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Influence of *Pseudomonas* and Biofertisol as a Foliar Spray on Soil Properties 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 conducted during the rabi season in the Experimental field, Department of Soil Science, JNKVV, Jabalpur (M.P.) under RBD design with four replications comprising five treatments of two types of biofertilizers: *Pseudomonas* and Biofertisol and scheduled combinations of inorganic fertilizers based on STCR (Soil Test Crop Response) for achieving targeted yield by using variety of vegetable pea, PSM-3. The best response was recorded from the application of treatment T_5 (TY120 q(87:147:74) +5tFYM) for increasing the content of soil available nutrients (N, P and K) by 7.89, 29.95 and 8.25%, respectively over that from control. Effect due to T_4 was significantly prominent on the proliferation of microorganisms viz., *Rhizobium* sp., *Pseudomonas*

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sp., and *Lactobacillus* sp. better by 6.79 log cfu (61.38 x 10^5 cfu g^{-1} soil), 6.44 log cfu (27.67 x 10^5 cfu g^{-1} soil) and 4.42 log cfu (26.18 x 10^3 cfu g^{-1} soil), respectively over control. The same treatment T_4 induced the enzyme activity of dehydrogenase by 86.99% as compared to that of the control (5.23 µg TPF hr^{-1} g^{-1}). Yields of the crop were best harvested due to T_4 by 81.91% over that of control 56.93 kg ha^{-1}. The vegetable pea (*Pisum sativum* L.), a cool-season crop and an important pulse crop in India. One of the impediments to supporting vegetable pea production and productivity is low soil fertility. Anthropogenic causes such as heavy use of fertilizer exacerbated the problem. A combination of fertilizers, biofertilizers and FYM are the solution to the problem since it makes use of available organic and inorganic nutrients and microbes to create an environmentally sound and economically sustainable farming system.

Keywords: Biofertisol; dehydrogenase; microorganisms; pseudomonas; STCR; vegetable pea.

1. INTRODUCTION

The vegetable pea (*Pisum sativum* L.) covers around 564 ha area in India, with a production of about 5694 tonnes (2019-20). Madhya Pradesh has coverage of 1.06 lakh ha area and production of 11.13 lakh mt production and 10.50 mt ha^{-1} productivity, that too in Jabalpur it is grown as a major crop on around 31.36 ha giving a yield of 52.50 MT (NHB 2019-20). 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 amino acids lysine and tryptophan [1].

Fertilizer is one of the most important but expensive inputs in achieving the yield potential of high-yielding pulse and oilseed cultivars. According to the latest concept of the STCR approach, the use of chemical fertilizer can be minimized with the inclusion of organic manure and biofertilizers, as thebest way in organic and sustainable agriculture [2]. Biofertilizers are a cost-effective and environmentally friendly input that has tremendous potential in organic and sustainable farming even under the STCR concept for supplying nutrients with curtailed doses of inorganic fertilizer up to 25-30% [3]. The bulky organic manure (FYM) has already been acknowledged for several advantages like supply and availability of plant nutrients including micronutrients, improved soil physical properties (i.e. structure, water holding capacity, etc.), optimized carbon sequestration, and a way to control parasitic nematodes and fungi with altered but balanced beneficial microorganisms for their source of carbon and energy.

Plant Growth Promoting Rhizobacteria (PGPR) *Pseudomonas* sp. Is future-oriented technology for sustainable agriculture. Biofertisol, a new introduction to organic manures, is a mixed product of enzymatic fish hydrolysate and sea weed (*Ascophyllum nodosum*), is a spectacularly rich source of essential nutrients and micronutrients along with phytohormones (such as cytokinins) and growth regulators.

2. MATERIALS AND METHODS

The research trial was laid out during the rabi 2020-21 on the vegetable pea crops. The field is located in the south eastern part of Madhya Pradesh at $23^0$13' North latitude, 79^0 57' East longitudes at an altitude of 393 meters above the mean sea level. The experimental field was well-drained with levelled topography. The soil of the experimental field is categorized as Vertisols, and they are from the Kheri series of fine montmorillonite and the Hyperthermic family of Typic Haplusterts, which is known as "black cotton soil". The initial basic properties of the soil are pH 7.31, EC 0.24 dSm^{-1} and organic carbon 4.9 g kg^{-1}. The available N, P, and K status in soil were 176, 11.6, and 218 kg ha^{-1}, respectively. The vegetable pea seeds (cv. PSM-3) were sown @ 100 kg ha^{-1} with inoculation as per the prescribed treatments in RBD with five treatment combinations and four replications. The crop was nourished with RDF 20:30:60 (N: P_2O_5: K_2O kg ha^{-1}) at basal dose through urea, single super phosphate, and muriate of potash, respectively. The treatment details are as **T_1:** Control; **T_2:** GRD (30:60: 30) + 3spray of *Pseudomonas* + 2spray 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 and **T_5:** T.Y.120q (87:147:74) + 5 t FYM.

The initial and post-harvest soil samples were taken from a depth of 0 to 15 cm for independent chemical and microbiological analysis in accordance with normal sampling practices. The soil pH was determined using the Glass

electrode pH meter method by the taking 1:2.5 ratio of soil and water suspension [4], EC by Electrical Conductivity meter method [4], available N was estimated by alkaline potassium permanganate method [5], available P was extracted with 0.5 *M* $NaHCO_3$ solution (pH 8.5) [6] and available K by neutral 1N ammonium acetate extraction and content was estimated as method described by [7].

The soil samples were used as fresh as possible without grinding, sieving or any modifications for microbial enumeration purposes. The low-density polyethylene (LDPE) bags that contained the collected samples were used as soon as feasible for microbiological tests and could be kept in the refrigerator at 4°C and these fresh soil samples were used for plating and counting of microbial populations by serial dilution method [8]. The dehydrogenase activity (DHA) was estimated by incubation with triphenyl tetrazolium chloride (TTC) as described by Burns [9].

The study was based on a randomized block design with four replications for each treatment. The data generated on soil analysis and yields were statistically analyzed to draw inferences as per the method described by Panse and Sukhatme [10].

3. RESULTS AND DISCUSSION

The result of field experiments with different levels of N, P, and K based on the targeted yield of vegetable peas along with foliar spray of *P. fluorescens* and Biofertisol on soil parameters in the form of data were recorded and statistically analyzed.

3.1 Available Nutrients (NPK) Status in Soil

Out comes of the study about available nutrients (N, P and K) in surface soil (up to 0-15 cm depth) at harvest of vegetable pea are presented in Table 1. The maximum uptake of nitrogen 104.19 kg N ha^{-1} was recorded due to the application of T_5 (TY120 q(87:147:74) +5tFYM) which was 7.89% better over the control treatment of T_1 (158.22 kg N ha^{-1}). This was followed by the influence from T_4, T_3 ,andT_2 for the available nitrogen content of 168.67, 166.22, and 162.22 kg N ha^{-1} along with responses 6.61, 5.06 and 2.53%, respectively. The increase in available N in the soil might also be attributed to the greater multiplication of soil microbes which converts organically bound N to inorganic forms reported by [11].

The treatment of T_5 (TY120q(87:147:74) +5tFYM) performed maximum representing 10.89 kg P ha^{-1} and 29.96% response relative to that from control (8.38 kg ha^{-1}). This was followed by performance from T_4, T_3 and T_2 for the available phosphorous content as 10.31, 9.71, and 9.46 kg P ha^{-1} with the respective response of 23.04, 15.88 and 12.89. These results are similar to the findings of [12] that the heterotrophic bacteria *P. fluorescens* might contribute to phosphate solubilization and free N_2-fixation. Similarly, other researcher's findings confirmed that certain bacteria, fungi and actinomycetes were capable of solubilizing nutrient minerals into soluble form by enzymatic oxidation-reduction reactions, formation of chelates and complexes with protein, aminoacids, organic acids, etc. Phosphate-solubilizing microorganisms are common, but their quantities vary by soil [13].

The highest content of 211.12 kg K ha^{-1} was obtained due to T_5 TY120 q(87:147:74) +5tFYM with 8.26% response over that of control (195.03 kg K ha^{-1}). This was followed by the effects from T_4, T_3 and T_2 presenting the available content of the nutrient of 207.73, 204.86, and 200.89 kg K ha^{-1} having with percentage responses of 6.52, 5.05 and 3.01, respectively. The availability of potassium might be increasing by many genera of bacteria such as *Pseudomonas, Bacillus*, *Achromobacter, Agrobacterium, Serratia* and several others solubilizing varying quantities of the nutrient depending on the efficiency of the strains. The most efficient and dominant solubilizers belong to the genera *Bacillus* and *Pseudomonas* [14]. Whereas, *Ascophyllum nodosum* improved both the growth and productivity of agricultural crops by increasing nutrient availability and its uptake [15,16,17]. *Ascophyllum nodosum* influenced natural chelation in the soil due to the presence of residual alginates present in the hydrolyzed extract, which allowed for an increase in plant-available minerals and increased soil aeration and water-holding capacity.

3.2 The population of Microorganisms in Rhizospheric Soil at the Crop Harvest

The populations of *Rhizobium* sp., *Pseudomonas* sp*., Lactobacillus* sp*.* in rhizospheric soil collected at the crop harvest were counted and shown in Table 2.

The maximum population of *Rhizobium* sp. in the rhizospheric soil due to use of T_4 (TY 100 q (58:110:47)+5 tFYM+1 spray of *Pseudomonas* + 1sprayofBiofertisol) as 6.788 log cfu ($61.38x10^5$ cfu g^{-1} soil) which was 1.54 log fold higher than that from control (4.418 log cfu = 26.19×10^3 cfu g^{-1} soil), but it was at par that from the application of T_3 (TY 80 q (29:72:20)+5 t FYM+2 spray of *Pseudomonas* +2 spray Biofertisol) representing the rhizobial population of 6.580 log cfu ($38.02x10^5$ cfu g^{-1} soil) with 1.49 log fold response. The treatments of the next performing group wereT_5 and T_2 for the bacterial population of 5.215 log cfu ($16.41x10^4$ cfu g^{-1} soil) and 5.873 log cfu (74.65×10^4 cfu g^{-1} soil) along with 1.18 and 1.33 log fold response. This might be due to the application of *Ascophyllum nodosum* and its organic fractions that induced rhizobial proliferation by regulating the legume-rhizobia signaling process [18]. Synergistic effect of the lactic bacteria and the endophytic bacteria in green gram; N-fixation, P-solubilization, and phytohormone production might also play a vital role in maintaining microbial population [19]. INM techniques significantly improved soil physical, chemical, and biological parameters compared to individual organic and chemical management approaches [20,21].

The response from T_4 (TY100q (58:11:47) + 5 t FYM + 1 spray of *Pseudomonas* + 1 spray of Biofertisol) was statistically best influencing the PGPR population by 6.44 log cfu ($27.67x10^5$ cfu g^{-1} soil) and 1.22 log fold response relative to that from control (5.293 logs cfu =19.64×10^4 cfu g^{-1}soil). But, the performance of T_4 was found statistically at par to that of T_3 (TY 80 q (29:72:20) +5 t FYM + 2 spray of *Pseudomonas* + 2 spray Biofertisol) which was 6.290 log cfu (19.50×10^5 cfu g^{-1}soil) with a response of 1.19 log fold. This was followed by the effects from T_5 and T_2 exhibiting the bacterial population of 5.923 log cfu ($83.76x10^4$cfu g^{-1} soil) and 6.015 log cfu (10.35×10^5cfu g^{-1}soil) with the respective response of 1.12 and 1.13 log fold. PGPR possessing the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase facilitated plant growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants [22]. Cell wall-degrading enzymes (β-1, 3-glucanase, chitinase, and cellulase) of rhizobacteria affected the structural integrity of the wall of the target pathogen, thus had a competitive survivability [23]. *Pseudomonas* members are extremely effective at competing for root resources in rhizobacterial communities [24]. The PGPR strains released different volatile blends and the difference in these volatile blends stimulated plant growth. The increase in the population of *Pseudomonas* could be attributed to the increased availability of N, P, and K through applied fertilizers. The population of *Pseudomonas* in soil ranges from 6.5 to $8.02x10^4$cfu g^{-1}. As compared to the control treatment, seed inoculation with *Pseudomonas* at 3 g kg^{-1} resulted in the highest number of *Pseudomonas* population with 8.5% numerical increase [25]. PGPR capable of swarming in close association with plant rhizosphere and maintain plant growth either by defending them from environmental stress or diseases or by providing essential nutrients and hormones through various mechanisms [26].

Among all the treatments, the effect from T_4 (TY 100 q (58:110:47) + 5 t FYM + 1 spray of *Pseudomonas* +1 spray of Biofertisol) was registered maximum for the population of lactic bacteria4.418 log cfu ($26.18x10^3$ cfu g^{-1} soil) with a response of 1.37 log fold relative to that from control (3.223 log cfu = $16.71x10^2$cfu g^{-1} soil).; but which was statistically at par to that from T_3 (TY 80 q (29:72:20)+5 t FYM+2 spray of *Pseudomonas* + 2 spray Biofertisol) with the bacterial population of 4.337 log cfu (21.73×10^3 cfu g^{-1} soil) and 1.34 log fold response. This was ensured by the performance from T_5 and T_2 with the population of 3.865 log cfu (73.28×10^2 cfu g^{-1} soil) and 4.163 log cfu (14.55×10^3cfu g^{-1} soil) which were 1.19, 1.29 log fold more over that of control. The findings of researchers stated that lactic acid bacteria when inoculated into soil amended with organic materials, could enhance the decomposition and release of plant nutrients and increase soil humus formation and proliferation of microorganisms with commensal relationship [27].

3.3 Dehydrogenase Activities in Soil

The treatment T_4(TY100q (58:110:47) + 5t FYM+1spray of *Pseudomonas* + 1 spray of Biofertisol) influenced biological system for increasing maximum activity of dehydrogenase by 9.78 µg hr^{-1} g^{-1} and 86.99% response relative to that from the control (5.23 µg TPF hr^{-1} g^{-1}). Performance from the remaining treatments of T_5, T_3 andT_2 followed the above representing the enzymatic activity of 8.57, 8.8 and 7.98 µg $hr^{-1}g^{-1}$ with the respective response of 63.87, 69.79 and 52.59%. The data on enzyme activity of dehydrogenase in the rhizospheric soil at harvest of the crop, as influenced by different treatments under study, were presented in Table 2.

Table 1. Effect of *Pseudomonas* and Biofertisol on available nutrients (N, P and K) in soil at harvest of the crop under STCR approach

Treatments	Soil available nutrient (kg ha^{-1})		
	N	P	K
T_1: Control	158.22	8.38	195.03
T_2:GRD(30:60:30) + 3sprayof*Pseudomonas* +2sprayof Biofertisol	162.22	9.46	200.89
T_3:TY 80 q(29:72:20) + 5tFYM+2 spray of *Pseudomonas* + 2 spray of Biofertisol	166.22	9.71	204.86
T_4:TY 100 q(58:110:47) + 5tFYM+1 spray of *Pseudomonas* + 1 spray of Biofertisol	168.67	10.31	207.73
T_5: TY 120 q(87:147:74) + 5tFYM	170.70	10.89	211.12
Mean	**165.21**	**9.75**	**203.93**
SE_m±	**0.33**	**0.19**	**0.46**
$CD_{5\%}$	**0.98**	**0.56**	**1.35**

Table 2. Effect of *Pseudomonas* and Biofertisol on population of microorganisms and Dehydrogenase activity in rhizospheric soil at harvest of the crop under STCR approach

Treatments	Population of microorganism (cfu g^{-1})			Dehydrogenase activity
	***Rhizobium* sp.**	***Pseudomonas* sp.**	***Lactobacillus* sp.**	**(µg TFP hr^{-1} g^{-1})**
T_1: Control	4.418 (26.19x10^3)	5.293 (19.64x10^4)	3.223 (16.71x10^2)	5.23
T_2:GRD(30:60:30) + 3sprayof *Pseudomonas*+ 2 spray of Biofertisol	5.873 (74.65x10^4)	6.015 (10.35x10^5)	4.163 (14.55x10^3)	7.98
T_3:TY 80 q(29:72:20) + 5tFYM+2 spray of *Pseudomonas* + 2 spray of Biofertisol	6.580 (38.02x10^5)	6.290 (19.50x10^5)	4.337 (21.73x10^3)	8.88
T_4:TY 100 q(58:110:47) + 5tFYM+1 spray of *Pseudomonas* +1spray of Biofertisol	6.788 (61.38x10^5)	6.448 (27.67x 10^5)	4.418 (26.18x10^3)	9.78
T_5:T Y 120 q(87:147:74) + 5tFYM	5.215 (16.41x10^4)	5.923 (83.76x10^4)	3.865 (73.28x10^2)	8.57
Mean	**5.774 (59.43x10^4)**	**5.993 (98.40x10^4)**	**4.001 (10.02x10^3)**	**8.09**
SE_m±	**0.074**	**0.124**	**0.063**	**0.13**
$CD_{5\%}$	**0.218**	**0.365**	**0.184**	**0.37**

Table 3. Effect of *Pseudomonas* and Biofertisol on pod yield of vegetable pea at harvest under STCR approach

Treatments	Pod Yield (q/ha)
T_1: Control	56.93
T_2:GRD(30:60:30) + 3sprayof*Pseudomonas* + 2sprayof Biofertisol	79.25
T_3:TY 80 q(29:72:20) + 5tFYM+2 spray of *Pseudomonas* + 2 spray of Biofertisol	89.67
T_4:TY 100 q(58:110:47)+5tFYM+1 spray of*Pseudomonas*+1 spray of Biofertisol	97.51
T_5: TY 120 q(87:147:74) + 5tFYM	103.56
Mean	**85.39**
SE_m±	**4.47**
$CD_{5\%}$	**13.71**

Microbial enzymes have vital role in soil and are used to measure the soil quality and influence of soil management [28,29]. Soil enzymes are important in catalyzing numerous essential reactions, necessary for existing processes of microorganisms in soils and the stabilization of soil structure, decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture. The benefits of sea weeds as sources of organic matter and fertilizer nutrients have led to their use as soil conditioners. The findings of [30] and [31] exhibited the significant increment in dehydrogenase, phosphatase activity and soil microbial biomass carbon by use of 100% RDF+FYM @10 t ha^{-1}.

3.4 Pod Yield of Vegetable Pea

The data on total yield of green pods (3 pickings) during the crop period and that of pods + straw at harvest was recorded and presented in Table 3. The maximum green pod yield of 103.56 kg ha^{-1} was harvested from the application of treatment T_5 (TY120 q(87:147:74)+5tFYM) which was computed as 81.91% relative that of control (56.93 kg ha^{-1}). However, it was statistically at par to the effect from application of T_4 (TY 100q (58:110:47) + 5tFYM + 1 spray of *Pseudomonas* +1 spray of Biofertisol) yielding 7.51 kg grain ha^{-1} with 71.28% response. This was followed by the effects due to T_3 and T_2 with grain yield of 89.67 and 79.25 kg ha^{-1} corresponding to 48.86 and 39.2% response, respectively. In the present study, an increase in yield could be attributed by various reasons that various cytokinins and cytokinin-like compounds were the most abundant plant growth regulators present in commercial extracts of *A. nodosum* [32]. Foliar application of PGR might have enhanced the CO_2 fixation and induced activity of carbohydrates synthesizing enzymes which is analyzed by an increase in a number of pods per plant and number of seeds per pod leading to balanced metabolism maintained continuously inside the plant for subsequent phases of growth. Findings of [33] also confirmed that *Pseudomonas fluorescens* increased grain yield by 33.8% and seed index (1000 grain wt.) by 12.9%.

4. CONCLUSION

The crop of vegetable pea (cv. PSM-3) thrived successfully with the balanced nutrients supplemented with the application of treatment T_4 (TY 100 q(58:110:47) + 5tFYM + 1 spray of *Pseudomonas* + 1 spray of Biofertisol) under STCR concept towards maximum soil available nutrients, the proliferation of beneficial microorganisms and their enzymatic activity (DHA) 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 (structure, water holding capacity etc.) and provided a better congenial environment for multiplication and activity of the beneficial microorganisms viz., *Rhizobium, Pseudomonas, Lactobacillus*. The microorganisms in biofertilizers *Pseudomonas* might have increased the availability of nutrients to the plant, provided phytostimulators (plant growth promoting, usually by the production of phytohormones: auxin, cytokinin, gibberellin), acted as rhizoremediators (degrading organic pollutants) and biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites). Biofertisol, an organic source of nutrients (N:P:K = 1.5:0.5:0.4 + 0.1:0.5:1.0) furnished with fish hydrolysate and extract of sea weed (*Ascophyllum nodosum*) increased the crop yield with plausible attributes of growth regulators viz., cytokinins and cytokinin-like compounds. In order to minimize the use of chemical fertilizer inclusion of organic manure and biofertilizers

might be the best way in organic and sustainable agriculture and 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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