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Research Article

Effect of Foliar Spray of *Pseudomonas fluorescens* on BNF, Nodulation Attributes, Leghemoglobin Content and Population of *P. fluorescens* on Phyllosphere of Soybean Under STCR Approach

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Abstract

The investigation entitled "Effect of foliar spray of *Pseudomonas fluorescens* on BNF, nodulation attributes, Leghemoglobin content and population of *P. fluorescens* on phyllosphere of soybean under STCR approach" was carried out at the Research Farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (India), taking soybean (variety JS 95-60). The experiment was laid out under randomized block design with 4 replications of 10 treatments based on targeted yields which consisted of control; T_2= control + foliar spray of *P. fluorescens*; T_3 = General Recommended Dose (20:60:20 kg N, P_2O_5 and K_2O ha^{-1}); T_4 = General Recommended Dose + foliar spray of *P. fluorescens*; T_5 = 23:62:27 kg N, P_2O_5 and K2O ha^{-1} (Targeted Yield 25 q ha^{-1}); T_6 = 23:62:27 kg N, P_2O_5 and K_2O ha^{-1} (Targeted Yield 25 q ha^{-1}) + foliar spray of *P. fluorescens*; T7= 49:88:47 kg N, P_2O_5 and K_2O ha^{-1} (Targeted Yield 30 q ha^{-1}1); T_8 = 49:88:47 kg N, P_2O_5 and K_2O ha^{-1} (Targeted Yield 30 q ha^{-1}); T9 = 73:114:66 kg N, P_2O_5 and K_2O ha^{-1} (Targeted Yield 35 q ha^{-1}); T_{10} = 73:114:66 kg N, P_2O_5 and K_2O ha^{-1} (Targeted Yield 35 q ha^{-1}) + foliar spray of *P. fluorescens*. Regarding the effect of foliar spray of *P. fluorescens* as influenced by different NPK levels results obtained on treatment T_6 (23:62:27 kg N, P_2O_5 and K_2O ha^{-1} + foliar spray *P. fluorescens*) has significantly increased nodule fresh weight (1.32 g), nodule dry weight (0.66 g), leghaemoglobin content (2.37mM at 45DAS) and maximum amount of BNF (8 kg ha^{-1}) when compared to nutrient applied at higher doses. The increase in population of Pseudomonas could be attributed to increased availability of N, P and K through applied fertilizers.

Keywords: *Pseudomonas fluorescens*; Targeted Yield; Nutrient Management; Sustainable Agriculture

Abbreviations

BNF: Biological Nitrogen Fixation; Lb: Leghaemoglobin; STCR: Soil Test Crop Response

Introduction

Soybean is a member of the Leguminosae family and is rich in nutrients, cultivated across the world wide approximately up to 97%, of the global pulse area [1]. In India, soybean cultivation spans 105.76 lakh hectares, with an annual production of 91.45 lakh tonnes. In India Madhya Pradesh is a leading soybean producer, accounting for 50 lakh hectares with an average annual production of 45.35 lakh tonnes and an average productivity of 905 kg ha^{-1}. Soybean is often called a miracle crop of the 21st century due to its dual purpose as both an oilseed crop and a pulse crop [2]. It is also considered a rich and inexpensive source of protein, as well as a source of amino acids, vitamins, minerals (5%), fats (15-20%), carbohydrates (30%), and dietary fibre [3]. The use of biofertilizers, which are both cost-effective and environmentally friendly, has tremendous potential for supplying nutrients and can reduce chemical fertilizer usage by 25-50% [4,5]. Microorganisms that serve as biofertilizers are crucial for promoting plant growth, sustaining soil fertility, and enhancing soil health. Foliar microbial populations can have either a positive, neutral, or negative impact on their hosts [6]. Phyllosphere bacteria have a significant impact on agriculture and the environment. *P. fluorescens*, for instance, is known to be a potential biocontrol agent that protects seeds and roots from fungal infection [7], Additionally, it also results in the production of secondary metabolites such as antibiotics, siderophores, and hydrogen cyanide [8]. Biological nitrogen fixation (BNF), both free-living and symbiotic, phosphate solubilization, siderophore production, and phytohormone synthesis are some of the most valuable features of these bacteria [9]. As the demand for sustainable agricultural practices rises, integrating PGPR with traditional inorganic fertilizers emerges as a promising approach. The integration aims to boost productivity while reducing chemical usage, maintaining soil ecology balance, and unlocking the potential of biota influencing plant growth. There is a clear need for further research to explore the efficacy of integrating PGPR with traditional fertilizers in soybean cultivation.

Material and Methods

Fertilizers were applied as per the established fertilizer adjustment equation for the targeted yield of soybean crop. Manurial schedule for Soybean are given in Table 1. The fertilizers were applied as Nitrogen, phosphorus and potassium per plot as basal doses through Urea, single super phosphate and murate of potash respectively before sowing of the crop. Where application of foliar spray of *Pseudomonas fluorescens* (30ml/liter) at three respective growing stages 25**,** 35 and 55 DAS as per the treatments.

Table 1: Manurial schedule for Soybean.

Treatments	Nutrients applied (kg ha^{-1})		
	N	P_2O_5	K_2O
T_1: Control	0	0	0
T_2: GRD	20	80	20
T_3: T.Y. 25 q ha^{-1}	23.31	62.31	27.65
T_4: T.Y. 30 q ha^{-1}	49.26	88.31	47.15
T_5: T.Y. 35 q ha^{-1}	75.21	114.3	66.65

Nodulation studies were done at 30, 45 and 60 DAS by uprooting 3 plants plot^{-1} by placing notch. The rhizosphere soil was washed in the running water. Nodules per plant were counted manually. Fresh weights of nodules were recorded after counting. The nodules were removed from the roots and kept in small paper bags then to record their fresh weight. Then the small paper bags were kept in a hot air oven at 70 ± 2^{0}C for 24 hours (till constant weight) to record their dry weight.

Leghaemoglobin (Lb) content in fresh nodules was determined as described by [9]. The solution's OD was recorded at 556 and 539 nm. The Lb content was calculated using the following formula:

$$\text{Lb concentration (Mm)} = \frac{OD556 - OD539}{23.4} \times 2D$$

Where, OD 556 and 539 represent absorbance (OD). Values recorded at 556, 539 IUU, respectively and D is the initial dilution.

BNF (kg ha^{-1}) = Total nitrogen uptake by crop in inoculated Plot (grain + straw) – Total nitrogen uptake by crop in fertilized uninoculated control (grain + straw)

Pseudomonas population counts in the phyllosphere (leaf surface)

Samples of leaves were used as fresh as possible without grinding or any modifications.

Preparation of leaf serial dilution.

Leaf samples were collected for pseudomonas population count and leaf-water dilution serial (1:1) by suspending 45 cm^2 of fresh leaf sample in 45 ml sterilized distilled water containing 1 drop of tween 80 in flasks and were shaken thoroughly which resulted in 10^{-1} dilution. Subsequent serial dilutions were made up to 10^{-5} dilution levels.

$$\text{Viable cells (cfu /cm}^2\text{ leaf)} = \frac{\text{No. of colonies}}{\text{Leaf area (cm)}} \times \text{dilution factor}$$

Results and Discussion

Nodulation attributes

The effect of foliar spray of *P. fluorescens* and their interaction with inorganic nutrients on nodulation attributes as influenced by different NPK levels based on targeted yield are presented in Table 2. The maximum number of root nodules, fresh and dry weight plant^{-1} recorded at 30 and 45 DAS. A significantly higher number of nodules (8.25 and 41.6 plant^{-1}) were recorded respectively under the treatment combination GRD + foliar spray of *P. fluorescens* but it was found at par with treatment T_3 (GRD, 8.20 and 40.3 plant^{-1}). The increase in nodules and their weight can be attributed to the positive response of soil inoculation enhancing rhizobial activity in the rhizosphere and hence such a response. The fresh weight of root nodules per plant (1.49 g plant^{-1}) and dry weight of root nodules per plant (0.78 g plant^{-1}) were recorded with T_6 (23:62:27 kg N+ kg P_2O_5 + kg K_2O ha^{-1} + foliar spray *P fluorescens*) at 45 DAS which is highly significant over only control while remaining treatments are on par with each other.

Soybeans contribute to soil fertility by fixing atmospheric nitrogen through root nodules and depositing nutrients through leaf fall upon maturity. However, exposure of roots to elevated levels of combined nitrogen is known to inhibit the development and nitrogen fixation activity of these nodules, aligning with the findings of [11]. Studies by [12-15], have reported that certain Plant Growth-

Table 2: Effect of foliar spray of *P. fluorescens* on nodulation at different growth stages of soybean under STCR approach.

Treatment	30 DAS			45 DAS		
	No of nodules plant^{-1}	Fresh weight of nodules (g)	Dry weight of nodules (g)	No of nodules plant-^{1}	Fresh weight of nodules (g)	Dry weight of nodules (g)
T_1: Control	7.53	0.29	0.23	36.4	0.62	0.31
$T_{2:}$ foliar spray *P. fluorescens*	7.68	0.32	0.25	37.3	0.79	0.39
T_3: GRD (N 20 + P_2O_5 80 + K_2O 20 kg ha^{-1})	8.20	0.38	0.30	40.3	1.28	0.65
$T_{4:}$ GRD + foliar spray *P. fluorescens*	8.25	0.41	0.32	41.6	1.35	0.67
T_5: TY 25 q ha^{-1}	8.40	0.45	0.36	35.2	1.40	0.70
T_6: TY 25 q ha^{-1} + foliar spray *P. fluorescens*	8.45	0.46	0.36	39.1	1.49	0.78
T_7: TY 30 q ha^{-1}	6.43	0.41	0.32	30.2	1.25	0.62
T_8 :TY 30 q ha^{-1} + foliar spray *P. fluorescens*	6.60	0.42	0.33	32.5	1.29	0.64
T_9: TY 35 q ha^{-1}	6.25	0.39	0.31	29.7	1.30	0.65
T_{10}: TY 35 q ha^{-1} + foliar spray *P. fluorescens*	6.30	0.40	0.32	31.5	1.32	0.66
Mean	7.41	0.39	0.31	35.38	1.21	0.61
SE m±	0.23	0.04	0.05	0.51	0.07	0.03
CD (p = 0.05)	0.69	0.12	0.15	1.56	0.21	0.09

Promoting Rhizobacteria (PGPR) enhance legume growth, nodulation, nitrogen fixation, root and shoot biomass, nodule dry weight, and ultimately, grain yield.

Leghaemoglobin content in nodules

The effect of foliar spray of *P. fluorescens* and their interaction with inorganic nutrients on leghemoglobin content in nodules as influenced by NPK levels was found significantly higher in the treatment T_6 (23:62:27 kg N + kg P_2O_5 + kg K_2O ha^{-1} + foliar spray *P. fluorescens*) Figure 1, having highest nodule leghaemoglobin content at 30, 45 and 60 DAS (0.65, 2.37 and 1.16 mM of fresh nodule) where minimum amount of leghemoglobin is found in treatment T_9 (0.32, 0.73, 0.63 mM) at all the stages of crop growth. However, an increase in N, P and K levels did not increase the nodule leghaemoglobin content significantly at all the growth stages of the crop.

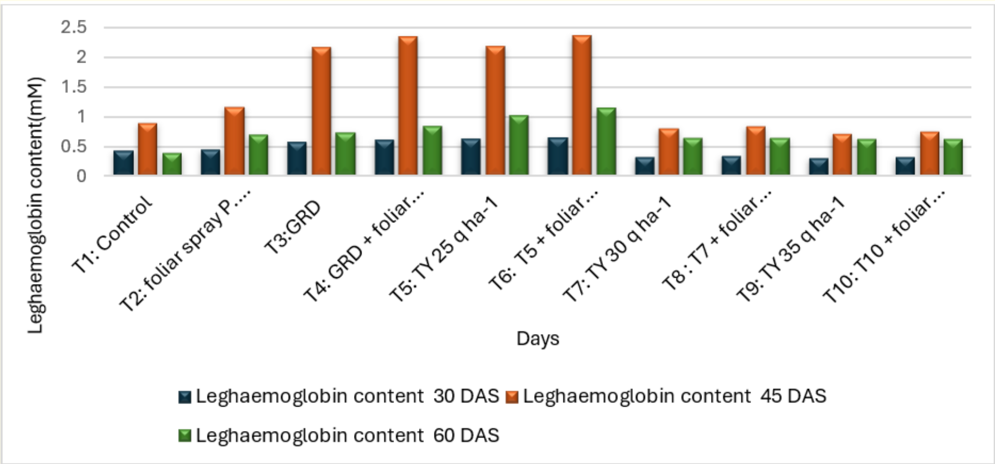

Figure 1: Effect of foliar spray of *P. fluorescens* on Leghemoglobin content in nodules at different growth stages of soybean under STCR approach.

Leghemoglobin (Lb) plays a crucial role in the N_2 fixation of leguminous nodules by facilitating O_2 supply to the bacteroid and protecting nitrogenase activity. Under high nitrogen levels, the concentration of Leghemoglobin (Lb) forms in nodules was decreased. This result indicates that nitrate can be absorbed from the nodule surface into the cytoplasm of the nodule epidermis, and it is transported by a symplastic pathway through plasmodesmata, and accumulated in the nodule cortex cells. Research on soybean Lb variations concerning plant age, nodule size, and nitrogenase activity has been conducted, shedding light on the dynamic changes in Lb levels under different conditions [16]. This highlights the intricate regulatory mechanisms involving Lbs in response to environmental cues and developmental stages within legume nodules. Studies by examined the changes in soybean leg haemoglobin concerning plant age, nodule size and nitrogenase activity.

Microbial population count

Data on *Pseudomonas* population counts in the phyllosphere presented in Table 3, shows a significant effect of foliar spray on *P. fluorescens*. Inorganic nutrients in the population are influenced by different N, P and K levels on the phyllosphere at 30, 45 and 60 DAS. Maximum total bacterial count was observed in T_{10} (75:114:66 kg N + kg P_2O_5 + kg K_2O ha^{-1} + foliar spray of *P. fluorescens*) {5.45(8.31x10^5), 6.14(1.38x10^6) cfu/cm^2 leaf} at 30 and 60 DAS which was found at par with T_4, T_6 and T_8 while minimum by control {5.23(1.69x10^5 cfu/cm^2 leaf)} at 60 DAS. Studies on the survival count of the *Pseudomonas* population increased at 60 days of leaf sampling compared to initial observation at 30 days on bacteria on leaves.

Table 3: Effect of foliar spray of *P. fluorescens* on population count on phyllosphere at different growth stages under STCR approach.

Treatment	*Pseudomonas* counts (cfu cm$^{2\ -1}$ leaf)		
	30 DAS	45 DAS	60 DAS
T_1: Control	4.21 1.31x10^4	4.75 5.24x10^4	4.52 3.31x10^4
$T_{2:}$ foliar spray *P. fluorescens*	5.23 1.69x10^5	5.32 2.08x10^5	5.97 9.33x10^5
T_3: GRD (N 20 + P_2O_5 80 + K_2O 20 kg ha^{-1})	4.31 1.41x10^4	4.92 8.31x10^4	4.45 2.81x10^4
$T_{4:}$ GRD + foliar spray *P. fluorescens*	5.36 2.88x10^5	5.4 2.63x10^5	6.11 1.28x10^6
T_5: TY 25 q ha^{-1}	4.38 1.47x10^4	5.11 1.25x10^5	4.72 5.24x10^4
T_6: TY 25 q ha^{-1} + foliar spray *P. fluorescens*	5.39 5.75x10^5	5.69 5.23x10^5	6.12 1.31x10^6
T_7: TY 30 q ha^{-1}	4.41 1.58x10^4	5.12 1.31x10^5	4.72 5.24x10^4
T_8:TY 30 q ha^{-1} + foliar spray *P. fluorescens*	5.39 6.60x10^5	5.72 6.03x10^5	6.13 1.34x10^6
T_9: TY 35 q ha^{-1}	4.41 1.69x10^4	5.13 1.34x10^5	4.81 6.45x10^4
T_{10}: TY 35 q ha^{-1} + foliar spray *P. fluorescens*	5.45 8.31x10^5	5.84 6.76x10^5	6.14 1.38x10^6
Mean	4.85	5.3	5.37
SE m±	0.03	0.01	0.05
CD (p = 0.05)	0.10	0.04	0.15

Studies on the survival count of the *Pseudomonas* population increased at 60 days of leaf sampling compared to initial observation at 30 days on bacteria on leaves. These results are similar to the findings of [17]. The *Pseudomonas fluorescens* which are phosphate solubilizers and free N_2-fixers, these microbes are heterotrophs and derive their nutrient either from soil reservoir or from applied fertilizers. The increase in population of *Pseudomonas* could be attributed to increased availability of N, P and K through applied fertilizers. These results are similar to the findings of [18].

BNF (Biological Nitrogen Fixation)

The study reveals a significant impact of foliar spray with *P. fluorescens* under varying N, P, and K levels on Biological Nitrogen Fixation (BNF), as depicted in Table 4 and Figure 2-3. Notably, the highest BNF of 8.7 kg ha^{-1} was observed in treatment T_6 (TY 25 q ha^{-1} + foliar spray of *P. fluorescens*), while the lowest occurred in treatment T_{10} (TY 35 q ha^{-1} + foliar spray *P. fluorescens*). The research findings indicate a significant influence of foliar spray with Pseudomonas fluorescens on Biological Nitrogen Fixation (BNF) under varying nitrogen (N) levels. The study demonstrates that foliar inoculation with *P. fluorescens*, along with *Azospirillum brasilense*, led to notable increases in tiller number, nitrogen accumulation in tissues, root system development, leaf blade percentage, and true digestibility *in vitro* of Megathyrsus maximus cv. BRS Zuri grass enhances overall nutritive value and yield [19]. Additionally, the investigation highlights the importance of phosphorus (P) availability in regulating BNF, showing that P deficiency can severely repress N fixation activity in diazotrophic bacteria like *Klebsiella variicola* W12, leading to adaptations such as lipid renovation to survive under P stress [20,21]. Additionally Consistent with findings, BNF facilitated by PGPR can contribute substantially, ranging from 12% to 70% of total N uptake in crops [22]. Furthermore, parallels are drawn with [23] observations, affirming that modest nitrogen levels stimulate plant growth, augment nodule parameters, and enhance nitrogen fixation, aligning with the current study outcomes.

Table 4: Effect of foliar spray of *P. fluorescens* on BNF of soybean under STCR approach.

Treatments	Total uptake (kg ha^{-1})	BNF
T_1: Control	58.2	0.0
$T_{2:}$ foliar spray *P. fluorescens*	62.8	4.6
T_3: GRD (20kg N+ 80 kg P_2O_5 + 20 kg K_2O ha^{-1})	102.0	0.0
$T_{4:}$ GRD + foliar spray *P. fluorescens*	108.4	6.7
T_5: TY 25 q ha^{-1}	160.8	0.0
T_6: TY 25 q ha^{-1} + foliar spray *P. fluorescens*	169.5	8.7
T_7: TY 30 q ha^{-1}	214.0	0.0
$T_{8:}$ TY 30 q ha^{-1} + foliar spray *P. fluorescens*	216.9	2.9
T_9: TY 35 q ha^{-1}	250.4	0.0
T_{10}: TY 35 q ha^{-1} + foliar spray *P. fluorescens*	252.3	1.9
Mean	159.53	2.48

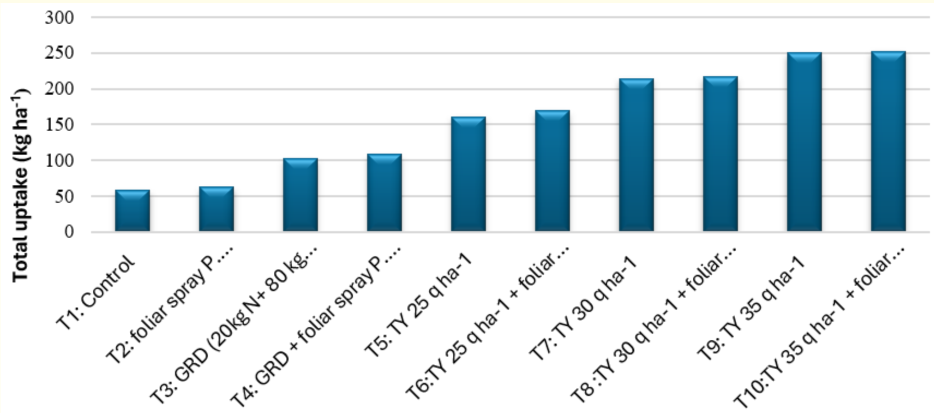

Figure 2: Effect of foliar spray of *P. fluorescens* on uptake of nutrients.

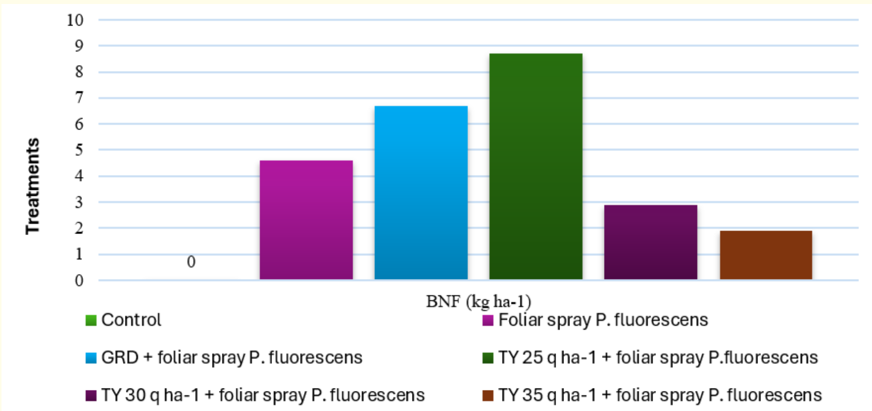

Figure 3: Effect of foliar spray of *P. fluorescens* on BNF of Soyabean under STCR approach.

Conclusion

The findings and discussion highlight that employing Treatment T_6 (TY 23:62:27 kg N, P_2O_5, and K_2O ha^{-1} + foliar spray *P. fluorescens*) significantly boosted nodulation, leghemoglobin content, and N2 fixation in nodules. Utilizing efficient Plant Growth-Promoting Rhizobacteria (PGPR) in the soil-plant environment emerges as an eco-friendly, cost-effective, and sustainable approach to enhance agricultural productivity. Additionally, the application of foliar biofertilizer holds promise for sustainable and organic farming, offering a potential solution to issues linked with chemical fertilizer use. This research underscores the significance of adopting environmentally conscious and economically viable practices for a resilient agricultural system.

Conflict of Interest

No conflict of interest.

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