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Influence of *Pseudomonas* and Biofertisol as Foliar Spray on Nodulation Attributes and Yield of Vegetable Pea under STCR Approach

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Authors' contributions

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

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ABSTRACT

A field experiment was carried out during the *rabi* season in the Experimental Field, Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur (M.P.) under RBD design with four replications consisting of five treatments of two types of biofertilizers: *Pseudomonas* and Biofertisol, and scheduled combinations of inorganic fertilizers based on STCR (Soil Test Crop Response) to achieve the desired yield using the vegetable pea variety PSM-3. The response due to treatment of T_4 (TY 100 q (58:110:47) + 5 t FYM+1 spray of Pseudomonas+1 spray of Biofertisol) was significantly effective in increasing nodulation attributes (nodulation enumeration, biomass and

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leghaemoglobin content) by 28.46, 47.15, and 35.29%; 78.33, 95.86, and 86.17%; and 21.70, 65.70, and 20.26%, respectively, relative to that of control. Yields of the crop were best harvested due to T_4 by 85.97 % over that of control 56.70 kg ha^{-1}. The continuous application of chemical fertilisers has decreased the fertility of the soil and degraded the soil. The reduction in the fertility of the soil has resulted in poor crop yields. The nutrient management of all the treatments involves the judicious use of organic and inorganic fertilizers, along with microbes, to meet the nutrient needs of the crop while minimizing environmental impacts.

Keywords: Biofertisol; leghaemoglobin; nodulation; Pseudomonas; STCR.

1. INTRODUCTION

Vegetable pea is commonly used in the human diet, and nutritionally, it is rich in protein, carbohydrates, vitamin A, calcium, and phosphorus and has high levels of the amino acid's lysine and tryptophan [1]. For high yielding pulse and oilseed cultivars to reach their full production potential, fertilizer is one of the most vital but expensive inputs. Nevertheless, it also leaves behind a considerable financial burden and a lot of environmental issues. The concept of Truog [2] as the basis for fertilizer application for targeted yields and the experimental proof provided by Ramamoorthy et al. [3] are effectively useful fertilizer recommendations for a targeted yield based on soil test value. Liebig's law of minimum states that plant growth is limited by the plant nutrient elements (N, P, and K) available in the smallest amount. The incorporation of organic manure and biofertilizers, however, as the best methods in organic and sustainable agriculture, can limit the usage of chemical fertilizer, according to the most recent idea of the STCR approach [4-8]. Even under the STCR concept for giving nutrients with a restricted dosage of inorganic fertilizer up to 25–30%, biofertilizers are a cost-effective and ecologically friendly input with enormous promise in organic and sustainable farming [9-14]. Bulky organic manure (FYM) has already been recognized for a number of benefits, including improved soil physical properties (such as structure, water holding capacity, etc.), optimized carbon sequestration, and a way to control parasitic nematodes and fungi with altered but balanced beneficial microorganisms as their source of carbon and energy.

A significant limiting factor for plant development and production is frequently the limited supply of nitrogen molecules in soils. Legumes circumvent this limitation by forming a symbiotic relationship with rhizobia that fix nitrogen for the plant. Legumes generate highly specialized structures called nodules in their roots through symbiotic associations with N-fixing soil bacteria of the genus Rhizobia [15]. The specialized root structures known as nodules, which are created by an organized and controlled process, are responsible for nitrogen fixation and nutrient exchange.

Plant growth-promoting rhizobacteria (PGPR) are slashing agricultural innovations. *Ascophyllum nodosum*, a seaweed, and enzymatic fish hydrolysate are combined to create Biofertisol, a novel organic manure that is a very rich source of micronutrients, phytohormones (including cytokinins), and growth regulators [16-21]. Additionally, beneficial for enhancing stress tolerance are organic inputs such volatile betaines and pH balancers [22]. The addition of recommended doses of fertilizers and plant growth regulators during foliage increases pulse productivity [23]. Organic manures (FYM, vermicompost and poultry manure etc.) also increase crop yields either by accelerating the respiratory process by increasing cell permeability or by hormone action and the application of living microorganisms to seeds [24-28]. They colonize the rhizosphere (the area around the roots of plants) or the interior parts of the plant and promote growth by increasing the supply or availability of primary nutrients to the pulse crop.

2. MATERIALS AND METHODS

The study trial was established on the vegetable pea crops during the rabi season. The field is situated 393 meters above mean sea level in the southeast of Madhya Pradesh at 23°13' North latitude and 79°57' East longitudes. The experimental field has a level topography and proper drainage. The soil in the experimental field is classified as Vertisol, and it is made of fine montmorillonite from the Kheri series and Typic Haplusterts from the Hyperthermic family, also referred to as "black cotton soil." Analyses of the soil's initial chemical properties included 7.31 pH, 0.24 ds m^{-1} electrical conductivity and the available soil nutrient status was 175.63, 11.56, and 218.41 kg ha^{-1} N, P, and K, respectively. The treatment combinations for a vegetable pea crop are given below as:

2.1 Treatment Combinations

T_1: Control
T_2: GRD (30:60: 30) + 3 spray of *Pseudomonas*+ 2 spray of Biofertisol
T_3: T.Y.* 80q (29:72:20) + 5 t FYM + 2 spray of *Pseudomonas*+ 2 spray of Biofertisol
T_4: T.Y.100q (58:110:47) + 5 t FYM +1 spray of *Pseudomonas* +1 spray of Biofertisol
T_5: T.Y.120q (87:147:74) + 5 t FYM
T.Y.* = Targeted Yield

At 30, 45, and 60 days after sowing, nodule enumeration was counted by gently removing five plants per plot without causing any nodule loss or damage. After counting, the nodules were put in little paper bags and stored in a hot air oven at 60 °C for 1–5 days (until a consistent weight) before being weighed using an electronic balance. Leghaemoglobin content in nodules was measured by Sadasivam and Manickam [29].

The harvested pods from all of the plots were weighed based on the net plot area, with the use of an electronic balance, the yields were measured in kilograms and converted to quintal per hectare (q ha^{-1}).

To assess the statistical significance of variation among various treatment means as impacted by the application of the treatments under research on various characteristics of vegetable pea, the recorded data were statistically analyzed using randomized block design.

3. RESULTS AND DISCUSSION

The result of the field experiment with different levels of N, P, and K based on the targeted yield of vegetable pea along with foliar sprays of *Pseudomonas fluorescens* and biofertisol with FYM on nodulation attributes and crop yield in the form of data was recorded and statistically analyzed.

3.1 Nodulation Studies

3.1.1 Nodule number

The nodule enumeration in vegetable peas was counted and tabulated in Table 1 and Fig. 1. At 30 DAS, the maximum number of nodules of 27.44 No. $plant^{-1}$ with a 28.5% response over that of the control (21.36 No. $plant^{-1}$) was recorded due to the application of T_4 (TY 100 q (58: 110:47) + 5 t FYM +1spray of *Pseudomonas*+1 spray of Biofertisol). This was followed by the effects from T_3, T_5 and T_2 exhibiting nodulation of 26.6, 25.3 and 23.8 No. $plant^{-1}$ and 24.5, 19.5 and 11.5% response, respectively. At 45 DAS of the crop, the maximum number of nodules (42.07 nodules $plant^{-1}$) was counted due to T_4 (TY100 q (58:110:47) +5t FYM+1 spray of *Pseudomonas* + 1 spray of Biofertisol) with the percentage response of 47.15 better over that of the control (28.59 No. $plant^{-1}$). This was followed by the response from T_3, T_5 and T_2 with nodulations of 40.4, 38.6 and 35.0 No. $plant^{-1}$ indicating 41.1, 34.9 and 22.2% increment, respectively. Similarly, the maximum number of nodules at 60 DAS range was 25.15 No. $plant^{-1}$ and 35.3% increment over that of control (18.59 No. $plant^{-1}$) which was observed due to the application of T_4 (TY100 q (58:110:47) +5 t FYM+1 spray of *Pseudomonas* +1 spray of Biofertisol). This was followed by the effects of T_3, T_5 and T_2 with nodulation of 24.5, 22.4 and 21.2 No. $plant^{-1}$ with the percent response of 31.7, 20.7 and 13.9, respectively.This may be attributed due to PGPS-colonized plants had improved nodulation with vigorous growth; Promoting the diazotrophic bacteria for better nodulation, nitrogenase activity, and nodular iron assimilation [30], thus helping the crops higher yield by supplying nitrogen particularly during the seed development stage [31].

3.1.2 Nodule biomass

At 30 DAS, treatment T_4 (TY100q (58:110:47) +5 t FYM+1spray of *Pseudomonas*+ 1 spray of Biofertisol) responded best by 56.44 mg nodule biomass $plant^{-1}$ with 78.33% increment as compared to that of control (31.65 mg $plant^{-1}$), followed by the effects of T_3, T_5 and T_2 with the nodule biomass of 51.26,45.23 and 38.56 mg $plant^{-1}$ and 62.0, 42.9 and 21.8% increment, respectively (Table1 and Fig. 2). Nodule biomass of vegetable pea at 45 DAS was 89.71 mg $plant^{-1}$ and an 89.7% response over that of control (47.29 mg $plant^{-1}$), which was envisaged due to T_4 (TY 100 q (58:110:47) + 5 t FYM +1 spray of *Pseudomonas*+1 spray of Biofertisol). The next group of nodule biomass was 87.29, 78.39 and 63.51 mg $plant^{-1}$ as the effects from T_3, T_5 and T_2 which were 84.58, 65.8 and 34.3% better in response, respectively. At 60 DAS of the crop, application of STCR based fertilizers for TY 100 q along with *Pseudomonas* and Biofertisol (T_4) performed best with 74.17 mg nodule biomass plant^{-1} and an 86.2% response over that of control (39.84 mg plant^{-1}). This was followed by the effects from T_3, T_5 and T_2 with the nodule biomass of 71.84, 62.30 and 51.25 mg plant^{-1} having the respective increments of 80.32, 56.38 and 28.64% respectively.

Singh [32] reported that application of FYM @ 15 t ha^{-1} was increased number of nodules and dry weight of the nodules plant^{-1} which gave a significantly higher grain yield of soybean than without FYM; nitrogen application decreased the number of nodules formed during the early growth stages of legumes.

3.2 Leghaemoglobin Content

The maximum content of leghaemoglobin in nodules at 30 DAS range 1.643 mg plant^{-1} representing a 21.7% response over that of control (1.350 mg plant^{-1}) for was estimated due to T_4 (TY100q (58:110:47) +5 t FYM+1spray of *Pseudomonas*+ 1 spray of Biofertisol). This was followed by the content of leghaemoglobin of followed by of 1.588 1.528 and 1.440 mg plant^{-1} with 17.6, 13.2 and 6.7% responses due to T_3, T_5 and T_2, respectively. The leghaemoglobin content in the nodules at different growth stages of vegetable pea is presented in Table 1 and Fig. 3. At 45 DAS, application of T_4 (TY100 q (58:110:47) +5 t FYM + 1 spray of *Pseudomonas*+1 spray of Biofertisol) increased leghaemoglobin content to 2.618 mg plant^{-1} which was 65.7% better over that of control (1.580 mg plant^{-1}). This was followed by the effects of T_3, T_5 and T_2 with leghaemoglobin content of 2.553, 2.483 and 2.388 mg plant^{-1} respectively with respective increments of 61.6, 57.2 and 51.1%. At 60 DAS, the maximum content of leghaemoglobin of 1.585 mg g^{-1} was estimated due to the treatment effect of T_4 (TY100 q (58:110:47) + 5 tFYM+1 spray of *Pseudomonas* + 1 spray of Biofertisol), which was 20.3% more relative to that of control (1.350 mg g^{-1}). The next group of was due to T_3, T_5 and T_2 for the leghaemoglobin content of 1.545, 1.493 and 1.430 mg plant^{-1} representing 17.2, 13.3 and an 8.5% response. The finding was in support to that of Eylenbosch et al. [33] that higher leghaemoglobin content was due to better root and nodule development. Dutta and Bandyopadhyay [34] reported that phosphorus and biofertilizer application increased significantly the nodulation, leghaemoglobin content, and nitrogenase activity.

3.3 Pod Yield

The maximum yield of a green pod (105.45 kg ha^{-1}) was harvested from the treatment T_5 (TY 120 q (87:147:74) +5 t FYM) which was found 85.97 % relative to that of control (56.70 kg ha^{-1}). However, it was statistically at par with the effect of application of T_4 (TY100 q (58:110:47) + 5 t FYM +1 spray of *Pseudomonas* +1 spray of Biofertisol), yielding 97.17 kg pod ha^{-1} with a 71.37% response. This was followed by the effects due to T_3 and T_2 with grain yield of 84.56 and 75.64 kg ha^{-1} respectively corresponding to 49.13 and 33.40% responses, respectively (Table 2 and Fig. 4). To ensure balanced fertilization of the appropriate kind and amount,

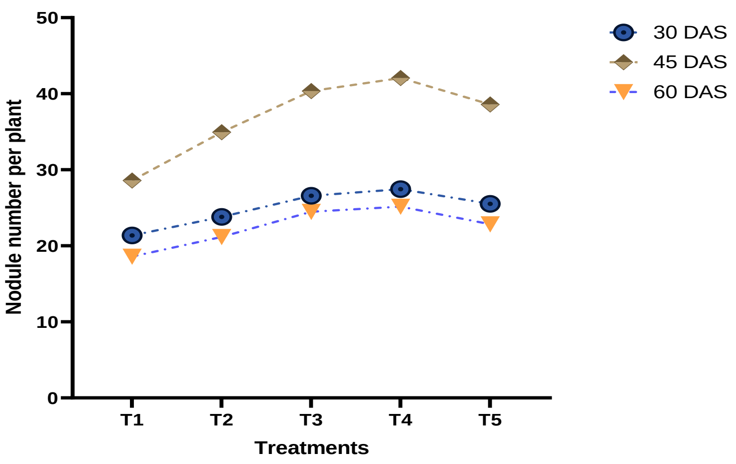

Fig. 1. Effect of *Pseudomonas* and Biofertisol on nodulation enumeration of vegetable pea at different growth stages under STCR approach

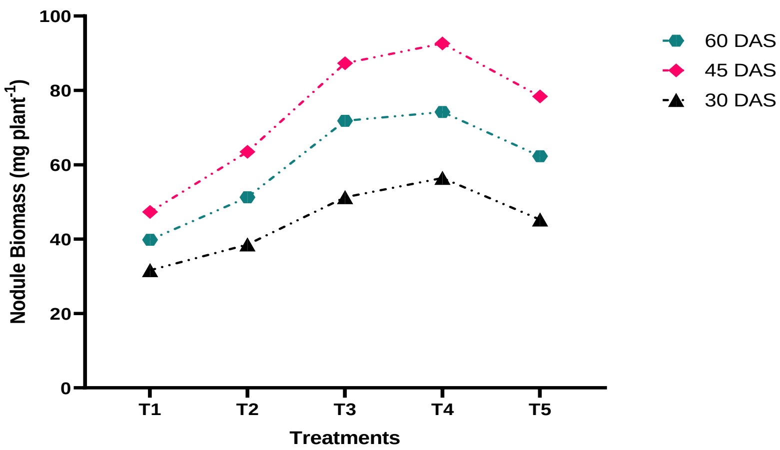

Fig. 2. Effect of *Pseudomonas* and Biofertisol on Nodule biomass of vegetable pea at different growth stages under STCR approach

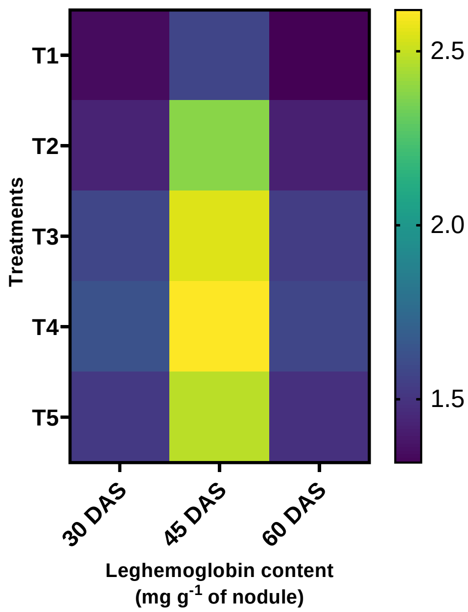

Fig. 3. Effect of *Pseudomonas* and Biofertisol on Nodule biomass of vegetable pea at different growth stages under STCR approach

soil test calibration aims to develop a relationship between the soil nutrient levels measured in the lab and the crop response to fertiliser nutrients applied in the field. As a result, the STCR idea of a targeted yield approach is important in recommending balanced fertilisation while taking the level of the soil's available nutrients and the crop's requirements into consideration [3]. The most prevalent plant growth regulators found in commercial extracts of *A. nodosum* in the current investigation were different cytokinins and cytokinin like substances. This increase in yield might be attributable to a number of factors [35]. Plant-PGPR interactions, according to Adesemoye and Kloepper [36], benefited seed germination, root growth, shoot and root weights, leaf area, chlorophyll content, protein content, nutrient absorption (like N and P), and yield. Remsangpuii and Mehera [37] also showed that application of integrated nutrients increases pod number per plant (19.09), number of seeds per plant (5.92), and seed yield (2.73 kg/ha).

Table 1. Effect of *Pseudomonas* and Biofertisol on nodulation attributes of vegetable pea at different growth stages under STCR approach

Treatment	Nodule enumeration (No. plant^{-1})			Nodule biomass (mg plant^{-1})			Leghaemoglobin content (mg g^{-1} of nodule)		
	30 DAS	**45 DAS**	**60 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**30 DAS**	**45 DAS**	**60 DAS**
T_1: Control	21.36	28.59	18.59	31.65	47.29	39.84	1.350	1.580	1.318
T_2: GRD (30:60:30) + 3 spray of *Pseudomonas*+ 2 spray of Biofertisol	23.81	34.95	21.17	38.56	63.51	51.25	1.440	2.388	1.430
T_3: T.Y. 80 q (29:72:20) + 5 t FYM+2 spray of *Pseudomonas*+2 spray of Biofertisol	26.59	40.35	24.48	51.26	87.29	71.84	1.588	2.553	1.545
T_4: T.Y.100 q (58:110:47) + 5 t FYM + 1 spray of *Pseudomonas* + 2 spray of Biofertisol	27.44	42.07	25.15	56.44	92.62	74.17	1.643	2.618	1.585
T_5: T.Y.120 q (87:147:74) + 5 t FYM	25.52	38.58	22.83	45.23	78.39	62.3	1.528	2.483	1.493
Mean	24.94	36.91	22.45	44.63	73.82	59.88	1.509	2.324	1.474
S.Em. ±	0.12	0.17	0.02	0.37	0.25	0.36	0.007	0.006	0.004
C.D. (P=0.05)	0.36	0.49	0.07	1.08	0.72	1.06	0.019	0.018	0.012

Table 2. Effect of Pseudomonas and Biofertisol on yields (green pod) of vegetable pea at harvest under STCR approach.

Treatment	Pod Yield (q ha^{-1})
T$_1$: Control	56.70
T$_2$: GRD (30:60:30) + 3 spray of *Pseudomonas* + 2 spray of Biofertisol	75.64
T$_3$: T.Y. 80 q (29:72:20) + 5 t FYM+2 spray of *Pseudomonas*+2 spray of Biofertisol	84.56
T$_4$: T.Y.100 q (58:110:47) + 5 t FYM+1 spray of *Pseudomonas* + 1 spray of Biofertisol	97.17
T$_5$: T.Y.120 q (87:147:74) +5 t FYM	105.45
Mean	82.78
S.Em. ±	5.13
C.D. (P=0.05)	15.09

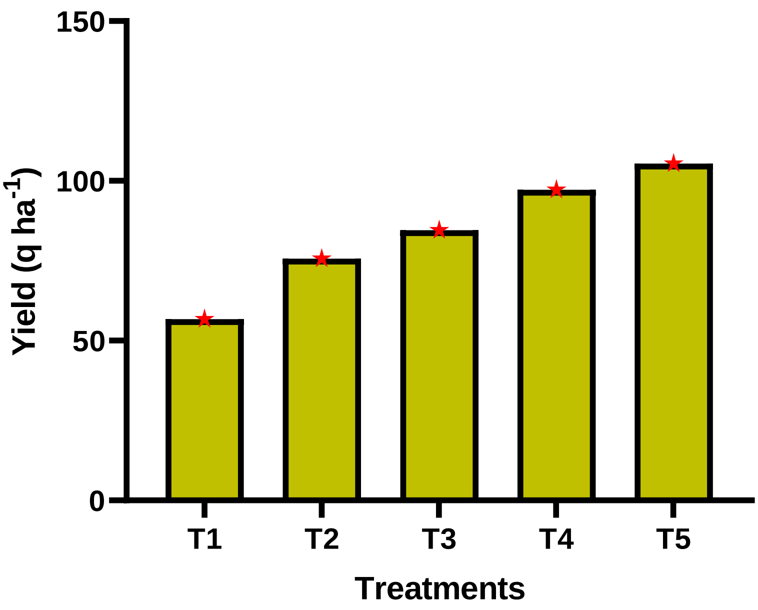

Fig. 4. Effect of Pseudomonas and Biofertisol on yields (green pod) of vegetable pea at harvest under STCR approach

4. CONCLUSION

The crop of vegetable pea (cv. PSM-3) thrived successfully with the balanced nutrients supplemented with the application of treatment T$_4$ (TY100q (58:110:47) + 5 t FYM+1 spray of *Pseudomonas*+1 spray of Biofertisol) under the STCR concept, which increased nodulation attributes and ultimately the yield of the crop. FYM increased the availability and supply of essential nutrients (0.5% N, 0.2% P_2O_5 and 0.5% K_2O) including micronutrients, improved soil physical conditions (like structure, water holding capacity etc.) and provided a better congenial environment for the multiplication and activity of the beneficial microorganisms viz., *Rhizobium*, *Pseudomonas*, and *Lactobacillus*. The microbial components of biofertilizers *Pseudomonas* may have improved the plant's access to nutrients, produced phytostimulators (hormones that promote plant development, usually auxin, cytokinin, and gibberellin), acted as rhizoremediators (degrading organic pollutants), and produced biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites). With potential characteristics of growth regulators, such as cytokinins and cytokinin-like substances, biofertisol, an organic source of nutrients (N: P: K = 1.5:0.5:0.4+0.1:0.5:1.0) supplemented with fish hydrolysate and an extract of seaweed (*Ascophyllum nodosum*), improved crop production. Taking a cue from Liebig's law of minimum, that plants growth is limited by the plant nutrient elements (N, P, and K) present in the smallest amount, the concept of Truog (1960) regarding the basis for fertilizer application for targeted yields and the experimental proof given by Ramamoorthy and co-workers [3] are effectively useful fertilizer recommendations for a targeted yield based on soil test value. In order to minimize the use of chemical fertilizer inclusion of organic manure and biofertilizers might be the best way to achieve organic and sustainable agriculture, as per the new concept of the STCR approach as well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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