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Impact of Different Bioinoculants on Growth and Biochemical Characteristics of *Senna alexandrina (Cassia angustifolia.* Vahl)

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ABSTRACT: The study sought to assess the effect of various bioinoculants on the growth and biochemical features of *Senna alexandrina* or *Cassia aungustifolia*, a medicinal plant recognized for its laxative qualities. The study looked at how nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, and PGPR affect plant development and biochemical properties, both separately and in combination. It measured shoot and root development, chlorophyll concentration, total proteins, and enzymatic activity. The findings shed light on how bioinoculants improve plant performance. *Senna alexandrina* was exposed to both single and combined applications of bioinoculants, such as PGPR, phosphate-solubilizing microorganisms, and nitrogen-fixing bacteria. Plant height, number of leaves, biomass, and root length were among the growth metrics that were noted. The amount of chlorophyll, total soluble proteins, and enzymatic activity (catalase and peroxidase) were measured using biochemical techniques. According to the study, Azospirillum considerably improved Senna alexandrina's development and biochemical characteristics. Plants treated with T4 exhibited the longest shoot (54.92 cm) and the most leaves, while other treatments showed a notable increase in root length between 30 and 90 days. T4 plants had the highest levels of reducing sugars and total chlorophyll, followed by T3 and T2, while T2, T3, and T4 plants also had significantly higher protein content in their roots than T1 plants. These results demonstrate Azospirillum's capacity to enhance Senna alexandrina's development and biochemical characteristics. The study emphasises the potential of bioinoculants as sustainable and environmentally friendly methods of enhancing Senna alexandrina agriculture and medicinal benefits. These results help sustainable agriculture and the rising demand for herbal goods by providing important information for creating bioinoculant-based methods to improve the production of medicinal plants.

Keywords: *Senna alexandrina*, Azospirillm, Biofertilizer, Nitrogen fixing, Phosphate solubilizing.

INTRODUCTION

The synthesis of chemical fertilizers to provide nitrogen and phosphorus, which are essential nutrients for plant growth and development, requires a lot of energy and is mostly dependent on fossil fuels (Ghosh *et al.,* 2016). Overuse of chemical fertilizers to restore soil phosphorus (P) and nitrogen (N) results in chemical spills, excessive expenses and serious environmental contamination (FAO, World Fertilizer Trends and Outlook to 2022; Ghosh *et al.*, 2016). On the other hand, biofertilizers offer a sustainable, economical, and renewable substitute for chemical fertilisers in agriculture (Biswas *et al*., 2009).

Plant growth-promoting rhizobacteria (PGPR), phosphate-solubilizing microorganisms, nitrogen-fixing bacteria and phytostimulators are all components of biofertilizers that improve soil fertility and crop yield (Bhaskar Tao *et al.,* 2015). They are essential to sustainable agriculture because they increase the availability of nutrients, encourage plant development, and lessen pollutants in the environment (Basak and Gajbhiye 2018). The process by which microorganisms transform inert atmospheric nitrogen (N_2) into organic forms that plants can use is known as biological nitrogen fixing (Biswas *et al.*, 2009). Both symbiotic microbes like Rhizobium, Frankia and Azolla as well as free-living bacteria like Azotobacter and Azospirillum are nitrogen-fixing organisms found in biofertilizers (Jackson, 1967; Jaiswal *et al.*, 2016).

Along with increasing root dry weight, PGPR and phosphate-solubilizing bacteria (PSB) including *Acetobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., and *Azospirillum* sp. also produce antibiotics and enhance soil nutrient availability (Javoreková *et al.*, 2015). These microorganisms improve soil quality and encourage plant growth, making them essential for sustainable farming practices (Jha and Subramanian 2016).

This study focusses on the use of biofertilizers to increase plant productivity and growth (Kayina *et al*., 2012). It specifically examines how phosphate-solubilizing microorganisms and nitrogen-fixing bacteria affect the growth and biochemical traits of Senna alexandrina or *Caasia angustifolia*, a medicinally significant plant that is frequently used for its therapeutic qualities (Muhr *et al.*, 1965; Singaravel *et al.*, 2016). The highly prized medicinal plant senna (*Cassia angustifolia* Vahl.) is said to have laxative qualities because of sennosides found in its leaves and pods (Farrar *et al.*, 2019; Chen, *et al.*, 2021). It contributes significantly to the export of medicinal herbs from India. With a pH of 7.0 to 8.5, senna can tolerate saline soils and thrives in semi-arid areas (Okon and Labandera-Gonzalez 1994). Plant productivity and soil fertility are enhanced by organic amendments such as FYM and compost, particularly in coarser soils. They have a promising but little-studied role in senna cultivation in saline soils (Muhr *et al.*, 1965). To ensure high-quality production and increase sustainable agriculture and economic returns, research on affordable amendments across a range of soil conditions is crucial (Singaravel *et al.*, 2016; Subbiah and Asija 1956). India's central and southern regions are home to Tinnevelly Senna (*C. angustifolia*) cultivation. The projected annual production of senna herbage is 7,500 tonnes. Like *Cassia angustifolia*, a few other senna species, the most significant of which being Alexandrian Senna, have laxative qualities in their pods and leaves. In North African nations like Ethiopia and Sudan, Alexandrian Senna grows naturally (Muhr *et al*., 1965; Farrar *et al.*, 2019).

MATERIALS AND METHODS

Selection of plant. Sennosides from the medicinal herb senna (*Senna alexandrina*) are used extensively to treat constipation since they are efficient laxatives. Additionally, it has anticancer, hepatoprotective, antioxidant, antibacterial, anti-inflammatory and antidiabetic qualities. Skin ailments, stomach problems, and liver disorders are all treated with this adaptable plant. Research on it is essential due to its wide range of medicinal possibilities and economic worth.

Collection of seeds. Senna alexandrina seeds were collected between August and October in the Seshachalam protected forest, near Rangampeta in Tirupati, Andhra Pradesh, India. After being carefully rinsed with tap water to eliminate contaminants, seeds of uniform size were chosen and surface-sterilized for 5 minutes with a 0.1% sodium hypochlorite solution. These sterilized seeds were then prepared for sowing to ensure optimal germination conditions.

Collection of biofertlizers inoculums. Azospirillum and Bacillus megatherium cultures were acquired from the Bangalore Division's Regional Biofertilizers Development Centre in India.

Preparation of mediums. Azospirillum and Bacillus megatherium were cultured in Ashby medium and Pikovskaia's broth, respectively.

Fig. 1. Senna leaves & Flowers.

Fig. 2. Senna Pods.

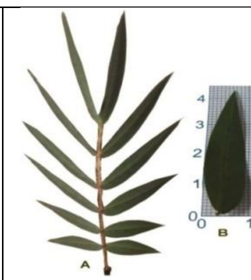

Fig. 3. Leaf length.

Experimental design. The experiment was conducted in a greenhouse to determine how the Senna alexandrina plant responded to the inoculation of PSB (*B. megaterium*) and nitrogen fixer (*A. brasilense*). The experiment's treatment specifics were broadly described as follows:

T1	::	Non- inoculated seeds (control) treated with deionized water
T2	::	Seeds inoculated with *A. brasilense (Nitrogen fixing bacteria)*
T3	::	Seeds inoculated with *B.megaterium (PSB)*
T4	::	Seeds co-inoculated with *A. brasilense* and *B.megaterium*

Cultivation of Plants. Senna alexandrina can be grown in controlled environments by using planting containers made of polythene bags with drainage holes. Fill each bag with a sterile soil mixture that has been prepared by combining clay, silt, and sand. Plant transplant seedlings or Senna alexandrina seeds, and establish six repetitions for each treatment. Apply inoculums in accordance with each treatment: for one treatment, add 20 ml of Bacillus megatherium culture to the soil; for another, add 20 ml of Azospirillum culture; and for a combined treatment, add 20 ml of each. Apply a solution of deionised water to the plants. Track plant growth over a three-month period and measure growth metrics to gauge how well bacterial treatments are working.

Physiological parameters. In all treatments, the growth parameters were measured on the 30th, 60th, and 90th days of plant growth. These measurements included shoot length, root length, number of leaves and leaf area.

Biochemical Parameters. Chlorophyll content (Arnon, 1949), starch (Mc Cready *et al.*, 1950), carbs (Highkin and Frankel 1962) and total proteins (Lowry *et al*., 1951) are estimated every 30 days.

Statistical analysis. The data was statistically analyzed of the by two way analysis of variance. **SPSS version 11.55** was used. The values were mentioned corresponding to each table in the results.

RESULT AND DISCUSSION

Shoot length (cms). All of the treated plants were found to have longer shoots than the untreated plants. The T4 plants (54.92) had the longest shoots after 90 days of growth. All of the treatments and the days showed a significant difference at the 5% level.

Root length (cms). On 90 days of plant growth, T4 treatment produced the longest root length (35.64) while T1 treatment produced the shortest root length (18.23). The root length increased significantly by 5% from 30 days to 90 days in all treatments.

Number of leaves. The numbers of leaves were found increased in all the treatments from 30 days to 90 days when compared to control. The maximum number of leaves were found in T4 treatment (76.41) followed by T3 (44.45), and T2 (54.13). Minimum numbers of leaves were found in T1 (26.27). It was observed that the number of leaves were significantly disserted between treatments at 5% level.

Leaf Number: At 90 DAS, the maximum number of leaves was recorded in T4-treated plants (76.41), followed by T2 (54.13) and T3 (44.45). The minimum leaf number was observed in T1-treated plants (26.27). A similar trend was observed at earlier growth stages, with T4 consistently producing the highest leaf numbers, followed by T2, T3, and T1. At 30 and 60 DAS, T4 plants had 21.19 and 44.80 leaves, respectively, while T1 recorded the lowest values of 7.86 and 15.31.

Leaf area (cm^2). Plant leaf area (cm²) was assessed at 30, 60, and 90 days post-sowing (DAS) for four treatments (T1, T2, T3, and T4). Significant changes in leaf area were detected between treatments, with T4 consistently having the highest values.

Leaf Area. At 90 DAS, T4-treated plants had the largest leaf area (54.00 cm²), followed by T2 (26.13 cm²) and T3 (22.82 cm²). T1-treated plants had the lowest leaf area (20.02 cm²). Earlier stages showed similar patterns, with T4 exhibiting 24.10 cm² and 38.56 cm² at 30 and 60 DAS, respectively, compared to T2 (11.66 cm² and 18.66 cm²), T3 (10.19 cm² and 16.30 cm²), and T1 (8.94 cm² and 14.30 cm²).

Table 1: Effect of Azospirillum and PSB on Shoot length of *Cassia angustifolia.*

Treatments	Shoot length (cms)		
	30DAS	60 DAS	90 DAS
T1	13.48	24.76	33.10
	(±0.20)	(±0.25)	(±0.30)
T2	18.59	35.84	46.82
	(±0.15)	(±0.18)	(±0.28)
T3	16.24	31.45	42.71
	(±0.18)	(±0.22)	(±0.26)
T4	29.65	42.86	54.92
	(±0.25)	(±0.35)	(±0.40)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

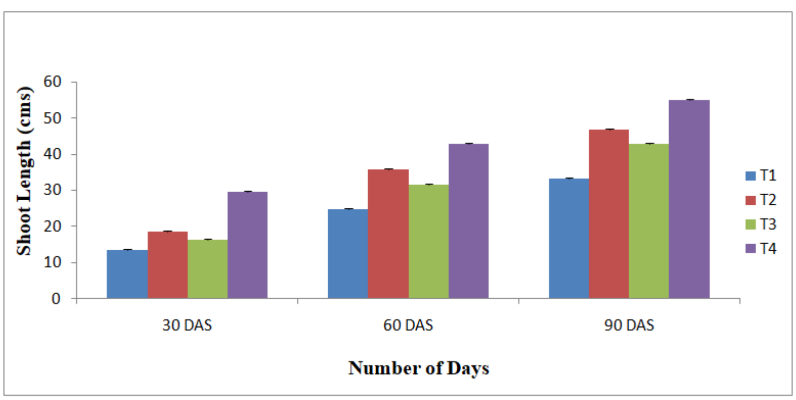

Fig. 4. Effect of Azospirillum and PSB on Shoot length of *Cassia angustifolia*.

Table 2: Effect of Azospirillum and PSB on Root length of *Cassia angustifolia*.

Treatments	Root length (cms)		
	30DAS	60 DAS	90 DAS
T1	6.34	12.58	18.23
	(±0.15)	(±0.20)	(±0.25)
T2	10.14	21.32	28.45
	(±0.18)	(±0.25)	(±0.30)
T3	9.87	20.11	27.76
	(±0.16)	(±0.22)	(±0.28)
T4	14.38	26.57	35.64
	(±0.22)	(±0.30)	(±0.40)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

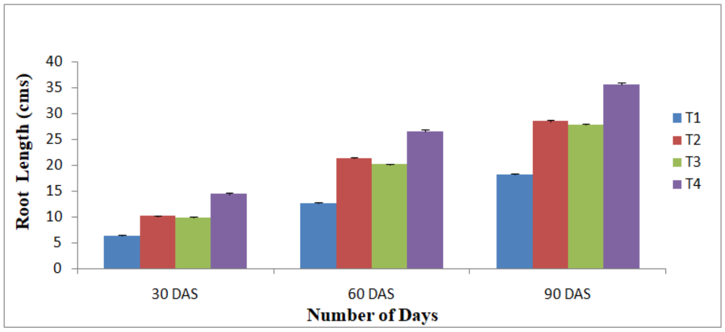

Fig. 5. Effect of Azospirillum and PSB on Root length (cms) of *Cassia angustifolia***.**

Table 3: Effect of Azospirillum and PSB on Leaf number of *Cassia angustifolia.*

Treatments	Leaf Number		
	Days after sowing (DAS)		
	30 DAS	60 DAS	90 DAS
	7.86	15.31	26.27
T1	(±0.55)	(±1.07)	(±1.84)
	10.25	30.82	54.13
T2	(±0.72)	(±2.16)	(±3.79)
	8.96	23.78	44.45
T3	(±0.63)	(±1.66)	(±3.11)
	21.19	44.80	76.41
T4	(±1.48)	(±3.14)	(±5.35)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$.

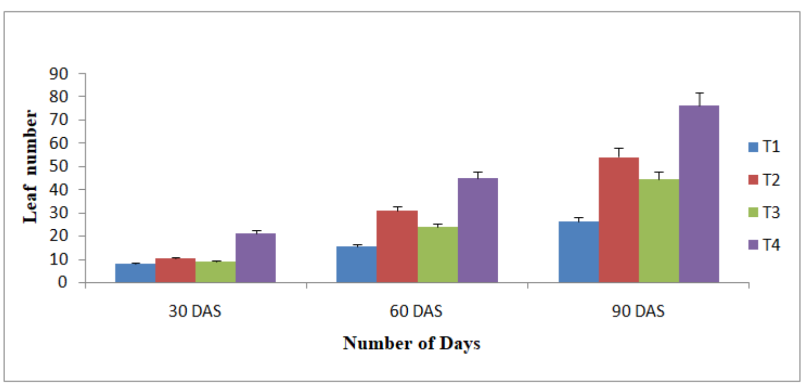

Fig. 6. Effect of Azospirillum and PSB on Leaf number of *Cassia angustifolia*.

Table 4: Effect of Azospirillum and PSB on leaf area (cm^2) of *Cassia angustifolia.*

Treatments	Leaf area (cm^2)		
	Days after sowing (DAS)		
	30DAS	60 DAS	90 DAS
T1	8.94 (± 1.34)	14.30 (± 2.15)	20.02 (± 3.00)
T2	11.66 (± 1.75)	18.66 (± 2.80)	26.13 (± 3.92)
T3	10.19 (± 1.53)	16.30 (± 2.45)	22.82 (± 3.42)
T4	24.10 (± 3.62)	38.56 (± 5.78)	54.00 (± 8.10)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

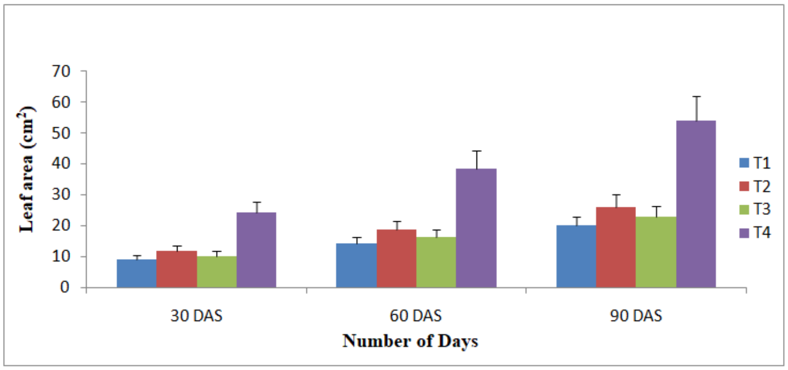

Fig. 7. Effect of Azospirillum and PSB on Leaf area of *Cassia angustifolia*.

BIOCHEMICAL STUDIES

Chlorophyll - a, Chlorophyll -b, and total Chlorophyll. Plant chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll) was measured at 30, 60, and 90 days after sowing (DAS) for four treatments (T1, T2, T3, and T4). T4 (60 Days 1.85 mg/g & 90 Days 1.73 mg/g) had the highest chlorophyll concentration at all stages, with significant differences detected at the 5% level of significance. T4 had the greatest chlorophyll a concentration, peaking at 1.25 mg/g at 60 DAS and slightly lowering to 1.18 mg/g at 90 DAS, surpassing T2 (1.00 mg/g), T3 (0.93 mg/g), and T1 (0.88 mg/g). Similarly, T4 had the highest chlorophyll b levels of 0.60 mg/g at 60 DAS and 0.55 mg/g at 90 DAS, which were significantly higher than T3, T2, and T1. Total chlorophyll in T4 peaked at 1.85 mg/g at 60 DAS and fell slightly to 1.73 mg/g at 90 DAS, outperforming T2 (1.43 mg/g), T3 (1.40 mg/g), and T1 (1.26 mg/g). These findings show that T4 promotes chlorophyll biosynthesis and retention, hence increasing photosynthetic efficiency through optimal light absorption.

Table 5: Effect of Azospirillum and PSB on Chlorophyll content of *Cassia angustifolia.*

Treatments	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Total Chlorophyll (mg/g)		
	Days after sowing (DAS)								
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	0.90 (±0.09)	0.92 (±0.08)	0.88 (±0.07)	0.40 (±0.04)	0.42 (±0.05)	0.38 (±0.04)	1.30 (±0.13)	1.34 (±0.14)	1.26 (±0.12)
T2	1.03 (±0.11)	1.06 (±0.12)	1.00 (±0.10)	0.46 (±0.05)	0.48 (±0.06)	0.43 (±0.04)	1.49 (±0.15)	1.54 (±0.16)	1.43 (±0.14)
T3	0.97 (±0.10)	0.99 (±0.11)	0.93 (±0.08)	0.50 (±0.05)	0.53 (±0.06)	0.47 (±0.05)	1.47 (±0.15)	1.52 (±0.17)	1.40 (±0.14)
T4	1.20 (±0.10)	1.25 (±0.13)	1.18 (±0.12)	0.58 (±0.06)	0.60 (±0.07)	0.55 (±0.05)	1.78 (±0.18)	1.85 (±0.20)	1.73 (±0.17)

Each value represents mean of six replications
Values within the parentheses indicate standard deviation
Values are significantly different from control at $p < 0.05$

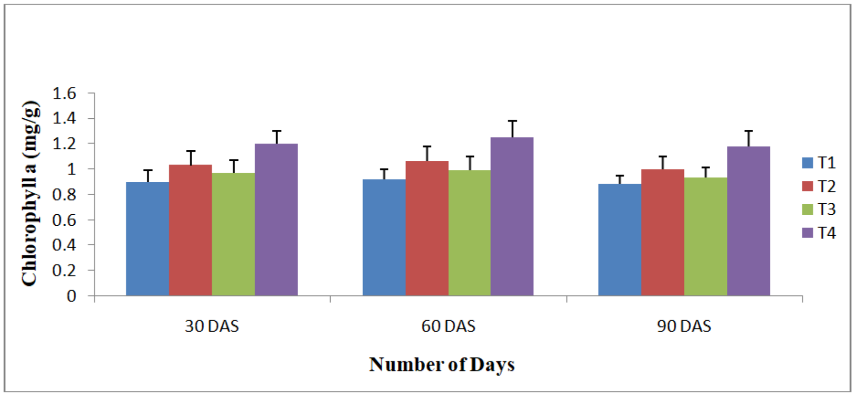

Fig. 8 (a) Effect of Azospirillum and PSB on Chlorophyll 'a' content of *Cassia angustifolia.*

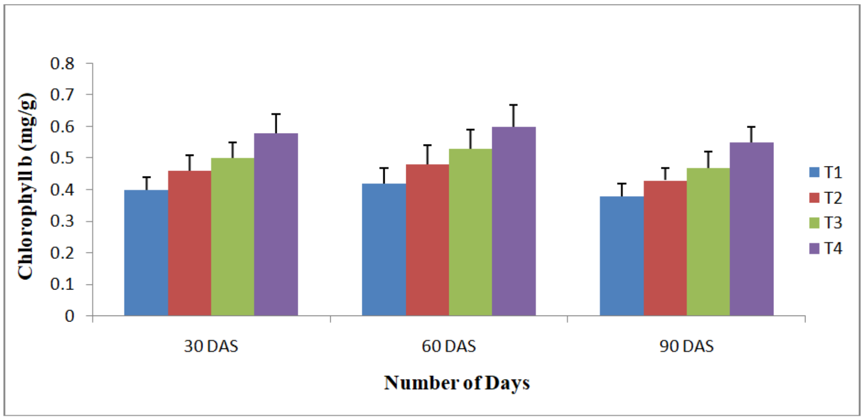

Fig. 8 (b) Effect of Azospirillum and PSB on Chlorophyll 'b' content of *Cassia angustifolia***.**

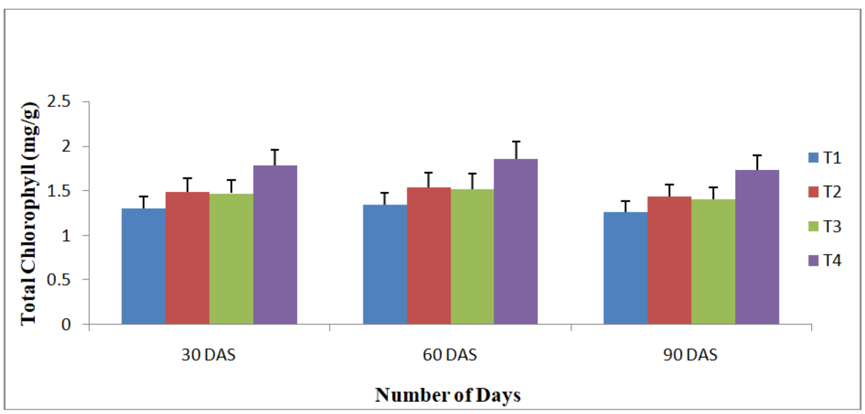

Fig. 8 (c) Effect of Azospirillum and PSB on Total Chlorophyll content of *Cassia angustifolia*.

Carbohydrate content of root: The levels of reducing sugars, non-reducing sugars, and starch were measured at 30, 60, and 90 days after sowing (DAS) in four treatments (T1, T2, T3, and T4), with T4 consistently exhibiting the highest values. Sugar reduction in T4 rose considerably from 2420.11 µg/g at 30 DAS to 4270.99 µg/g at 90 DAS, surpassing T2 (3600.55 µg/g), T3 (3450.42 µg/g), and T1 (3078.09 µg/g). T4 achieved 5390.03 µg/g at 90 DAS, higher than T2 (4824.02 µg/g), T3 (4580.23 µg/g), and T1 (4034.22 µg/g). The largest starch content was found in T4, which increased from 7.20 mg/g at 30 DAS to 11.53 mg/g at 90 DAS, exceeding T2 (9.24 mg/g), T3 (8.87 mg/g), and T1 (7.65 mg/g). These findings underscore T4's role in carbohydrate production and accumulation, most likely via increased enzymatic activity, photosynthesis, and carbohydrate partitioning. T1 typically had the lowest results, with T2 and T3 showing moderate improvements. Overall, T4 was more effective in increasing carbohydrate metabolism, resulting in robust growth and productivity.

Table 6: Effect of Azospirillum and PSB on Carbohydrate content in root of *Cassia angustifolia.*

Treatments	Reducing sugars (µg/g)			Non-reducing sugars (µg/g)			Starch (mg/g)		
	Days after sowing (DAS)								
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	1810.96 (±180.03)	2503.74 (±251.02)	3078.09 (±304.06)	2760.96 (±295.02)	3560.99 (±363.45)	4034.22 (±410.83)	5.10 (±0.07)	6.58 (±0.09)	7.65 (±0.12)
T2	2100.89 (±210.07)	3020.12 (±325.48)	3600.55 (±361.08)	3002.17 (±330.05)	4083.95 (±421.89)	4824.02 (±480.07)	6.37 (±0.08)	8.51 (±0.10)	9.24 (±0.13)
T3	2016.45 (±200.05)	2860.14 (±290.03)	3450.42 (±460.15)	2900.95 (±296.42)	3830.87 (±394.02)	4580.23 (±468.50)	5.85 (±0.07)	7.59 (±0.09)	8.87 (±0.12)
T4	2420.11 (±240.02)	3567.09 (±375.05)	4270.99 (±450.07)	3598.03 (±371.45)	4570.92 (±470.06)	5390.03 (±552.08)	7.20 (±0.09)	9.74 (±0.11)	11.53 (±0.14)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

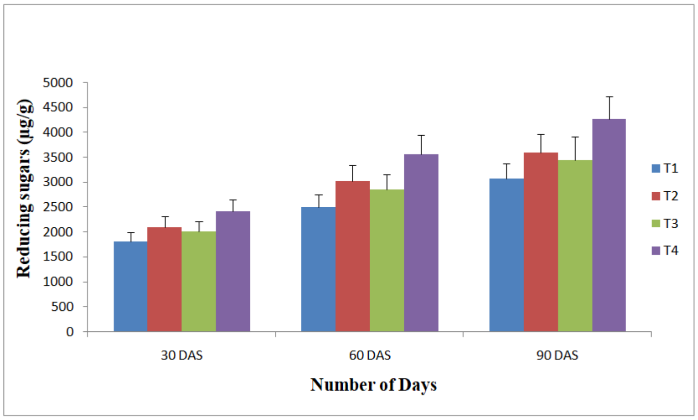

Fig. 9 (a) Effect of Azospirillum and PSB on Reducing sugars content in root of *Cassia angustifolia***.**

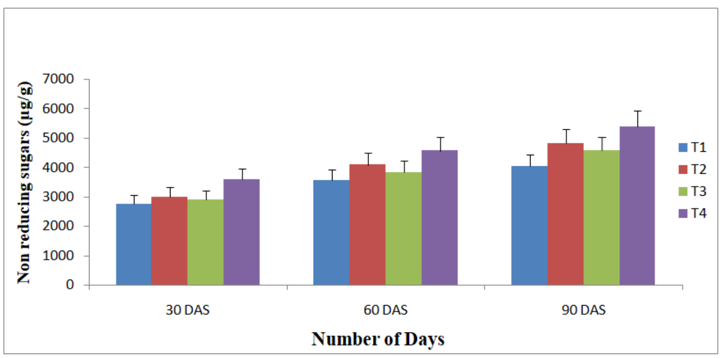

Fig. 9 (b) Effect of Azospirillum and PSB on Non reducing sugars content in root of *Cassia angustifolia.*

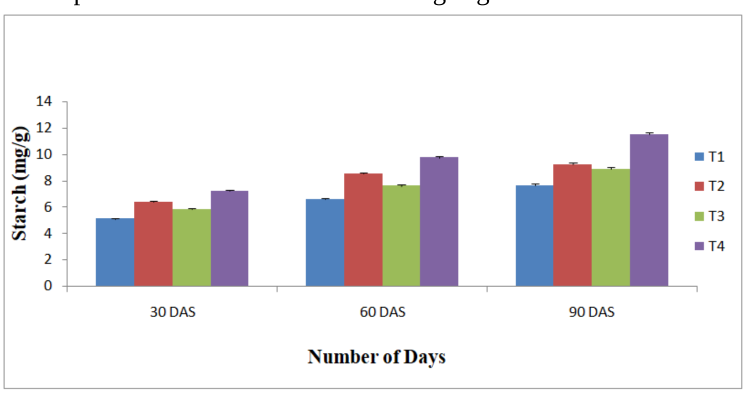

Fig. 9 (c) Effect of Azospirillum and PSB on Starch content in root of *Cassia angustifolia.*

Protein estimation (mg/g). The protein level (mg/g) of samples treated with T1-T4 varied significantly at 30, 60, and 90 DAS. T4 consistently had the highest protein content, beginning at 3.91 mg/g at 30 DAS, peaking at 5.30 mg/g at 60 DAS, and gradually decreasing to 4.92 mg/g at 90 DAS. T2 (3.16-4.32 mg/g) and T3 (2.84-3.80 mg/g) shown moderate improvements over T1, which consistently had the lowest protein content (2.35-3.10 mg/g). Despite a modest drop at 90 DAS, T4 outperformed other therapies in terms of protein accumulation. These findings show that T4 effectively increases protein synthesis, whereas T1 is the least effective.

Table 7: Effect of Azospirillum and PSB on Protein content in root of *Cassia angustifolia.*

Treatments	Protein content(mg/g)		
	Days after sowing (DAS)		
	30days	60days	90days
T1	2.35	3.10	2.82
	(±0.27)	(±0.36)	(±0.34)
T2	3.16	4.32	3.90
	(±0.31)	(±0.43)	(±0.47)
T3	2.84	3.80	3.53
	(±0.33)	(±0.46)	(±0.42)
T4	3.91	5.30	4.92
	(±0.46)	(±0.63)	(±0.59)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

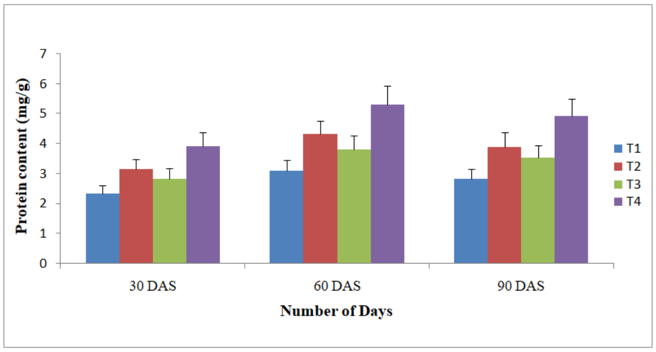

Fig. 10. Effect of Azospirillum and PSB on Protein content in root of *Cassia angustifolia.*

Lipid estimation (mg/g). The lipid content (mg/g) of samples treated T1-T4 varied significantly at 30, 60, and 90 DAS, with T4 consistently having the highest values. At 30 DAS, T4 measured 7.53 mg/g, substantially higher than T2 (6.15 mg/g), T3 (5.89 mg/g), and T1 (4.20 mg/g). Lipid content rose at 60 DAS, with T4 reaching 12.10 mg/g, followed by T2 (9.30 mg/g), T3 (8.90 mg/g), and T1 (6.80 mg/g). At 90 DAS, lipid content peaked with T4 at 17.80 mg/g, which was substantially greater than T2 (14.30 mg/g), T3 (13.60 mg/g), and T1 (10.50 mg/g). Lipid content increased gradually over time in all treatments, with T4 showing the greatest rise, followed by moderate improvements in T2 and T3, and T1 consistently shows the lowest values.

Table 8: Effect of Azospirillum and PSB on Lipid content in root of *Cassia angustifolia.*

Treatments	Lipid content (mg/g)		
	Days after sowing (DAS)		
	30 DAS	60 DAS	90 DAS
T1	4.20	6.80	10.50
	(±0.45)	(±0.75)	(±1.21)
T2	6.15	9.30	14.30
	(±0.67)	(±1.03)	(±1.60)
T3	5.89	8.90	13.60
	(±0.59)	(±1.03)	(±1.81)
T4	7.53	12.10	17.80
	(±0.88)	(±1.36)	(±2.22)

Each value represents mean of six replications.
Values within the parentheses indicate standard deviation.
Values are significantly different from control at $p < 0.05$

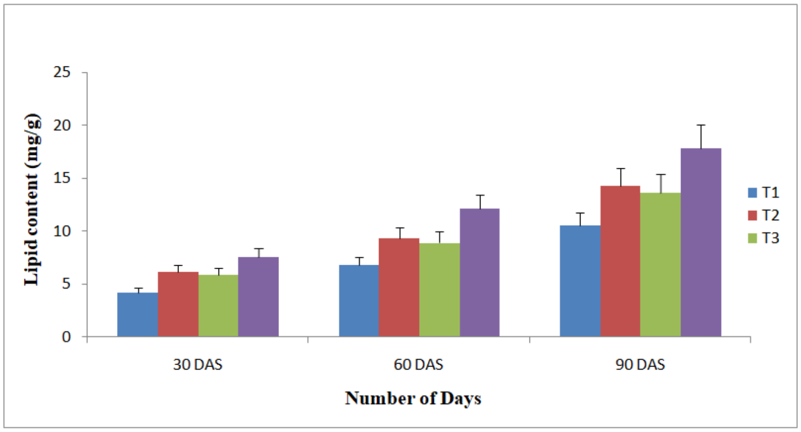

Fig. 11. Effect of Azospirillum and PSB on Lipid content in root of *Cassia angustifolia.*

CONCLUSIONS

Azospirillum has the ability to fix molecular nitrogen, which increases soil fertility and promotes plant development. Azospirillum is frequently used in agriculture, particularly in nitrogen-based fertilizers using crops. We concluded that Azospirillum had and PSB a considerable effect on *Cassia angustifolia* instead of Senna alexandrina's root, shoot and leaf growth. Furthermore, Azospirillum and PSB had a significant effect on Senna's carbohydrate and protein content. At the 5% level of significance, chlorophyll-a, chlorophyll-b, and total chlorophyll concentration differed significantly between treatments and over different days of a given treatment.

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