroides*, *Serratia*, *Bacillus*, *Paenibacillus*, *Arthrobacter*, *Micrococcus*, *Curtobacterium, Pleomorphomonas* and *Azospirillum*, are the genera of bacterial endophytes which were isolated from roots of different crops and most common bacterial genera in roots are usually *Bacillus, Pseudomonas and Micrococcus.*

Table 2: Morpho-biochemical and physiological characterization of endophytic bacterial isolates

Sr. No.	Morpho-biochemical and physiological characters	Root endophytic isolate											
		SB13	**SB14**	**SB28**	**SB51**	**SB58**	**SB64**	**SB26**	**SB43**	**SB46**	**SB55**	**SB67**	**SB73**
1	Colony morphology	Brownish, entire, irregular, slightly raised	Light brown, entire, circular, convex	Light creamy white, entire, circular, convex	Faint yellow, entire, circular, convex	Brown, entire, circular, flat	Waxy white, entire, circular, raised	Deep orange, entire, circular, convex	Gummy white, serrate, irregular, raised	White, lobate, irregular, flat	Brown, undulate, irregular, umbonate	Light brown, entire, circular, convex	Light orange, entire, circular, convex
2	Pigment production	-	-	-	-	-	-	-	-	-	-	-	-
3	Gram reaction	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
4	Bacteria morphology	Medium rods	Medium rods	Long rods	Long to medium rods	Long rods	Medium rods	Small rods	Long rods	Medium rods	Small rods	Small rods	Minute cocci
5	Bacteria arrangement	Pairs	Pairs & chains	Singly	Singly	Singly	Pairs & chains	Singly	Pairs & chains	Pairs	Singly	Singly	Pairs
6	Endospore position	Central	Central	-	-	-	Terminal	-	Central	Central	-	-	-
7	Indole production	-	-	+	-	-	-	-	-	-	-	-	-
8	Methyl red test	-	-	-	+	-	-	+	-	-	-	-	-
9	Voges-Proskauer reaction	-	-	-	-	-	-	-	-	-	-	-	-
10	Citrate utilization	+	-	-	-	+	-	+	-	+	+	-	-
11	Oxidase	+	+	+	+	+	+	+	+	-	+	+	+
12	Catalase	+	+	+	+	+	+	+	+	+	+	+	+
13	H_2S production	-	-	-	-	-	+	-	-	-	-	-	-
14	Starch hydrolysis	-	-	+	+	-	+	-	-	-	-	-	-
15	Cellulose hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-
16	Acid production	-	+	-	-	+	-	-	+	-	-	-	-
	Probable genus	***Bacillus***	***Bacillus***	***Pseudomonas***	***Pseudomonas***	***Pseudomonas***	***Bacillus***	***Pseudomonas***	***Bacillus***	***Bacillus***	***Pseudomonas***	***Pseudomonas***	***Micrococcus***

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***In vivo* studies**

Effect on plant nutrient status

The plant inoculation of selected endophytes resulted into change in soil pH from 6.12 to 6.85. The isolate SB51 resulted in highest fall in pH (6.12) and least fall was observed in control (6.85). Similarly, the observations on equivalent conductivity (EC) revealed that the significantly highest fall in EC was observed upon inoculation with SB51 (0.11 dSm^{-1}) against the control (0.17 dSm^{-1}). The significantly least fall in EC was observed upon inoculation with SB67 and SB73 (0.15 dSm^{-1} each. There was significant improvement in activities with highest activity in SB51 and less in SB46 (Table 3). The NPK content increased significantly in all the inoculated plants in comparison to uninoculated control (except SB26 for P), with highest N and K content observed in plants inoculated with SB51. The higher P content was reported in SB46. The least NPK content was observed in plants inoculated with SB28, SB26 and SB55 respectively. The calcium content increased significantly in all the inoculated plants except SB43 in comparison with uninoculated control (1.22%), with highest calcium content observed in the plants inoculated with SB28 (1.37%) and least calcium content among all the inoculated plants in SB43 (1.24%). The magnesium content increased significantly in all the inoculated plants except SB43 and SB67 in comparison to with uninoculated control (0.12%), with highest magnesium content observed in the plants inoculated with SB51 (0.25%) followed by SB28 (0.23%). The least magnesium content among all the inoculated plants was observed in the plants inoculated with SB43 (0.13%) followed by SB67 (0.14%). The sulphur content increased significantly in all the inoculated plants except SB55 and SB67 in comparison with uninoculated control (0.21%), with highest sulphur content observed in the plants inoculated with SB51 (0.39%). The least sulphur content among all the inoculated plants was observed in plants inoculated with SB43 (0.23%) followed by SB55 and SB67 [0.25% both (Table 4)]. The zinc, copper, iron and manganese, content in inoculated plants showed significant increase as compared to uninoculated control (22.38 ppm). Highest zinc and iron was found in plants inoculated with SB28 (47.57 and 187.30 ppm respectively). While least zinc and iron content was seen in plants inoculated with SB43 (27.16 ppm). The copper content was highest in plants inoculated with SB28 (18.01 ppm) followed by SB51 (17.28 ppm) and SB14 (17.21) respectively, least copper content among all the inoculated plants was observed in plants inoculated with SB43 (10.77 ppm). Highest iron content was found in the plants inoculated with SB28 (187.30 ppm) followed by SB51 (167.52 ppm) and SB14 (161.24 ppm), least iron content among all the inoculated plants was noted in plants inoculated with SB43 (101.63 ppm). Highest manganese content was observed in plants inoculated with SB51 (125.52 ppm) followed by SB28 (117.73) and SB14 (112.52 ppm), least manganese content among all the inoculated plants was seen upon inoculation with SB43 [(75.53 ppm) (Table 5)]. In inoculated plants chlorophyll 'a', 'b', total chlorophyll and carotenoid content ranged from 1.54-2.06, 0.83-1.11, 2.37-3.17 and 0.39-0.55 mg g^{-1} fresh weight in comparison to 1.48, 0.76, 2.24 and 0.337 mg g^{-1} fresh weight in uninoculated control. The highest chlorophyll 'a', total chlorophyll and carotenoid content was observed in the plants inoculated with SB51 followed by SB28, SB14 and SB58 (Fig. 1). The changes observed in present study suggest a direct effect of bacterial isolates, as well as indirect effect through changes in microbial composition in the rhizospheric soil. Rana *et al*[36]., reported that endophytic bacterial inoculation increased plant N, P uptake as well as microbial biomass carbon and soil biological properties like dehydrogenase activity, phosphatase activity etc. over uninoculated control. The major reason for favorable change appears increase in bacterial population in the soil which enhanced organic

matter decomposition releasing minerals and are metabolized to form cell constituents. Dutta and Neog[7] observed increase in phosphatase, dehydrogenase and urease activates and soil carbon content due to bacterial inoculation. Hassan and Bano[14] reported that *Pseudomonas* sp. inoculation in wheat resulted in increased grain yield, availability of N, P, Ca, and K contents availability in soil.The bacterial inoculants of endophytic bacteria are among the most important plant growth promotors which work through a number of mechanisms[11]. Some species are known to supply plants with nutrients nitrogen, phosphorus, iron, etc.[40]. El-Ghany *et al*[8]., reported that bacterial inoculations improve soil physical properties like EC, bulk density, pH, etc. through organic matter degradation products, microbial gums (EPS) and root growth promoting substances enhance soil aggregation process, subsequently soil penetrability resistance decreases. The net result is less cohesion relation to adhesion forces between soil particles, which may be one of the reasons of improved soil physical properties. Ardebili *et al*[2]., reported that the beneficial bacterial endophytes enhance plant growth in tomato. Endophytic microorganisms enhance plant growth through production of plant hormones and antimicrobial metabolites, as well as through solubilization and mobilization of the soil nutrients[26]. Hoon *et al*[16]., too observed enhanced nutrient uptake and overall yield in pepper as a result of *Pseudomonas* sp. inoculation. Endophytic bacteria are more often capable of triggering physiological changes that promote the growth and development of plants[33]. Similar to our investigation, Padder *et al*[34]., reported that *Pseudomonas* sp. enhanced chlorophyll and carotenoid content and other growth parametersMany bacteria capable are able to promote plant growth by solubilizing sparingly soluble inorganic phosphates in the soil[38]. Moreover *P. fluorescens* strains are considered to be good plant growth promoters through the production of growth-stimulating hormones[39] and this could be another attribute affecting growth, nutrient uptake and finally the crop yield.

Table 3: Effect of endophytic bacterial inoculation on soil* physical properties

Isolate	Soil pH	EC (dSm^{-1})
Control	6.85	0.17
SB13	6.35	0.13
SB14	6.37	0.13
SB26	6.57	0.14
SB28	6.32	0.13
SB43	6.57	0.14
SB46	6.55	0.14
SB51	6.12	0.11
SB55	6.45	0.14
SB58	6.22	0.13
SB64	6.45	0.14
SB67	6.55	0.15
SB73	6.60	0.15
C.D ($p \leq 0.05$)	0.081	0.008
SE(m)	0.028	0.003
C.V%	0.876	4.057

*The soil was silty clay loam

Table 4: Effect of selected bacterial endophytes on macro-nutrient content (%) in brown sarson

Isolate	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur
Control	1.34	0.13	1.06	1.22	0.12	0.21
SB13	1.83	0.16	1.25	1.29	0.21	0.27
SB14	1.55	0.17	1.38	1.34	0.21	0.36
SB26	1.68	0.13	1.15	1.28	0.16	0.26
SB28	1.38	0.16	1.45	1.37	0.23	0.32
SB43	1.48	0.18	1.15	1.24	0.13	0.23
SB46	1.45	0.27	1.18	1.25	0.17	0.24
SB51	2.06	0.16	1.55	1.36	0.25	0.39
SB55	1.53	0.18	1.14	1.28	0.15	0.25
SB58	1.95	0.24	1.33	1.34	0.19	0.32
SB64	1.75	0.15	1.25	1.26	0.17	0.36
SB67	1.50	0.16	1.24	1.28	0.14	0.25
SB73	1.44	0.19	1.21	1.26	0.16	0.26
C.D (p≤0.05)	0.043	0.004	0.037	0.036	0.020	0.040
SE(m)	0.015	0.002	0.013	0.012	0.007	0.014
C.V.%	1.861	1.702	2.017	1.920	7.946	1.923

Table 5: Effect of selected endophytic bacterial inoculation on plant micro-nutrient status (ppm)

Isolate	Zinc	Copper	Iron	Manganese
Control	22.38	7.78	81.59	69.50
SB13	38.50	14.49	139.79	102.28
SB14	46.13	17.21	161.24	112.52
SB26	35.93	11.20	125.39	86.94
SB28	47.57	18.01	187.30	117.73
SB43	27.16	10.77	101.63	75.53
SB46	37.06	13.10	128.51	91.95
SB51	53.27	17.28	167.52	125.52
SB55	29.16	11.33	113.17	78.63
SB58	41.71	14.96	149.64	105.28
SB64	36.12	14.40	134.28	97.34
SB67	33.10	12.21	121.51	85.75
SB73	35.15	13.32	129.14	91.14
C.D (p≤0.05)	0.427	0.266	0.462	0.521
SE(m)	0.149	0.093	0.161	0.181
C.V.%	0.801	1.369	0.240	0.380

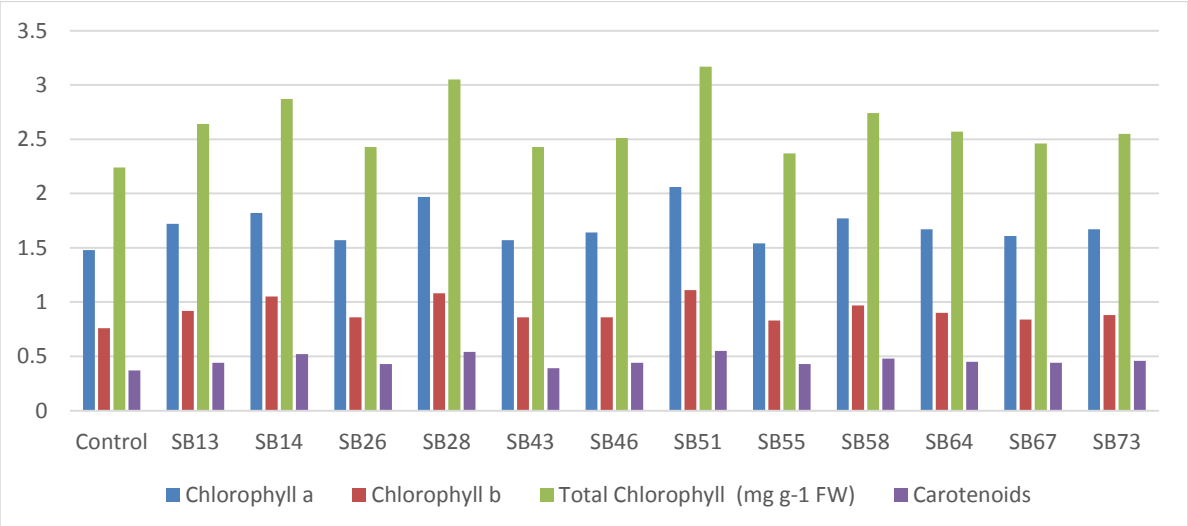

Fig. 1: Effect of endophytic bacterial inoculation on leaf pigments of brown sarson

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