

Original Research Article

Identification of endophytes and evaluation of their impact on growth of Chia

(*Salvia hispanica* L.)

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 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

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. X

Comment [D1]: Kindly include the source of citation

The plant growth promotion route involves the phytohormone production, nitrogen fixation, siderophore production, solubilization of inorganic substances (P, K, Zn etc.) and making it conveniently available to the plants. 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)

Comment [D2]: Please re-check/examine the grammatical structure of the English language

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 (Varban *et al.*, 2022).

Comment [D3]: Pls clarify, what did authors mean?

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. (Prasanna *et al.*, 2021)

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.

MATERIALS AND METHODS

Isolation of endophytic microorganisms 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, Tumakur district and brought to the laboratory and was kept in refrigerator at 4° 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), Kuster's agar, and Potato Dextrose Agar (PDA) 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.

Comment [D4]: What is the purpose of utilizing PDA media?

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Identification of efficient endophytic microorganisms involved in plant growth promotion

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 °C for 4 - 7 days. The isolates which were able to form colonies in the nitrogen free medium were designated as positive for N fixation. (Wafula *et al.*,2020)

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 (Gour, 1980).

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. (Parmar *et al.*, 2013)

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 (1987) 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.

IAA (Indole Acetic Acid) production

IAA production was estimated using nutrient broth. Inoculation was done in 15ml nutrient broth and incubated at 28°C 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 FeCl₃). Production of pink colour indicated IAA production. (Aung *et al.*, 2011)

Molecular characterization of endophytic microorganisms using 16S rRNA

Bacterial isolates were grown in nutrient broth and incubated at 28°C 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 37°C. **Again**, 100µl of 5 M NaCl and 80 µl of CTAB solution were added and incubated for ten minutes at 65°C. 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 4°C. 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 -20° C overnight for precipitation of DNA. Later it was centrifuged at 10,000 rpm for 20 min at 4°C 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 (16SF5'-AGAGTTTGATCCTGGCTCAG-3'; 16SR 5'-CTACGGCTACCTTGTACGA-3'). Amplification was carried out in Nexus Mastercycler at the Department of Plant Biotechnology UAS, GKVK with initial denaturation (95°C), annealing (55°C) and extension temperature (72°C). 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 were 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 (Kumar *et al.*, 2016).

Evaluation of selected endophytic microorganisms for growth promotion in Chia (*Salvia hispanica* L.) 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 °C 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 × 10⁶ cells mL⁻¹) of 5 ml (OD₅₆₀=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.

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

Treatment	Seedlings treated with solution
T ₁	Control (No treatment)
T ₂	<i>Pseudomonas fluorescens</i> CEPB2
T ₃	<i>Enterobacter cloacae</i> CEPB8
T ₄	<i>Bacillus cereus</i> CEPB5
T ₅	<i>Stenotrophomonas maltophilia</i> CEPBA
T ₆	CEPB2+CEPB8
T ₇	CEPB5+CEPBA
T ₈	CEPB2+CEPBA
T ₉	CEPB5+CEPB8
T ₁₀	CEPB2+ CEPBA+CEPB5+CEPB8

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.

RESULTS AND DISCUSSION

Isolation of endophytic microorganisms 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.

Morphological characterization of endophytic microorganisms

The shape of the colony was observed of which 83.33% of them were round and 16.6% of them had dome shaped colonies. Among them 16.6% were green, 33.3% were cream, 41.6% were opaque, 8.3% were yellowish in colour. Majority of isolates were cream in colour. 25% of them were coccus-shaped, and 75% of them were rod-shaped bacteria. The cell shape, colour and shape of the colonies are shown in the **Table 2**. Microscopic analysis was conducted using Gram staining under 100X magnification to investigate distinctions among the isolates. 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.* (2016) 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

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.

Husseinyet *al.*(2021) 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.

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

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*.

Table 4: Similarity percentage of the isolate with organism

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

Similar procedure was followed by Ashmawy *et al.* (2020) 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.* (2021) 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.

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.

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

Treatment	Number of branches			Number of leaves		
	30 DAT	60 DAT	90 DAT	30DAT	60DAT	90DAT
T1	2.66 ^c	5.66 ^h	8.66 ^h	8.00 ^h	21.33 ^j	43.66 ^j
T2	5.33 ^c	8.33 ⁱ	12.00 ⁱ	15.60 ^{ei}	32.00 ^g	61.33 ^g
T3	5.00 ^c	9.60 ^e	13.30 ^e	15.00 ⁱ	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 ^c	74.66 ^c
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

Treatment	Root length			Root dry weight		
	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 maltophilia* 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 $p \leq 0.05$ level by DMRT.

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). Treatment10 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.* (2018). 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.

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.

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Comment [D6]: References: Alphabetical order

UNDER PEER REVIEW