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Zero Tillage in Combination with Seed Treatment by Different Biofertilizers Increased Soil Organic Carbon, Macro and Micro-nutrients Status and Nodulation under Faba Bean (*Vicia faba* L.)

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ABSTRACT

Background: Symbiotic association with different microbes is an integral and ecologically vital component of legumes. Most previous studies focused on sole application of microbial agents, with limited studies investigating the effects of co-inoculation of different microbial agents under different tillage practices on soil properties. The current study aimed to assess the effect of tillage practice and seed treatment with different biofertilizers inoculations on the soil chemical properties and nodulation under faba bean (*Vicia faba* L.) crop.

Methods: The study was conducted in factorial randomized block design (FRBD) with two tillage system *viz*., T_1: Conventional tillage (CT), T_2: Zero tillage (ZT) and eight biofertilizer inoculation combinations of *Rhizobium spp*., phosphorus solubilizing bacteria (PSB) and vesicular arbuscular mycorrhizae (VAM) replicated three times during *rabi* 2019-20.

Result: Zero tillage and biofertilizer inoculations significantly increased organic carbon, available nitrogen and phosphorus content of the soil. The number of nodules/plant, nodules fresh and dry weight/plant were recorded significantly higher in ZT as compared to CT and in seed treatment with *Rhizobium spp.* + PSB + VAM over other inoculations except *Rhizobium spp.* + VAM. The content of DTPA (Diethylenetriamine pentaacetate)-extractable Fe and Mn in soil was significantly enhanced by seed treatment with biofertilizer.

Key words: Available nutrients, Organic carbon, PSB, *Rhizobium spp*., VAM, Zero tillage.

INTRODUCTION

Pulse crops are of primary importance to human beings due to their high nutrition value and soil health-improving capabilities (Bhardwaj *et al*., 2021). They play a significant role in improving soil fertility and maintaining sustainability through their multi-pronged services like carbon sequestration, lowering greenhouse gases (GHG) emissions, solubilization of insoluble soil phosphorus, biological nitrogen fixation, enhancing soil physical and microbial environment (Stagnari *et al*., 2017). However, the real potential of only a few species from such a varied group has been exploited in India so far, *e.g.,* during 2013-14, Bengal gram dominated with over 48.1% stake of entire pulse production followed by red gram (16.0%), black gram (8.5%), green gram (8%), lentil (5.9%) and other legumes (13.5%) (IIPR Vision-2050, 2015). There is a need for more research to be carried out to explore other pulse crops and faba bean is one of such crops with good future prospects. Faba bean is a potential multipurpose short-duration grain legume crop grown in the cool season (Etemadi *et al*., 2018). Faba bean is well known for its biological nitrogen fixation capabilities and can fix 50-330/ kg N ha^{-1} (Etemadi *et al*., 2018). It is rich in minerals, proteins, dietary fibre, complex carbohydrates, lecithin, choline and secondary metabolites. It also has great therapeutic potential, as it contains a large amount of L-3, 4 dihydroxyphenylalanine (L-DOPA), a precursor to neurotransmitter catecholamine used to treat Parkinson's disease (Etemadi *et al*., 2018).

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Despite being the largest producer of pulses in the world with a net production of 23.15 million tonnes in 2019-20 accounting for 23.62% of the world, the country imported pulses worth `102.21 billion in 2020 itself to meet the domestic need (Ministry of Agriculture and Farmers Welfare, Government of India, 2021). Thus, a three-pronged approach *i.e.*, diversification, increase in productivity and improvement in soil characteristics when combined together

will help in meeting the demand and to overcome this huge gap between demand and supply of pulses. Faba bean nicely fits into this scheme of things for crop diversification as it is legume crop with very low water requirement. Seed treatment with biofertilizers is a cost-effective, eco-friendly way to increase the productivity of pulse crops. The notion that seed inoculation with biofertilizer is a very lucrative and successful agronomic method has been widely researched and established by the academic community. The role of rhizobium inoculants is well studied and researched in various legumes including faba bean but meagre efforts have been made to enquire the effect of co-inoculation of different microbial inoculants like *Rhizobium*, PSB and VAM for improving the soil fertility under faba bean crop. A study conducted by Sharma *et al*. (2019) showed a significant increase in DTPA-extractable micronutrients with co-inoculation of biofertilizer in chickpea as compared to single inoculation indicating a synergistic interaction.

Soil tillage is a basic and an important input to alleviate soil related constraints in crop production. It has long-term effects on sustainability through its effect on nutrient availability, soil properties and crop growth (Dhiman and Dubey, 2017). However, the long-term negative effects of intensive conventional tillage, such as soil compaction, disruption of soil structure and decreased hydro-stability of aggregates leads to faster soil erosion and infertility, are now well understood (Dayou *et al*., 2017). The conventional tillage was found to decrease soil microbial biomass, active carbon, total nitrogen and aggregate stability, in contrast, no-till significantly increased the same in a study conducted by Aziz *et al*. (2013). Numerous studies have also indicated beneficial interactions between microbial inoculation and tillage. Thus, in order to apply the above explained three prolonged approach, an attempt has been made to examine the effect of zero tillage in combination with seed treatment by different biofertilizers on soil organic carbon, macro and micro-nutrients status and nodulation under faba bean crop.

MATERIALS AND METHODS

This field study was carried out at the experimental farm of Medicinal, Aromatic and Potential Crop Section (MAP), Department of Genetics and Plant Breeding of CCS Haryana Agricultural University, Hisar, Haryana, India during *rabi* 2019-20. The soil characteristics determined at beginning of the experiment from 0-15 cm top layer are shown in Table 1. The climate of the experimental site was tropical monsoon with a mean annual temperature of 25.3°C and mean annual rainfall of 450 mm concentrated mostly during South-West Monsoon (July-September: 80%). Factorial randomized block design (FRBD) with three replications was used for conducting the experiment. The experimental area was divided in 48 plots with individual plot size of 5.4 × 5 m (27 m^2). Seedbed was prepared with tractor-drawn disc harrow and cultivator when the field attained sufficient moisture conditions for tillage operation, followed by planking in the case of conventional tillage blocks. In contrast, no-tillage operations were done in zero-tillage blocks. To control weeds, ZT plots received a herbicidal treatment of Roundup Glyphosate 41 per cent SL @ 3 L/ha. Seeds were treated with *Rhizobium spp*., PSB and VAM using the jaggery (adhesive). Seed treatment was done in the evening while sowing was done next morning. The source of biofertilizers used was Rhizotica for *Rhizobium spp*., Phosphotica for PSB obtained from biofertilizer lab of CCS HAU and spore culture containing *Glomus mosseae* mycorrhizae for VAM inoculation. Seeding (cv. HFB-1) was done at recommended rate of 100 kg/ha maintaining a row to row spacing of 45 cm. Only a recommended starter dose of 40 kg N/ha and 60 kg P_2O_5/ha was applied in each of the plots as basal dose at the time of sowing. Two irrigations, first at flowering and second at pod filling stage using canal water were applied. The weeds were mechanically controlled by two hand weeding's in CT blocks at 30 and 60 DAS. Whereas one selective/ patch weeding was carried out for ZT blocks.

To determine the soil physico-chemical properties, representative soil samples up to a depth of 0-15 cm were randomly collected from six different places of experimental site just before the beginning of investigation. The procedure followed for physical and chemical characterisation of soil and results obtained are presented in Table 1. Four random samples were collected from each plot of the experimental area post-harvest and were processed for analysis of organic carbon (OC), electrical conductivity (EC), pH, available nitrogen, phosphorus, potassium and DTPA extractable micronutrients content in soil. For nodulation studies, three random plants per plot were carefully dug out with help of a sickle at 50% flowering from a wet field to get an intact root system. Roots were gently washed with water and number of nodules was counted and weighed to get nodules fresh weight. Then mean was computed to get the number of nodules/plant. After counting number of nodules per plant at 50% flowering, nodules were detached and oven-dried at 70°C up to constant weight was obtained. The dry weight of nodules was measured and expressed in g/plant. The statistical analysis and interpretation of results were done using statistical method given by Panse and Sukhatme (1985). For evaluating comparative performance of different treatments, the data were analyzed using analysis of variance technique described by Fisher (1950). Mean values of replicated observations have been used in study. All the tests of significance were done at 5% level of significance. Treatment means were compared for critical difference (C.D.) to determine the significance of the treatments.

RESULTS AND DISCUSSION

EC, pH and OC

Different tillage and biofertilizer treatments did not result in significant variation in EC and pH of soil (Table 2). However, numerically higher EC and lower pH were observed in ZT (0.29 and 7.82) as compared to CT (0.28 and 7.83), respectively (Table 2) which may be attributed to the

formation of organic acids and mineralization of plant residues in soil under zero tillage (Chatterjee and Lal, 2009). Electrical conductivity (ds/m) was recorded minimum in control (0.26), while maximum in *Rhizobium spp.* + VAM and *Rhizobium spp.* + PSB + VAM (0.30) biofertilizer inoculations. As the EC of soil is directly related to the ions present in it; so the probable reason is the production of acids or their intermediates through the decomposition of organic compounds, which reacted either with the partially soluble salts already present in the soil or converted them into soluble salts (Kaur *et al.*, 2017). Among different biofertilizer inoculations, the maximum pH was noticed in control (7.89), while minimum with *Rhizobium spp.* + PSB + VAM (7.75) which may be attributed to organic acids and extracellular secretions made by the microbes (Kaur *et al.*, 2017). Zero tillage markedly improved organic carbon (%) over conventional tillage from 0.33 to 0.35 per cent (Table 2). The probable reason may be better root growth and biomass as well as physical protection of organic matter due to increased aggregation in ZT resulting in improved soil physical environment and soil OC content (Parihar *et al.*, 2016). Among different biofertilizer inoculations, maximum soil OC was recorded with *Rhizobium spp.* + PSB + VAM (0.36) which was statistically indifferent to all the biofertilizers treatments except control (0.32).

Table 1: Soil characteristics determined at beginning of the experiment from top 0-15 cm soil.

Soil properties	Values	Method used
A. Mechanical composition of the soil (%)		
Sand	71.4	International Pipette Method (Piper, 1966)
Silt	17.7	
Clay	10.9	
B. Chemical composition of soil		
pH (1:2)	7.90	Glass electrode pH meter (Jackson, 1973)
EC (dSm^{-1}, 1:2 at 25°C)	0.26	Conductivity bridge meter (Richards, 1954)
Organic carbon (%)	0.33	Walkley and Black Wet oxidation method (Jackson, 1973)
C. Available nutrients		
Available nitrogen (kg/ha)	125	Alkaline permanganate method (Subbiah and Asija, 1956)
Available phosphorus (kg/ha)	12	Olsen's method (Olsen *et al.*, 1954)
Available potassium (kg/ha)	311	Flame photometric method (Richards, 1954)
DTPA-Fe (mg kg^{-1})	2.02	Atomic Absorption Spectrophotometer (Lindsay, 1978)
DTPA-Cu (mg kg^{-1})	0.56	
DTPA-Zn (mg kg^{-1})	1.64	
DTPA-Mn (mg kg^{-1})	1.79	

Table 2: Effect of tillage and biofertilizers on pH, EC, OC, available nitrogen, phosphorus and potassium in soil post-harvest.

Treatments	pH	EC (dS/m)	OC (%)	Available nitrogen (kg/ha)	Available phosphorus (kg/ha)	Available potassium (kg/ha)
Tillage method						
T_1: Conventional tillage	7.83	0.28	0.33	126.7	13.1	302.0
T_2: Zero tillage	7.82	0.29	0.35	131.8	13.9	302.9
SEm ±	0.02	0.01	0.01	0.9	0.2	3.5
CD ($p \leq 0.05$)	NS	NS	0.01*	2.8*	0.5*	NS
Biofertilizers inoculations						
B_1: Control (No inoculation)	7.89	0.26	0.32	125.5	12.2	301.0
B_2: *Rhizobium spp.*	7.87	0.27	0.34	129.2	12.5	302.0
B_3: PSB	7.84	0.28	0.33	125.9	13.5	301.2
B_4: VAM	7.86	0.28	0.33	127.5	13.2	301.3
B_5: *Rhizobium spp.* + PSB	7.80	0.29	0.35	130.0	13.9	302.2
B_6: *Rhizobium spp.* + VAM	7.82	0.30	0.36	133.2	13.6	303.4
B_7: PSB + VAM	7.79	0.29	0.34	128.4	14.4	304.4
B_8: *Rhizobium spp.* + PSB + VAM	7.75	0.30	0.36	134.1	14.5	303.9
SEm ±	0.04	0.01	0.01	1.9	0.3	6.9
CD ($p \leq 0.05$)	NS	NS	0.03*	5.7*	1.0*	NS
Initial	7.90	0.26	0.33	125	12	311

*Significant at $p \leq 0.05$; NS- Non-significant at $p > 0.05$; PSB- Phosphorus solubilizing bacteria; VAM- Vesicular arbuscular mycorrhizae.

Table 3: Effect of tillage and biofertilizers on DTPA-Fe, Cu, Zn and Mn in soil post-harvest and nodulation.

Treatments	DTPA-Fe (mg kg^{-1})	DTPA-Cu (mg kg^{-1})	DTPA-Zn (mg kg^{-1})	DTPA-Mn (mgkg^{-1})	Nodules per plant (no.)	Nodules fresh weight per plant (g)	Nodules dry weight per plant (g)
Tillage method							
T_1: Conventional tillage	2.52	0.55	1.67	2.08	46.69	27.10	4.05
T_2: Zero tillage	2.62	0.54	1.70	2.16	50.36	29.43	4.37
SEm ±	0.03	0.09	0.02	0.03	0.84	0.40	0.06
CD (p≤0.05)	NS	NS	NS	NS	2.43	1.15	0.16
Biofertilizers inoculations							
B_1: Control (No inoculation)	2.13	0.51	1.63	1.77	38.85	21.43	3.16
B_2: *Rhizobium spp.*	2.51	0.53	1.66	2.05	50.68	30.40	4.66
B_3: PSB	2.22	0.51	1.64	1.82	40.35	22.54	3.38
B_4: VAM	2.33	0.52	1.66	1.94	41.96	23.34	3.50
B_5: *Rhizobium spp.* + PSB	2.55	0.55	1.68	2.14	53.30	31.76	4.70
B_6: *Rhizobium spp.* + VAM	3.01	0.57	1.74	2.48	58.23	34.19	5.12
B_7: PSB + VAM	2.58	0.56	1.72	2.20	44.61	25.62	3.84
B_8: *Rhizobium spp.* + PSB + VAM	3.11	0.57	1.75	2.58	60.26	36.47	5.33
SEm ±	0.07	0.02	0.03	0.05	1.68	0.79	0.11
CD (p≤0.05)	0.19*	NS	NS	0.15*	4.87*	2.30*	0.32*
Initial	2.02	0.56	1.64	1.79	-	-	-

*Significant at p≤0.05; NS- Non-significant at p>0.05; PSB- Phosphorus solubilizing bacteria; VAM- Vesicular arbuscular mycorrhizae.

Available nitrogen, phosphorous and potassium

Zero tillage (131.8 kg/ha) resulted in significantly higher available nitrogen in comparison to conventional tillage (126.7 kg/ha) with a net increment of 4% (Table 2). The increase in nitrogen under ZT might be due to reduced soil disturbance, enhanced soil aggregation, maintaining crop residue on the surface. Under CT soil organic matter is more exposed to humification which is conducive for mineralization, causing sudden loss of nitrogen from soil (Lopez-Fando and Pardo, 2009). Among different biofertilizers treatments, the highest available nitrogen (kg/ha) was recorded in *Rhizobium spp.* + PSB + VAM (134.1) followed by *Rhizobium spp.* + VAM (133.2) and *Rhizobium spp.* + PSB (130) which were statistically at par with each other.

Maximum available phosphorus (kg/ha) resulted in seed inoculation with *Rhizobium spp.* + PSB + VAM (14.5) which was statistically at par with all other combined inoculations. The increased available P in soil under zero tillage might be owing to release of organic acids (Piscidic acid) in course of microbial decomposition of residue resulting in release of native phosphorus of soil (Ahlawat *et al*., 2005). Both tillage as well as biofertilizer treatments did not result any significant change in available potassium (kg/ha) of soil post-harvest.

DTPA extractable micronutrients (Iron, copper, zinc and manganese)

Tillage had no-significant effect on micronutrients (Table 3). However, the content of DTPA extractable micronutrients except copper was recorded numerically higher under ZT as compared to CT probably due to addition of more crop residue under ZT which directly increases the content of these micronutrients (Kaushik *et al*., 2018). DTPA-Cu was found higher under CT as compared to ZT which might be due to higher affinity of copper with organic matter and hence higher content of organic matter in ZT leading to less availability. These results are in accordance with Kaushik *et al*. (2018). Among different DTPA extractable micronutrients, DTPA-Fe and DTPA-Mn were increased significantly by different biofertilizers inoculations, while observed non-significant for DTPA- Cu and DTPA-Zn. The maximum content for these micronutrients were recorded in seed treatment with *Rhizobium spp.* + PSB + VAM, while minimum with control.

Nodulation

The number of nodules per plant, nodule fresh weight per plant (g) and nodules dry weight per plant (g) observed at 50% flowering were found significantly higher in ZT over CT (Table 3). It could be ascribed to improved soil conditions under ZT that deliver a congenial surrounding for both the host plant and microbes for a symbiotic relationship. Number of nodules per plant, nodules fresh weight and nodules dry weight per plant in *Rhizobium spp.* + PSB + VAM was recorded significantly higher than all the other treatments except *Rhizobium spp.* + VAM (Table 3).

CONCLUSION

Overall results demonstrated that zero tillage was proved superior than conventional tillage in terms of enhancing organic carbon, macro and micronutrients status of the soil and nodulation of faba bean. Similarly, the results highlighted the importance of seed treatment with different biofertilizer inoculations in improving soil chemical properties. The study demonstrated that application of zero tillage technology in

combination with seed treatment with co-inoculation of *Rhizobium spp.* + PSB + VAM is beneficial for improving the soil macro and micro-nutrients status and nodulation under faba bean crop.

Conflict of interest

On the behalf of all authors, I declare that no authors has any conflict of interest regarding this article submitted in the journal of Legume Research.

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