J. Soils and Crops 35 (2) 354-358, December 2025 ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

EFFECT OF BIO-INOCULANTS ON MORPHO-PHYSIOLOGICAL AND CHEMICAL PARAMETERS OF *Sesbania sesban* (L.) Merr.

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ABSTRACT

An experiment was conducted at Dept. of Botany, S.G.R.G. Shinde Mahavidhyalaya Paranda, Dist. Dharashiv during year 2023-2024.*Sesbania sesban* (L.) Merr. seedlings were taken in eight different pots, in which soil was treated with *Glomus fasciculatum*(*Gf*). In eight different pots soil was treated by *G. fasciculatum* along with *Trichoderma viride, Trichoderma harzianum, Trichoderma viride + Trichoderma harzianum, Rhizobium meliloti,* Bacterial Combination of *Bacillus megatherium + Fraturia aurentia*, *Bacillus megatherium + Fraturia aurentia + Rhizobium meliloti* and *Bacillus megatherium + Fraturia aurentia + Rhizobium meliloti + Trichoderma* Spp. Observations on number of leaves, number of rachis, shoot length, shoot diameter, root length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight were taken after six months of sowing of the plants. Observations on macro and micro elements i.e. Total nitrogen, ammonical nirogen, nitratre nitrogen, phosphorus, potash, calcium, magnesium, sulphur, zinc, copper, ferrous, manganese, boron, molybdenum, and sodium were estimated and calculated.Data revealed that number of leaves and rachis were found highest in *Gf +Rhz* treatment; shoot length and shoot diameter, fresh and dry shoot weight, sulphur, zinc and copper in *Gf + Bacterial combination*; root length and ammonical nitrogen in *Gf+Rhz+* bacterial combination treatment; fresh root weight, phosphorus, potash, and manganese in *Gf* treatment;dry root weight and calcium in *Gf+Tv+th* treatment; molybdenum and ferrous in *Gf + Tv*, treatment; total nitrogen, magnesium and boron in *Gf +Tv+Th+Rhz* + bacterial combination treatment; sodium in *Gf+Th* treatment.

(Key words: (*Sesbani asesban* (L.) Merr., *Glomus fasciculatum,* AMF, *Trichoderma* Spp., *Rhizobium meliloti*, *Bacillus megatherium* (PSB) and *Fraturia aurentia* [KMB])

INTRODUCTION

Sesbania sesban (L.) Merr. is from the family Fabaceae under angiosperms. This family has 730 genera, 19,400 species and is important in agriculture and economic point of view. Various parts of the plant used in traditional medicine as anti-inflammatory, anti-diarrheal, anthelmintic, and anti-oxidant (Goswami *et al*., 2016). It has economical values e.g. soil improvement, nutrient enhancement, growth enhancer, bio-fertilizer, yield improvement and pharmacological activities. It is rich in phytochemicals and are important in treatments like inflammation, diabetes, fungal infection and bacterial etc. It also fixes atmospheric nitrogen through its symbiotic association with soil bacteria, like *Rhizobium, Bradyrhizobium, Azorhizobium, and Sinorhizobium* (Lahdachi *et al.,* 2015). It is also used in agroforestry in tropical regions, as forage legume, including sub-Saharan Africa. It has other uses as fuelwood, stakes and it reduces soil erosion (Sileshi *et al.,* 2014).

AM fungi can enhance wheat growth by improving phosphorus uptake, especially in nutrient-poor soils (Abdul and Wahab, 2023). Effect of different AM -fungi on growth, nutrition and forskolin (active ingredient) content of C. forskohlii after AM inoculation was recently reported by Borkotoki *et al*. (2019). Several legumes showed poor growth and failed to nodulate in sterilized soil unless mycorrhizal. This was likely due to phosphorus deficiency, as adequate phosphorus is essential for nodule formation and nitrogen fixation (Saad Sulieman and Lam-Son Phan Tran*,* 2015). Inoculation with VA mycorrhizal fungi and *Rhizobium meliloti* can synergistically enhance nodulation, nitrogen fixation, and plant growth (Athanasia Kavadia *et al*., 2021). Most agricultural soils contain native AMF spores, whose role in crop productivity has been extensively studied (Pushpa Soti, 2023). Therefore, appropriate host-endophyte combinations are necessary to achieve optimal results. This can be achieved by better understanding the interaction between *Gf*, AMF fungi and *Trichoderma viride, Trichoderma harzianum*, *Rhizobium meliloti*, *Bacillus megatherium* and *Fraturia aurentia*.

MATERIALS AND METHODS

Rhizosphere sample of *Sesbania sesban* (L.) Merr. was collected from the Yedshi Ramling Wildlife Sanctuary.

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Root segments were cleared with 10% KOH, stained with Trypan blue, and assessed for mycorrhizal colonization using the methods of (Phillips and Hayman,1970) and (Giovanetti and Mosse,1980). AMF spore density was determined using the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). AMF fungal species were identified using the keys of (Schenck and Perez, 1990).

Soil was sterilized in autoclave. This sterilized soil is used for pot experiments. Seeds of *Sesbania sesban* (L.) Merr. were planted in pots containing sterilized soil. These pots were maintained in a greenhouse and watered as per the need. *Sesbania sesban* (L.) Merr. seeds were inoculated with *G. fasciculatum, Trichoderma viride, Trichoderma harzianum*, *Rhizobium meliloti*, *Bacillus megatherium* + *Fraturia aurentia* and *Rhizobium meliloti*, (*PSB and KMB*) with *G. fasciculatum.*

A mycorrhizal inoculum (native) containing spores was thoroughly mixed and applied to each pot. Eight treatments were applied including non-mycorrhizal treatment. For treatment, 5 g of *Trichoderma* Spp. and 25g of bacteria (*PSB and KMB*) were applied to kg^{-1} of seeds. Plants were harvested six months after sowing. Number of leaves, size of leaves, shoot height, shoot diameter, root length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weights were recorded at harvest. After harvest, all the samples were sent to laboratory for bio-chemical analysis. Total N, Ammonical Nitorgen, Nitrate N and Phosphorus were estimated as per method suggested by Jackson (1973), Potash by Davy (1807) Calcium and magnesium by Davy (1808), sulphur, Zinc, Copper, Ferrous and Manganese by Issac and Kerber (1971), Boron by Capelle (1961), Molybdenum by Robinson (1948), Sodium by Volhard (1858).

RESULTS AND DISCUSSION

Experiment was conducted on *Sesbania sesban* (L.) Merr. using eight different treatments. The readings on 9 different parameters were noted for the period of six months as shown in Table 1.

Table 1.Effect of bio-inoculants on morpho-physiological and chemical parameters of *Sesbania sesban* (L.) Merr.

Sr. No.	Parameters	Treatments							
		Gf	*Gf+ Tv*	*Gf+ Th*	*Gf Tv+ Th*	*Gf + Rhz*	*Gf+ bacterial* combination	*Gf+Rhz +bacterial* combination	*Gf+Tv+Th+Rhz+ bacterial* combination
1.	No. of leaves	1826	2090	1782	1892	2112	1672	1452	1144
2.	No. of rachis	166	190	162	168	192	152	132	104
3.	Shoot length (in cm)	119.2	121.3	106.6	105.2	117.1	126.3	101.2	77.8
4.	Shoot diameter (in cm)	3.8	3.7	3.1	3.2	3.2	3.8	3.1	2.3
5.	Root length (cm)	22.3	34.2	44.2	44	30.1	46.2	46.7	35.2
6.	Fresh root weight (g)	25.1	21.25	22.54	16.63	17.49	21.1	10.67	10
7.	Dry root weight (g)	14.76	12.67	12.74	15.08	11.77	11.92	8.77	8.18
8.	Fresh shoot weight (g)	59.94	65.7	44.1	46.4	56.6	73.61	38.67	21.95
9.	Dry shoot weight (g)	23.17	26.09	18.83	21.74	23.67	32.04	16.23	9.17

Gf: *Glomus fasciculatum*, Tv: *Trichoderma viride*, Th: *Trichoderma harzianum*, Rhz: *Rhizobium meliloti*, Bacterial Combination: *Bacillus megatherium*, *Rhizobium meliloti* and *Fraturia aurentia*

Data recorded that number of leaves and rachis were found highest in *Gf+Rhz* treatment and lowest in *Gf+Tv+Th+Rh+* bacterial combination. Shoot length and shoot diameter was highest in *Gf* + bacterial combination treatment and lowest in *Gf+Tv+Th+Rh+* bacterial combination. Root length was highest in *Gf + Rhz+* bacterial combination treatment and lowest in *Gf* treatment. Fresh root weight recorded highest in *Gf* treatment while dry root weight was found highest in *Gf+Tv+Th* treatment and both found lowest in *Gf+Tv+Th+Rh+* bacterial combination treatment. Fresh shoot weight and dry shoot weight were found highest in *Gf* + bacterial combination and lowest in *Gf+Tv+Th+Rh+* bacterial combination treatments when compared with remaining treatments.

Table 2. Effect of bio-inoculants on chemical parameters of *Sesbania sesban* (L.) Merr.

Sr. No.	Parameters	Treatments								
		Unit	*Gf*	*Gf+ Tv*	*Gf+ Th*	*Gf Tv+ Th*	*Gf+ Rhz*	*Gf+ bacterial* combination	*Gf+Rhz +bacterial* combination	*Gf+Tv+Th+Rhz+ bacterial* combination
1	Total nitrogen	%	0.84	0.62	1.23	1.12	1.01	1.12	0.62	1.29
2	Ammonical Nitrogen	ppm	641	658	681	658	633	632	684	631
3	Nitrate Nitrogen	ppm	418	413	451	430	414	431	438	412
4	Phosphorus	%	0.14	0.11	0.07	0.07	0.11	0.08	0.01	0.02
5	Potash	%	2.56	2.23	1.13	1.60	1.93	1.83	2.26	0.77
6	Calcium	%	1.00	0.96	1.00	1.12	0.96	1.00	0.60	0.68
7	Magnesium	%	0.37	0.44	0.37	0.19	0.51	0.37	0.12	0.80
8	Sulphur	%	0.01	0.02	0.11	0.09	0.11	0.13	0.11	0.08
9	Zinc	ppm	22	24	22	27	14	28	11	18
10	Copper	ppm	28	31	35	31	28	35	15	18
11	Ferrous	ppm	146	186	158	148	121	136	111	88
12	Manganese	ppm	63	62	45	45	32	28	12	34
13	Boron	ppm	61.30	62.90	63.80	65.40	61.30	71.50	70.40	78.50
14	Molybdenum	ppm	0.71	0.84	0.64	0.48	0.58	0.71	0.71	0.59
15	Chloride	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	Sodium	%	0.07	0.05	0.09	0.06	0.08	0.04	0.02	0.05

Gf: Glomus fasciculatum, Tv: Trichoderma viride, Th: Trichoderma harzianum, Rhz: Rhizobium, Comb: Rhizobium meliloti + *Bacillus megatherium* + *Fraturia aurentia*

Chemical analysis of *Sesbania sesban* (L.) Merr. showed highest in total nitrogen, magnesium and boron in *Gf + Tv+ Th + Rhz* + bacterial combination treatment and lowest total nitrogen in *gf + Tv* treatment and magnesium and boron in*gf+ Rhz* + bacterial combination treatment. While highest ammonical nitrogen was found in *Gf + Rhz* + bacterial combination and lowest in *gf + Tv +Th* + bacterial combination. *Gf* treatment was found highest in phosphorus, potash and manganese and lowest phosphorus was recorded in treatment *Gf + Rhz*+ bacterial combination, lowest potash in *Gf +Th* and lowest manganese in *Gf+ Rhz* + bacterial combination. Plant treated with *Gf + Tv* showed highest in ferrous and molybdenum and lowest ferrous was noted in *Gf +Tv + Th + Rhz* + bacterial combination treatment and lowest molybdenum in *Gf +Tv + Th* treatment. Highest nitrate nitrogen, copper and sodium was recorded in *Gf + Th* treatment and lowest nitrate nitrogen was observed in *Gf +Tv* treatment and lowest copper and sodium was found in *Gf + Rhz* +bacterial combination treatment. Calcium was found highest in *Gf + Tv+ Th* treatment and lowest in *Gf + Rhz* + bacterial combination treatment, while sulphur, zinc and copper were found highest in *Gf* + bacterial combination treatment and lowest sulphur was showed in *Gf* treatment and lowest zinc and copper was recorded in *Gf+ Rhz* + bacterial combination treatment when compared with other remaining treatments.

By observing the parameters studied it is seen that, combined application of different microorganisms (fungi and bacteria) resulted in synergistic effects on plant growth. It had positive impact on growth and development of leaves and shoot. Roots were seen aggressively grown when treated with *Gf* and combination of *Rhizobium meliloti* + *Bacillus megatherium* + *Fraturia aurentia*. Therefore, by observing the data recorded in the first six months, *Gf* + bacterial combination treatment had positive impact on growth and development of *Sesbania sesban* (L.) Merr.in its initial stage. However, more extensive study and research is required to observe the impact of the said treatment throughout the life cycle of *Sesbania sesban* (L.) Merr.

The results of this study demonstrate the positive impact of mycorrhizal fungi on the growth and development of *Sesbania sesban* (L.) Merr. To establish legume root nodules, it requires root-modulating bacteria in the soil or it can be provided during sowing (Thilakaranthna and Raizada,2017). This study showed that certain indigenous *Rhizobium* strains significantly improved nodulation, shoot biomass, and nitrogen content in *Sesbania sesban*. Eight strains boosted growth by ~50% over nitrogen-fertilized controls, highlighting their strong symbiotic efficiency (Wolde-Meskel *et al.*,2016). To establish a functioning N, -fixing symbiosis, an ample number of root-nodulation bacteria in the soil is required, or they must be provided during sowing (Bhat and Bhatt, 2020)**.**Further research is needed to elucidate the specific mechanisms underlying the observed growth responses and to explore the potential for optimizing the combined application of these fungi.

The study established that PSBs are active bio-fertilizers and found a sustainable alternative to chemical phosphorus fertilizers in recent agriculture (Kumar *et al*., 2023). Phosphate solubilizing bacteria have the capability to convert insoluble phosphorus compounds into bioavailable forms, helping better nutrient uptake and crop development (Sharma *et al*., 2023). PSB play a vital role in mobilizing insoluble phosphorus in the soil, assembly it nearby to plants and improving complete soil fertility (Rao *et al*., 2023). Phosphate solubilizing bacteria improve the bioavailability of phosphorus in soils by flouting down complex inorganic phosphate compounds over biological processes (Patel *et al*., 2023). PSBs are key bio-fertilizers that increase plant phosphorus uptake by changing insoluble forms into plant-available forms over microbial activity (Begum *et al*., 2023).

From pot experiment it has been observed that, *Sesbania sesban* (L.) Merr.showed good growth and development when *G. fasciculatum* was used with consortium of bacteria like *Rhizobium meliloti*, (*PSB and KMB*).This study demonstrated that dual inoculation with *Rhizobium* and *Glomusf asciculatum* significantly enhanced biomass, nitrogen, and phosphorus content, as well as nodule nitrogenase activity and chlorophyll content in *Sesbania grandiflora* compared to controls (Lalitha, 2015). The association of AMF helps nutrient uptake, soil stability, control root diseases and lowers water stress problems (Begum *et al*., 2019). There are reports of enhanced correlation in between spore number and root colonization (Visen *et al*., 2016). Keserwani *et al*. (2018) reported that seed borne mycoflora i. e.*Rhizopus* sp. and *Fusarium* sp. reduced the seedling vigour index of pea varieties whereas, fungicidal and biocontrol agent *Trichoderma* sp. increased the seedling vigour index by keeping seed borne mycoflora under check.Therefore, further study is required on association of *G. fasciculatum* with *Rhizobium meliloti*, other bacteria and *Trichoderma* Spp.

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Rec. on 02.04.2025 & Acc. on 17.04.2025