Journal of Advances in Microbiology

Volume 24, Issue 3, Page 8-16, 2024; Article no.JAMB.114429
ISSN: 2456-7116

Identification of Rhizosphere Endophytes and Evaluation of Their Impact on Growth of Chia (*Salvia hispanica* L.)

Sumarani D V [a*], Lakshmeesha R [a], J Harish [b], K M Harini Kumar [a] and Babu C V [a]

[a] Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, Karnataka- 560065, India.
[b] Department of Plant Pathology, University of Agricultural sciences, Bangalore, Karnataka- 560065, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2024/v24i3802

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114429

Original Research Article

Received: 04/01/2024
Accepted: 09/03/2024
Published: 13/03/2024

ABSTRACT

Endophytic microorganisms promote root growth, improve nutrient uptake efficiency and produce more yields. By collaborating with endophytic microorganisms, plants experience enhanced growth. This study was focused on endophytes from Chia plants in Bhukkapatna, Tumakur with the objective of molecular characterization of efficient endophytic microorganisms and its impact on growth of Chia. Twelve bacterial isolates were identified, with eight displaying nitrogen fixation, phosphate solubilisation, potassium fixation, production of IAA, GA, siderophore and exopolysaccharide production. Molecular characterization using 16S rRNA revealed their identities

**Corresponding author: E-mail: sumaranidv23@gmail.com;*

as *Pseudomonas* fluorescens, Bacillus cereus, Stenotrophomonas maltophilia, and Enterobacter cloacae. Among these, Enterobacter cloacae (CEPB8) exhibited highest positive impact on Chia plant growth. In greenhouse conditions, treatments involving combinations of all four demonstrated the highest plant height (35.467cm) and biomass (158.37 grams) at harvest, surpassing the control group. Combined treatments outperformed individual strains, highlighting synergy. The study affirms that endophytic microorganisms significantly enhanced growth in Chia plant.

Keywords: Endophytes; plant growth; 16S rRNA; Chia yield.

1. INTRODUCTION

Agricultural output levels have increased dramatically as a result of the green revolution, but at a tremendous environmental cost. The increasing use of chemical fertilizers and crop varieties with high yields has resulted in the deterioration of the soil and reduction in its fertility. Alternatives to chemical-based techniques are currently being researched by scientists. The beneficial bacteria of the rhizosphere and endophytic microorganisms are essential for improving plant growth, health, and nutrient availability [1].

The plant growth promotion involves the phytohormone production, nitrogen fixation, siderophore production, solubilization of inorganic substances (P, K, Zn etc.) and making it conveniently available to the plants (Timofeeva et al. 2023). Through the symbiotic association with plants, endophytic microorganisms promote root growth and improve nutrient uptake efficiency. This produces stronger, healthier plants that can tolerate stress and produce more yields. (Pandey *et al*., 2019)

Chia (*Salvia hispanica* L.) is an annual short-day herbaceous species from the family Lamiaceae. There is an increase in the demand for this crop because of its anti-inflammatory, antioxidant properties and its potential use in controlling phytopathogenic bacteria and fungi. Chia seeds are considered a “superfood” due to nutritional characteristics, Superfoods’ are claimed to prevent diseases as well as improving overall health, though the lack of explicit criteria means that any food can be labelled ‘super’ without support from scientific research. Typically, these ‘superfoods’ are rich in a particular nutrient for example antioxidants or omega-3 fatty acids [2-3].

In comparison to other traditional cereal crops like finger millet and maize, the cultivation costs for chia typically amount to approximately 15,000/acre. The average seed yield of chia per acre falls within the range of 500-600 kg. However, with the application of appropriate agronomic practices, some reports have indicated a higher yield potential, reaching up to 850 kg per acre [4].

By optimizing agricultural practices and endophytic microorganisms in the form of biofertilizers, there is the potential to significantly increase the growth of Chia plants, thereby making it a more attractive and lucrative crop for farmers. The rhizosphere and roots of Chia plants harbor a diverse community of endophytic microorganisms. The following study was done to identify and investigate the influence of endophytic microorganisms on the growth and yield of chia plants.

2. MATERIALS AND METHODS

2.1 Isolation of Endophytic Micro-organisms from the Roots and Soil

The healthy Chia plants and the rhizosphere soil associated with them were collected in polythene bags from Bhukkapatna village, Sira taluk, Tumkur district and brought to the laboratory and was kept in refrigerator at 4^{o} C for one day. Plant roots were cleaned by washing them under tap water to remove soil and most epiphytes. They were then cut into approximately 1 cm segments and surface sterilized using 70% ethanol for 1 minute, followed by a 3-minute treatment with a 2% sodium hypochlorite solution. The sterilized root fragments were thoroughly rinsed with sterile water. These prepared root bits were placed onto King’s B, Nutrient agar (NA) and Kuster’s agar plates with gentle pressure applied. The plates were then incubated at 28 °C for 48 hours. Serial dilution was performed for the rhizosphere soil and fractions 10^{-3} and 10^{-4} dilution were plated on media and incubated at 28 °C for 3 days. The isolates were picked and studied for colony morphology, pigmentation and Gram staining was performed.

2.2 Identification of Efficient Endophytic Microorganisms Involved in Plant Growth Promotion

2.2.1 Nitrogen fixation

The nitrogen fixing ability of the selected bacterial endophytes was estimated by utilising Norris's glucose nitrogen free medium. The isolates were streaked on the this medium and incubated at 28 ºC for 4 - 7 days. The isolates which were able to form colonies in the nitrogen free medium were designated as positive for N fixation [5].

2.2.2 Phosphate solubilization

The qualitative evaluation of tricalcium phosphate solubilization in the isolated strains was done using Pikovskaya's agar. The bacterial isolates were spot inoculated on the surface of Pikovskaya's agar medium and was incubated at 28 °C for 72 hours. The formation of a clear zone around the colony indicated phosphate solubilization [6].

2.2.3 Potassium solubilization

The selected bacterial isolates were spot inoculated on the Aleksandrov's medium and incubated at 28 °C for 72 hours. The formation of clear zone around the growth of colony is positive for potassium solubilization [7].

2.2.4 Siderophore production

The bacterial isolates were tested for their ability to produce siderophores. Supernatant extracted from 3 days old culture was grown in nutrient broth. One mL of culture was centrifuged at 10,000 rpm for 10 min. The supernatant and the universal Chrome Azurol S (CAS) reagent were taken in the ration of 1:1 as described by Schwyn and Neilands [8] to detect the siderophore production kept under room temperature for 1 h. The change of colour from blue to yellow indicated the production of siderophores.

2.2.5 IAA (Indole Acetic Acid) production

IAA production was estimated using nutrient broth. Inoculation was done in 15ml nutrient broth and incubated at 28ºC for 4-5 days. After this period, cultures were collected by centrifugation for 10 mins at 5000 rpm. In 10µl supernatant, two drops of orthophosphoric acid and 5ml Salkowski's reagent were mixed (Salkowski's reagent= 50ml, 35 per cent perchloric acid+1ml 0.5M $FeCl_3$). Production of pink colour indicated IAA production [9].

2.3 Molecular Characterization of Endophytic Microorganisms using 16S rRNA

Bacterial isolates were grown in nutrient broth and incubated at 28ºC for overnight under shaking. About 1.5 ml of culture was taken in a microcentrifuge tube, spun for 7 minutes and the supernatant was decanted. To the pellet 567µl of TE Buffer, 3µl of 20 mg/mL proteinase-k, 30µl of 10 per cent SDS was added and incubated for one hour at 37ºC. Again, 100µl of 5 M NaCl and 80 µl of CTAB solution were added and incubated for ten minutes at 65ºC. Further, it was extracted with an equal volume of chloroform: isoamyl alcohol and the aqueous phase were transferred to the fresh tube and to this equal volume of phenol: chloroform: isoamyl alcohol was added and subjected to centrifugation at 8,000 rpm for 5 min at 4ºC. It was washed with chloroform: isoamyl alcohol until the clear supernatant was obtained. Then an equal volume of chilled propanol was added, mixed gently and kept at -20º C overnight for precipitation of DNA. Later it was centrifuged at 10,000 rpm for 20 min at 4ºC to pellet the DNA. The pellet was washed with 70 per cent ethanol and air-dried. The DNA was dissolved in TE buffer. The DNA purity was analysed by estimating the ratio between 260 nm and 280 nm in microplate reader. The DNA samples between the ratio 1.8 to 2.0 were diluted to a final concentration of 40 ng/µL using TE buffer. (Sambrook *et al*.,1989).

The 16S rRNA gene (~1.5 kb) was amplified employing universal primers (16SF 5´-AGAGTTTGATCCTGGCTCAG-3´; 16SR 5´-CTACGGCTACCTTGTTACGA-3´). Amplification was carried out in Nexus Mastercycler at the Department of Plant Biotechnology UAS, GKVK with initial denaturation (95ºC), annealing (55ºC) and extension temperature (72ºC). Amplification products were analysed by gel electrophoresis at 100 V (in 0.8 per cent agarose gel, 0.2 µg of ethidium bromide mL^{-1}) in a 1X TBE, and visualized under UV light in a gel documentation unit. Sequencing of amplified PCR product was carried out on commercial basis from Barcode Biosciences, Bangalore, Karnataka. The partial sequences of nucleotides were compared with available sequences from NCBI databases and sequences showing >99 % similarity was

retrieved by Nucleotide Basic Local Alignment Search Tool (BLASTN) program available at the National Center for Biotechnology Information (NCBI) BLAST server. 10 sequences having more than 95% query cover and percent identity with 0 E value were selected and FASTA file of those sequences were downloaded. Multiple sequence alignment was done using MUSCLE algorithm to align DNA. The aligned sequences were saved in MEGA format and using this phylogenetic tree was constructed using maximum likelihood method with 100 bootstrap replications and nucleotide substitution. The multiple sequence alignment and phylogenetic tree was constructed using MEGAX 11 software using the maximum likelihood method [10].

2.4 Evaluation of Selected Endophytic Microorganisms for Growth Promotion in Chia under Greenhouse Condition

Chia (*Salvia hispanica* L.) seeds were collected from AICRP for dryland crops, UAS, GKVK, Bengaluru. The potting mixture was prepared and sterilized (121 ºC at 15 psi pressure for 15 minutes). The potting mixture had red sandy loam soil: sand: farmyard manure (FYM) in the ratio of 2:1:1. The potting mixture was filled into surface sterilized (wiped with 70 % ethanol) 30 pots of 10 Kg capacity and were arranged in the greenhouse in the Department of Plant Biotechnology, UAS, GKVK, Bengaluru.

Uniformly grown seedling roots were dipped in bacterial suspension (~ 2×10^6 cells mL^{-1}) of 5 ml (OD_{560}=2.0) for 3 hours through seedling dip method. The 15 days old seedling roots were dipped in the endophyte solution and kept for 15– 20 min. Then, the seedlings were kept under the shed for 20 minutes to dry up. These treated seedlings were transplanted in the pots and were kept in the greenhouse conditions. The following 10 treatments with 3 replications were evaluated under the greenhouse condition.

Data on different characters like number of leaves, number of branches, plant height, root dry weight, root length and plant biomass were analysed as outlined for CRD using the WASP 1.0 software (https://ccari.icar.gov.in/waspnew.html) from ICAR website. The Duncan's Multiple Range Test (DMRT) was followed to find out the significant differences between the treatments.

Table 1. Allocation of treatments using selected endophytic microorganisms to Chia plants

Treatment	Seedlings treated with solution
T_1	Control (No treatment)
T_2	*Pseudomonas fluorescens* CEPB2
T_3	*Enterobacter cloacae* CEPB8
T_4	*Bacillus cereus* CEPB5
T_5	*Stenotrophomonas maltophia* CEPBA
T_6	CEPB2+CEPB8
T_7	CEPB5+CEPBA
T_8	CEPB2+CEPBA
T_9	CEPB5+CEPB8
T_{10}	CEPB2+ CEPBA+CEPB5+CEPB8

3. RESULTS AND DISCUSSION

3.1 Isolation of Endophytic Micro-organisms from Root and Soil

The growth of endophytes surrounding the incubated root bits and from rhizosphere soil was observed in nutrient agar media. Twelve different isolates were chosen from the three colonies surrounding the root bits on each petri plate for further investigation. Among them nine isolates (CEPB1, CEPB2, CEPB3, CEPB4, CEPB5, CEPB6, CEPB7, CEPB8 and CEPB9) having different colour and colony morphology were chosen from root and three isolates (CEPBA, CEPBB and CEPBC) were chosen from rhizosphere soil.

3.2 Morphological Characterization of Endophytic Microorganisms

The cell shape, colour and shape of the colonies are shown in the Table 2. These isolates exhibited a mixed composition, comprising 33.33% Gram-positive and 66.6% Gram-negative types.

Similar results were observed by Suhandono *et al*. [11] (Lu et al. 2018) where Gram-positive bacilli bacteria with a white colony morphology, nonmotility, circular form, whole edge, and convex elevation were identified as the Sp.2 isolate. Gram-negative cocci bacteria with a white colony morphology, nonmotility, circular form, whole edge, and convex elevation were identified as Sp.4 isolates isolated from Rambutan fruits (*Nephelium lappaceum* L.)

Table 2. Morphological and physiological characteristics of endophytic isolates CEPB1, CEPB2, CEPB3, CEPB4, CEPB5, CEPB6, CEPB7, CEPB8, CEPB9, CEPBA, CEPBB and CEPBC from Chia root and rhizosphere soil

Sl no.	Isolates	Color	Shape	Cell shape	Gram reaction
1	CEPB1	Green	Round	Rod	Negative
2	CEPB2	Green	Round	Rod	Negative
3	CEPB3	Cream	Round	Rod	Positive
4	CEPB4	Opaque	Round	Rod	Negative
5	CEPB5	Cream	Round	Rod	Positive
6	CEPB6	Opaque	Dome	Cocci	Positive
7	CEPB7	Opaque	Round	Rod	Negative
8	CEPB8	Opaque	Round	Rod	Negative
9	CEPB9	Cream	Dome	Cocci	Positive
10	CEPBA	Yellowish	Round	Rod	Negative
11	CEPBB	Opaque	Round	Rod	Negative
12	CEPBC	Cream	Round	Cocci	Negative

3.3 Identification of Efficient Endophytic Microorganisms for Plant Growth Promotion

CEPB1, CEPB2, CEPB4, CEPB5, CEPB7, CEPB8, CEPB9, CEPBB and CEPBC formed colonies when streaked on Norris's glucose nitrogen free medium and showed their ability to fix nitrogen. CEPB4, CEPB5, CEPB7, CEPB8, CEPB9, CEPBA and CEPBB formed clear zones in Pikovskaya's media and were efficient in phosphate solubilization. Among the 12 bacteria isolated, 75% of them solubilised potassium and formed clear zones in Aleksandrov's agar media around the inoculated spot area. 41.6% (CEPB2, CEPB4, CEPB7, CEPB8 and CEPBC) were efficient in production of IAA. 66.6% of the isolates produced yellow precipitation in ethanol and showed exopolysaccharide production. Eight of the isolates CEPB1, CEPB3, CEPB4, CEPB5, CEPB6, CEPB8, CEPBA and CEPBB changed the colour of CAS reagent to yellow and produced siderophore.

Husseiny *et al*. [12] performed *in-vitro* screening to evaluate the potential of the bacterial isolates in promoting plant growth through various attributes, including Indole acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, and nitrogen fixation. Among them 95% of the isolates were capable of producing IAA, 7.5% produced ACC deaminase and 15% exhibited nitrogen-fixing abilities. Based on their plant growth promoting potential, a total of 25 bacterial isolates belonging to 16 different genera and distributed across four phyla were sequenced and identified. Therefore, from the above discussed results CEPB1, CEPB2, CEPB4, CEPB5, CEPB7, CEPB8, CEPBA and CEPBB were found to have plant growth promoting properties and were further selected for molecular characterization using 16S rRNA.

3.4 Molecular Characterization of Endophytic Microorganisms using 16s rRNA

The PCR products having an amplicon size of about 1450 bp were sent for sequencing and the sequences acquired were compared to sequences on the GenBank at the NCBI website. The sequenced data obtained were subjected to nucleotide BLAST in NCBI website in FASTA format. The isolates were identified as *Pseudomonas fluorescens*, *Bacillus cereus*, *Stenotrophomonas maltophilia* and *Enterobacter cloacae*.

Similar procedure was followed by Ashmawy *et al*. [13] where characterized 14 bacterial isolates recovered from seeds of different varieties of tomato, eggplant, black nightshade and tobacco. The bacterial isolates were identified at the molecular level by PCR reactions utilizing the 16S rRNA gene. Partial DNA sequences analysed using BLAST tool revealed that the inferred 16S rRNA partial sequences of the 7 isolates showed similarity to *Pantoea ananatis* (3 isolates), *Pseudomonas syringae* pv. tomato (2 isolates) and *Xanthomonas vesicatoria* (2 isolates). Dubey *et al*. [14] extracted genomic DNA from the selected bacterial isolates and performed PCR amplification of the 16S rRNA gene. Based on 16S rRNA gene sequencing,

endophytic bacterial strain AKAD A1-1 showed a close resemblance to *Bacillus cereus*; AKAD A1-2 and AKAD A1-16 were found to be affiliated with members of the *Pseudomonas* spp.

Table 3. Identification of efficient endophytic microorganisms based on plant growth promoting characters

Isolate	Nitrogen fixation	Phosphate solubilization	Potassium fixation	IAA	Exopolysaccharide production	Siderophore production
CEPB1	+	-	+	-	+	+
CEPB2	+	-	+	+	+	-
CEPB3	-	-	-	-	+	+
CEPB4	+	+	+	+	-	+
CEPB5	+	+	-	-	-	+
CEPB6	-	-	+	-	+	+
CEPB7	+	+	+	+	-	-
CEPB8	+	+	+	+	+	+
CEPB9	-	-	+	-	-	-
CEPBA	-	+	+	-	+	+
CEPBB	+	+	+	-	+	+
CEPBC	+	-	-	+	-	-

Legend: Nitrogen fixation: (+) growth and (-) no growth on Norris glucose nitrogen free medium; Phosphate solubilization: (+) formation of clear zones (-) absence of clear zones; Potassium fixation: (+) formation of clear zones (-) absence of clear zones; IAA (Indole acetic acid) production: production of pink colour (-) no change in the colour; Siderophore production: (+) Change of colour to yellow (-) no change; Exopolysaccharide production: (+) yellow precipitation (-) no precipitation

Table 4. Similarity percentage of the isolate with organism

Isolate	Similarity percentage (%)	Organism
CEPB1	98.81	*Pseudomonas fluorescens*
CEPB2	98.63	*Pseudomonas fluorescens*
CEPB4	98.83	*Enterobacter cloacae*
CEPB5	99.28	*Bacillus cereus*
CEPB7	99.17	*Enterobacter cloacae*
CEPB8	98.55	*Enterobacter cloacae*
CEPBA	97.00	*Stenotrophomonas maltophilia*
CEPBB	98.95	*Enterobacter cloacae*

Table 5. Effect of inoculation of endophytic microorganisms on number of branches and number of leaves of Chia plant at 30 DAT, 60 DAT and 90 DAT

	Number of branches			Number of leaves		
Treatment	30 DAT	60 DAT	90 DAT	30DAT	60DAT	90DAT
T1	2.66^{e}	5.66^{h}	8.66^{h}	8.00^{h}	21.33^{j}	43.66^{j}
T2	5.33^{c}	8.33^{f}	12.00^{f}	15.60^{ef}	32.00^{g}	61.33^{g}
T3	5.00^{c}	9.60^{e}	13.30^{e}	15.00^{f}	34.33^{f}	68.00^{f}
T4	4.00^{d}	7.00^{g}	10.00^{g}	10.33^{g}	25.33^{i}	50.00^{i}
T5	5.00^{c}	8.00^{f}	11.00^{g}	12.00^{g}	28.33^{h}	56.33^{h}
T6	7.66^{b}	16.00^{b}	20.00^{b}	25.33^{b}	46.66^{b}	91.00^{b}
T7	7.00^{b}	12.00^{d}	15.30^{d}	17.00^{e}	37.00^{e}	74.66^{e}
T8	5.33^{c}	14.30^{c}	17.30^{c}	19.67^{d}	39.66^{d}	79.60^{d}
T9	5.33^{c}	15.00^{b}	19.00^{b}	23.30^{c}	44.00^{c}	85.33^{c}
T10	11.00^{a}	17.60^{a}	22.60^{a}	32.60^{a}	54.66^{a}	97.30^{a}
CD (0.05)	0.88	0.76	0.98	1.86	2.17	1.24

Table 6. Effect of inoculation of endophytic microorganisms on root length and root dry weight of Chia plant at 30 DAT, 60DAT and 90DAT

	Root length			Root dry weight		
Treatment	**30 DAT**	**60 DAT**	**90 DAT**	**30 DAT**	**60 DAT**	**90 DAT**
T1	9.03^{i}	12.80^{j}	28.93^{j}	0.931^{j}	3.08^{h}	5.40^{j}
T2	12.36^{g}	19.33^{g}	39.73^{g}	1.98^{g}	4.97^{e}	6.68^{g}
T3	14.86^{f}	22.06^{f}	42.20^{f}	2.06^{f}	5.08^{e}	6.95^{f}
T4	11.00^{h}	14.30^{h}	33.70^{h}	1.23^{i}	3.79^{g}	5.72^{i}
T5	11.23^{gh}	16.73^{h}	35.93^{h}	1.59^{h}	4.64^{f}	5.96^{h}
T6	26.76^{b}	35.86^{b}	53.20^{b}	3.24^{b}	7.66^{ab}	11.62^{b}
T7	17.66^{e}	25.56^{e}	45.50^{e}	2.10^{e}	6.65^{d}	7.22^{e}
T8	20.20^{d}	27.73^{d}	47.80^{d}	2.36^{d}	7.01^{c}	8.27^{d}
T9	24.10^{c}	30.43^{c}	50.76^{c}	3.04^{c}	7.53^{b}	10.63^{c}
T10	28.96^{a}	38.86^{a}	57.13^{a}	3.98^{a}	7.90^{a}	12.31^{a}
CD (0.05)	1.23	0.97	0.98	0.04	0.24	0.23

Table 7. Effect of inoculation of endophytic microorganisms on plant height of Chia plant at 30 DAT, 60 DAT and 90 DAT and plant biomass at 90 DAT

Treatment	**30 DAT**	**60 DAT**	**90 DAT**	**Plant Biomass at 90 DAT**
T1	13.36^{j}	67.33^{j}	75.43^{j}	40.44^{j}
T2	17.66^{fg}	81.93^{g}	94.92^{g}	65.87^{g}
T3	18.30^{f}	84.86^{f}	97.19^{f}	74.00^{f}
T4	15.00^{i}	74.46^{i}	88.36^{i}	52.09^{i}
T5	16.76^{g}	78.90^{h}	90.02^{h}	57.51^{h}
T6	30.80^{b}	100.60^{b}	121.66^{b}	120.79^{b}
T7	21.06^{e}	88.50^{e}	99.30^{e}	85.83^{e}
T8	22.50^{d}	90.76^{d}	112.60^{d}	94.44^{d}
T9	24.66^{c}	94.86^{c}	118.20^{c}	105.12^{c}
T10	35.46^{a}	123.33^{a}	128.90^{a}	158.37^{a}
C.D (0.05)	1.14	2.21	1.54	2.05

Legend: T1= Control (No treatment); T2 =Treatment using Pseudomonas fluorescens (CEPB2); T3= Treatment using Enterobacter cloacae (CEPB8); T4= Treatment using Bacillus cereus (CEPB5) T5= Treatment using Stenotrophomonas maltophia CEPBA; T6 = CEPB2+CEPB8; T7= CEPB5+CEPBA; T8= CEPB2+CEPBA; T9= CEPB5+CEPB8; T10= CEPB2+ CEPBA+CEPB5+CEPB8

DAT = Days After Transplantation. Mean values followed by the same superscript in each column do not differ significantly at p ≤ 0.05 level by DMRT.

3.5 Evaluation of the Effectiveness of Selected Endophytic Microorganisms on Plant Growth

The application of treatments significantly influenced the number of leaves, number of branches, plant height, root length, root dry weight, root length and plant biomass of the plant.

The number of branches were recorded the highest in the plants treated with T10 with average of 11, 17.66, 22.67 branches/plant at 30DAT, 60 DAT, 90DAT respectively followed by T6 (CEPB2+CEPB8) during 60DAT and 90 DAT. Treatment T10 showed more average number of leaves (54.66) compared to T1(control) with average of 21.33 number of leaves. T10 had highest root length of 28.97cm, 38.86 cm, 57.13 cm and highest root dry weight with an average of 3.97g, 7.9g, 12.31g at 30DAT, 60 DAT, 90 DAT respectively. Highest plant height was exhibited by Treatment 10 with the average height of 35.467cm, 123.33 cm and 128.96 cm respectively at 30DAT, 60DAT and 90DAT followed by T6 (CEPB2+CEPB8). Treatment 10 demonstrated the highest plant biomass, measuring 158.37 grams. In contrast, the control group (T1) displayed the lowest plant biomass, merely amounting to 40.443 grams.

Among the 10 treatments, the best treatment was treatment 10 i.e., the combination of 4 endophytic microorganisms (*Pseudomonas fluorescens* CEPB2+ *Enterobacter cloacae* CEPB8+ *Bacillus cereus* CEPB5+,

Stenotrophomonas maltophilia CEPBA). All these isolated microorganisms were efficient in nitrogen fixation, phosphate solubilisation, potassium fixation, production of IAA, GA, siderophore and exopolysaccharide production and the Treatment 3 i.e., CEPB8 (*Enterobacter cloacae*) performed best among individual treatments as CEPB8 was also efficient in all the plant growth promoting characters. Similar results were reported by Taghinasab *et al*. [15]. He stated that colonization of wheat roots by *Trametes versicolor* and *Piriformospora indica* increased plant biomass. In tomato and brinjal, Dileep Kumar and Dube (1991) investigated the *Pseudomonas fluorescens* strain RBT-13's ability to promote plant development. When compared to the uninoculated control, they noticed that both crops had longer roots and increased plant height [16-17].

4. CONCLUSION

The findings of the study showed that among the isolated 12 strains, 8 strains had efficient plant growth promoting traits and improved growth and biomass content of Chia under the greenhouse conditions. Maximum growth and biomass were observed in the treatment having combination of the 4 endophytic microorganisms. *Pseudomonas fluorescens*, *Enterobacter cloacae*, *Bacillus cereus*, and *Stenotrophomonas maltophilia* helped in increasing the root growth, plant height and plant biomass indicating that the endophytic microorganisms have a major impact on growth of Chia plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saeed Q, Xiukang W, Haider FU, et al Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. International Journal of Molecular Sciences 22:10529. Sambrook J, Fritsch FF, Maniatis T. 1989. Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, New York. 2021;931-957.
2. Barsby JP, Cowley JM, Leemaqz SY, et al. Nutritional properties of selected superfood extracts and their potential health benefits. PeerJ 9:e12525Dileepkumar BS, Dube HC. Plant growth promoting activity of fluorescent pseudomonads from tomato rhizoplane. Indian Journal of Experimental Biology. 2021;29:366-370.
3. Varban R, Crisan I, Varban D, Muresan C. Cultivation Potential of Chia (*Salvia hispanica* L.). Cluj County; 2022.
4. Prasanna HS, Maruthi Prasad BN, Shankarappa TH, Vishnuvardhana SM. Economics of chia (*Salvia hispanica*) cultivation as influenced by foliar application of different elicitors. *Journal of Pharmaceutical Innovation*. 2021;10(4): 245-249.
5. Wafula EN, Murunga SI, Nalianya Wafula E, Murunga SI, Wafula EN. Isolation and identification of phosphate solubilizing and nitrogen-fixing bacteria from lake Ol'Bolossat sediments, Kenya. Modern Applied Science. 2020;14:37.
6. Gour AC. Phosphate solubilizing microorganisms as biofertilizers. Omega scientific publishers, New Delhi; 1980.
7. Parmar P, Sindhu SS. Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. Journal of Microbial Research. 2013;3(1):25-31.
8. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry. 1987;160(1):47-56.
9. Aung TN, Nourmohammadi S, Sunitha EM Myint M. Isolation of endophytic bacteria from green gram and study on their plant growth promoting activities. International Journal of Applied Biology and Pharmaceutical Technology. 2011;2:525-536.
10. Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. Springer. 2016;6(1):1-8.
11. Suhandono S, Kusumawardhani MK, Aditiawati P. Isolation and molecular identification of endophytic bacteria from Rambutan fruits (*Nephelium lappaceum* L.) cultivar Binjai. HAYATI Journal of Biosciences. 2016;23(1):39-44.
12. Husseiny S, Dishisha T, Soliman HA, Adeleke R, Raslan M. Characterization of growth promoting bacterial endophytes isolated from *Artemisia annua* L. South African Journal of Botany. 2021;143:238-247.

13. Ashmawy NA, Shoeib AA, Youssef HF, Mahmoud SM. Pathological, biochemical and molecular characterization of the seed-borne bacteria *Pantoea* spp., *Xanthomonas* spp. and *Pseudomonas* spp. from solanaceous plants in Egypt. Journal of Microbiology, Biotechnology and Food Sciences. 2020;10(2):289-295.
14. Dubey A, Saiyam D, Kumar A, Hashem A, Abd Allah EF, Khan ML. Bacterial root endophytes: characterization of their competence and plant growth promotion in soybean (*Glycine max* L. Merr.) under drought stress. International Journal of Environmental Research and Public Health. 2021;18(3): 931.
15. Taghinasab M, Imani J, Steffens D, Glaeser SP, Kogel KH. The root endophytes Trametes versicolor and Piriformospora indica increase grain yield and P content in wheat. Plant and Soil. 2018;426(1);3339-348
16. Pandey S, Pandey S and Alam A. Isolation and characterization of endophytic bacteria associated with gametophytes of bryophytes in Mount Abu (Rajasthan). Rhizosphere. 2022;24:p.1005 92.
17. Patel H A, Patel R K, Khristi S M, Parikh K and Rajendran G. Isolation and characterization of bacterial endophytes from *Lycopersicon esculentum* plant and their plant growth promoting characteristics. Nepal Journal of Biotechnology. 2012;2(1):37-52.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114429