INFLUENCE OF TILLAGE AND SEED TREATMENT ON FODDER PRODUCTION, NPK CONTENT, UPTAKE AND PROTEIN YIELD OF FABA BEAN (*VICIA FABA* L.)

SUMIT BHARDWAJ*1, JHABAR MAL SUTALIYA1,2, RAVI KUMAR2 AND AKSHAY PAREEK1

¹Department of Agronomy, CCS Haryana Agricultural University, Hisar-125004 (Haryana), India ²MAP Section (GPB), CCS Haryana Agricultural University, Hisar-125004 (Haryana), India *(e-mail : sumitbhardwaj909@hau.ac.in)

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SUMMARY

The present investigation was undertaken to study the effect of zero tillage and seed treatment with different biofertilizers inoculations on fodder yield, nutrients and protein content of faba bean (Vicia faba L.). The field experiment was carried out in factorial randomized block design (FRBD) with two levels of tillage system viz., T.: Conventional Tillage (CT), T.: Zero Tillage (ZT) and eight levels of biofertilizers inoculations viz., B₁: Control (No Inoculation), B₂: Rhizobium spp., B₃: Phosphorus Solubilizing Bacteria (PSB), B₄: Vesicular Arbuscular Mycorrhizae (VAM), B₅: *Rhizobium spp.* + PSB, Be: Rhizobium spp. + VAM, Be: PSB + VAM, Be: Rhizobium spp. + PSB + VAM replicated three times during Rabi 2019-20. The source of biofertilizers used for seed treatment was Rhizotica containing Rhizobium spp., Phosphotica containing Pseudomonas spp. obtained from biofertilizer lab of CCS HAU and spore culture containing *Glomus mosseae* mycorrhizae for VAM inoculation. Dry matter accumulation (g/m.r.l.) in zero tillage was observed 2%, 3.6% & 5.45% higher than conventional tillage at 60 DAS, 90 DAS and harvest, respectively. The maximum dry matter accumulation (g/m.r.l.) was recorded in seed treatment with Rhizobium spp. + PSB + VAM at all the stages viz., 60 DAS (39.60), 90 DAS (89.05) and at harvest (305.67). Faba bean exhibited significantly higher nitrogen (0.80, 4.28) and phosphorus (0.12, 0.21) content of straw and seed respectively in ZT as compared to CT, however, no significant variation was observed concerning potassium content of straw as well as seed. NPK uptake of straw and seed was found significantly higher under ZT over CT. A significantly higher protein content (26.74%), protein yield (881 kg/ha) and straw yield (4839 kg/ha) were recorded under ZT in comparison to CT. Biofertilizers significantly affected nitrogen content, phosphorus content and NPK uptake of straw as well seed, whereas the effect on potassium content of straw and seed was nonsignificant. Rhizobium spp. + PSB + VAM exhibited maximum values for NPK content and uptake of straw as well as seed. Protein content, protein yield and straw yield were significantly affected by biofertilizer treatments, maximum been recorded in *Rhizobium spp.* + PSB + VAM.

Key words : Biofertilizer, fodder, nutrients, protein content, zero tillage

Pulse crops are of paramount importance to human beings as well as livestock because of their high nutrition value and soil health-improving capabilities. Faba bean is a potential multi purpose pulse crop used for both food and fodder (hay, silage and straw) that can grow to a height of 1.5-2.0 m. In Egypt and Sudan, straw from faba bean harvest gets a premium and is considered a cash crop (Hulse, 1994). It has been recognized for its efficient biological nitrogen fixation capabilities which is maximum amongst cool season grain legumes (Mekkei, 2014). Studies show that it can fix 50-330 kg N/ha (Etemadi *et al.*, 2018). In Europe, faba beans have been proposed as a protein source for cattle as an alternative to soybean (Smith *et al.*, 2013). Faba bean seeds are a valuable source of protein and energy for animals since they are high in protein (25-33 percent DM) and starch (40-48 percent DM). They have a significant amount of fibre (crude fibre 7-11 percent DM). Fresh faba bean fodder is of good quality, with protein levels ranging from 14 percent to more than 20 percent dry matter (DM). In ruminants, faba bean seeds are highly digested (OM digestibility: 91%) and are equivalent to peas (Micek *et al.*, 2012). Therefore, faba bean is a potential emerging crop having wider adaptability under varied agro-climatic conditions for providing nutritionally rich fodder to livestock (Arya, 2018 & Arya *et al.*, 2021). But it is still an underutilized crop and has not gained much popularity and area under cultivation. One of the most important reasons for this is its low productivity due to cultivation on marginal lands having very low fertility.

A significant way to increase the productivity of pulse crops is use of bio-inoculants, which are costeffective, eco-friendly, promising renewable source of plant nutrition for supplementing synthetic fertilizers. The academic community has extensively explored and established the fact that seed inoculation with biofertilizer is a very remunerative and profitable agronomic practice. Most importantly role of rhizobium inoculants in legumes is well known but meager efforts have been made to study the effect of co-inoculation of different microbial inoculants for improving the fodder yield, nutrients content and protein content of faba bean (Vicia faba L.). A study conducted by Sharma et al., 2019 in chickpea showed a significant increase in nitrogen, phosphorus, potassium uptake and straw yield in seed treatment with co-inoculation of biofertilizers over un-inoculated control. The protein content as well as NPK uptake by faba bean was found significantly higher in seed treatment with dual inoculation of Rhizobium + PSB over all the other treatments in a study conducted by Bhat et al., 2013 indicating a synergistic interaction. The dual inoculation of rhizobia and mycorrhizae induced a more significant increase in plant dry weight, N and P content of faba bean than single inoculation with either VAM or Rhizobium spp. alone (El-Din and Moawad, 1988). Increased growth attributes in combined inoculation occur due to highly synergistic effects of releasing of growth substances (Kumariet al., 2009).

Moreover, the long-term negative impacts of intensive conventional tillage like soil compaction, disruption of soil structure, reduction of hydro-stability of aggregates leading to accelerated soil erosion and making soil infertile are now well known (Dayou et al., 2017). Conservation tillage practice, such as zero tillage may offset these consequences associated with land preparation (Jatet al., 2011).ZT as opposed to CT provides better growth conditions to crop by increasing soil water storage, stabilizing the soil aggregates (Montgomery, 2007), greater infiltration and reduced evaporation improving soil structure (Borieet al., 2006) and other physical properties. Thus improved field conditions under ZT lead to increased nutrient availability and uptake (Singh et al., 2020). The study conducted by Roy et al., 2014 showed a higher straw yield, NPK uptake and protein content of chickpea under ZT as compared to CT. Similarly, Romaneckas et al. (2018) reported that among

different tillage systems highest protein content of faba bean was achieved under ZT. Furthermore, many studies have shown positive interaction effects of microbial inoculation with tillage (Mulas *et al.*, 2015). Thus keeping in view the above aspects, an attempt has been made to study theeffect of zero tillage and seed treatment with different biofertilizers inoculations on fodder yield, nutrients and protein content of faba bean (*Vicia faba* L.).

MATERIALS AND METHODS

The field experiment was performed at an experimental farm of Medicinal, Aromatic and Potential Crop Section (MAP), Department of Genetics & Plant Breeding of Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana, India, 29º10' N, 75º46' E; 215.2 m a.s.l.) during Rabi 2019-20.Based on mechanical and chemical analysis, the soil was categorized as sandy loam in texture and alkaline in nature. The experiment was conducted in a Factorial Randomized Block Design (FRBD) with three replications. The experimental area consisted of 48 plots with an individual plot size of 5.4 x 5m (27m²). When the field reached appropriate moisture conditions for carrying out tillage operation, seedbed was prepared with tractor-drawn disc harrow and cultivator followed by planking in case of conventional tillage blocks. While no-tillage operation was carried out in zero tillage blocks.Herbicidal application of Roundup Glyphosate 41% SL @ 3L/ha was done in ZT plots to knock down the weeds. Seeds were treated with Rhizobium spp., PSB & VAM using the jaggery (adhesive). 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 isolated using wet sieving & decanting method (Gerdemann and Nicolson, 1963).

Dry matter accumulation was recorded at 60, 90 DAS andat harvest. For measurement of dry matter production, three random samples of 1m row length of plants were cut down near to the base from each plot. Samples were first air-dried at room temperature and then dried in the oven at 70° C until a constant weight was attained. Dry samples were weighed and the dry weight obtained was expressed in g.Straw yield per m² was computed by subtracting the seed yield per m² from biological yield (total above-ground biomass) per m² and permuted to kg/ha.For determination of N, P, K content in straw/seed, 0.2 g ground material of straw/seed samples were digested with 10 ml of diacid mixture of H₂SO₄ and HClO₄ in a ratio of 9:1 on a hot plate to obtain a clear colorless solution (3-4 ml). It was transferred in a 50 ml conical flask and distilled water was added to make volume up to mark. It was passed through Whatman filter paper No. 42 and stored in a plastic bottle for further analysis. (i) Colorimetric (Nessler's reagent) method (Lindner, 1944) was used to determine nitrogen in straw taking 0.2ml of aliquot. (ii) Phosphorus in straw samples measured was by the Vanadomolybdophosphoric yellow color method using 5ml of aliquot (Jackson, 1973). (iii) Potassium in straw samples was directly determined in aliquot using a flame photometer (Richards, 1954). NPK uptake was computed using the following formula,

	% NPK content × Yield of faba bean
NPK uptake of -	(Seed/Straw)
faba bean (kg/ha)	100

The protein content (%) of seed was calculated by multiplying nitrogen percentage in seed by 6.25, a conversion factor for estimating protein content.Protein yield was calculated by multiplying protein content (%) with the seed yield of faba bean. The data from the study were analyzed by the ANOVA (analysis of variance) technique described by Fisher (1950). Mean values of replicated observations have been used in the study. All the tests of significance were done at a 5% level of significance. Treatment means were compared for Critical difference (C.D.) to determine the significance of the treatments.

RESULTS AND DISCUSSION

Dry matter Accumulation : The perusal on data of dry matter accumulation shown in Table 1 unveiled that the effect of tillage and biofertilizer treatments were found significant at all the observed stages except for tillage at 60 DAS. Dry matter accumulation (g/m.r.l.) in zero tillage was observed 2%, 3.6% & 5.45% higher than conventional tillage at 60 DAS, 90 DAS and harvest, respectively. The probable reason may be better growth conditions like soil moisture, temperature etc. under ZT for more vegetative growth(Malhi and Lemke, 2007). Faba bean crop has a shallow root system with little osmoregulation which makes it very sensitive to water stress and high temperature (Bond *et al.*, 1993). Underwater stress faba bean adapts coping mechanisms like reducing rate of

TABLE 1
Effect of tillage and biofertilizers on periodic dry matter
accumulation (g/m.r.l.) of faba bean

Treatments	Dry matter accumulation (g/m.r.l.)			
	60 DAS	90 DAS	At harvest	
Tillage method				
T ₁ : Conventional Tillage	38.10	81.10	261.16	
T_2 : Zero Tillage	38.87	84.07	275.40	
SĒm ±	0.27	0.52	2.30	
CD (p≤ 0.05)	NS	1.52	6.67	
Biofertilizers inoculations				
B ₁ : Control (No inoculation)	37.00	76.90	236.37	
B ₂ : <i>Rhizobium</i> spp.	38.41	82.10	259.01	
B ₃ : PSB	37.71	77.46	238.26	
B_{4} : VAM	38.23	81.32	251.66	
B_{s} : <i>Rhizobium</i> spp. + PSB	38.68	83.06	274.26	
B ₄ : <i>Rhizobium</i> spp. + VAM	39.31	86.30	294.96	
B_7 : PSB + VAM	38.98	84.55	286.04	
B_{s}' : <i>Rhizobium</i> spp. + PSB + VAM	39.60	89.05	305.67	
SËm±	0.55	1.05	4.60	
CD (p≤ 0.05)	1.58	3.04	13.34	

*Significant at p≤ 0.05; NS- Non-significant at p>0.05; PSB-Phosphorus Solubilizing Bacteria; VAM- Vesicular Arbuscular Mycorrhizae.

height increase, leaf area expansion, producing leaves of the small specific area (Husain et al., 1990). The maximum dry matter accumulation (g/m.r.l.) was recorded in seed treatment with Rhizobium spp. + PSB + VAM at all the stages viz., 60 DAS (39.60), 90 DAS (89.05) andat harvest (305.67). At 90 DAS and harvest Rhizobium spp. + PSB + VAM was significantly superior to rest of the treatments except Rhizobium spp. + VAM, while at 60 DAS it was statistically at par with all the other treatments except PSB and control.At all the stages viz., 60 DAS, 90 DAS and harvest minimum dry matter accumulation was found in control which was statistically at par with PSB at 90 DAS and harvest. This might be due to the synergistic effect of co-inoculation of biofertilizers on growth-promoting factors. Rhizobium produces phytohormones such as IAA, cytokinins and gibberellic acid which impart growthpromoting effects on crops (Hayashi et al., 2014).PSB and VAM increase phytohormones, phosphorus and root length & root hairs (Sandhya et al., 2013).

N content & uptake of straw and seed : It is evident from the data given in Table 2 that nitrogen content (%) in straw and seed was significantly higher in zero tillage (0.80, 4.28) as compared to conventional tillage (0.71, 4.13) respectively. The increased N content may be due to uniform distribution of roots and more numbers of nodules in ZT compared to CT (Roy et al., 2014). Seed inoculation with Rhizobium spp. + PSB+ VAM (0.87) resulted in significantly higher nitrogen content (%) in straw over rest of the treatments except Rhizobium spp. + VAM (0.85) and *Rhizobium spp.* + PSB (0.82) which were statistically at par. Whereas, the maximum nitrogen content (%) of seed was observed in Rhizobium spp. + PSB + VAM (4.36) which was statistically at par with all the Rhizobium spp. containing treatments viz., Rhizobium spp. + VAM (4.34), *Rhizobium* spp. + PSB (4.30) and Rhizobium spp. (4.27). A significantly higher N uptake by straw and seed (kg/ha) was observed under zero tillage (38.88, 140.95) over conventional tillage (28.24, 110.65) respectively (Table 3). N uptake of straw and seed (kg/ha) in Rhizobium spp. + PSB + VAM (43.29, 149.55) was significantly superior to all the other treatments except Rhizobium spp. + VAM (42.08, 145.37) respectively. Higher nodulation and nitrogen fixation might have resulted in more N content and uptake in all the Rhizobium spp. containing treatments over other treatments. Similar findings have been reported by Sharma et al., 2019.

P content & uptake of straw and seed : It is clear from data in Table 2 that P content (%) of straw &seed was significantly superior in zero tillage (0.12, 0.21) in comparison to conventional tillage (0.11, 0.20) respectively. With respect to biofertilizers inoculations, the highest P content (%) of straw & seed was found in *Rhizobium spp.* + PSB + VAM *i.e.* 0.13 & 0.23 respectively. Phosphate-solubilizing microorganisms like PSB and VAM play a vital role in phosphorus solubilization and uptake. VAM has capabilities to boost the uptake of inorganic nutrients in almost all plants, specifically phosphate (Nell et al., 2010). Pulses form beneficial relationships with VAM that aid in extending root system and further enhance phosphorus uptake (Dotaniya et al., 2014). A probe into data shown in Table 3 revealed that P uptake by straw and seed (kg/ha) under zero tillage (5.71, 7.06) was significantly higher over conventional tillage (4.27, 5.36) by 1.44 and 1.70 respectively. The highest P uptake by straw and seed (kg/ha) was recorded in seed inoculation with *Rhizobium spp.* + PSB + VAM (6.58, 7.89) which was significantly higher over all the other treatments except PSB + VAM (6.06, 7.34) respectively. The higher phosphorus uptake in combined inoculated treatment might be due to improved availability of phosphorus in the presence of PSB and VAM with phosphorus solubilizing abilities (Sharma et al., 2019).

K content and uptake of straw and seed : K content (%) in straw and seed was not significantly

Treatments	N content (%)		P content (%)		K content (%)	
	Straw	Seed	Straw	Seed	Straw	Seed
Tillage method						
T ₁ : Conventional Tillage	0.71	4.13	0.11	0.20	2.49	1.16
T ₂ : Zero Tillage	0.80	4.28	0.12	0.21	2.51	1.21
SĒm±	0.01	0.03	0.002	0.003	0.04	0.02
CD (p≤ 0.05)	0.03	0.10	0.005	0.01	NS	NS
Biofertilizers inoculations						
B ₁ : Control (No inoculation)	0.65	4.05	0.09	0.18	2.45	1.15
B ₂ : <i>Rhizobium</i> spp.	0.77	4.27	0.09	0.19	2.46	1.17
B ₃ : PSB	0.68	4.06	0.11	0.20	2.48	1.19
B ₄ : VAM	0.70	4.11	0.10	0.20	2.49	1.15
B_{s} : <i>Rhizobium</i> spp. + PSB	0.82	4.30	0.12	0.22	2.51	1.18
B _c : <i>Rhizobium</i> spp. + VAM	0.85	4.34	0.12	0.21	2.53	1.20
B_7 : PSB + VAM	0.73	4.14	0.13	0.23	2.54	1.21
B_{\circ} : <i>Rhizobium</i> spp. + PSB + VAM	0.87	4.36	0.13	0.23	2.56	1.23
SËm±	0.02	0.07	0.004	0.01	0.08	0.04
CD (p≤ 0.05)	0.06	0.20	0.01	0.02	NS	NS

 TABLE 2

 Effect of tillage and biofertilizers on NPK content of straw and seed (%)

*Significant at p≤ 0.05; NS- Non-significant at p>0.05; PSB- Phosphorus Solubilizing Bacteria; VAM- Vesicular Arbuscular Mycorrhizae.

influenced by both tillage and biofertilizers treatment (Table 2). The data shown in Table 3 implied that K uptake by straw and seed (kg/ha) was significantly higher in zero tillage (121.54, 39.89) as compared to conventional tillage (98.02, 31.10) respectively. K uptake by straw (kg/ha) was significantly higher in Rhizobium spp. + PSB + VAM (127) over rest of the treatments except *Rhizobium spp.* + VAM (124.13) and PSB + VAM (118.04) (Table 3). K uptake by seed with Rhizobium spp. + PSB + VAM was 12.16 kg/ha higher than control.Straw and seed's nutritional status could have improved significantly as a result of increased nutrient availability in the soil environment, as well as extraction and translocation to plant system. The increase in concentration and uptake of nutrients with application of different bio-inoculants might be attributed to synthesis of some growth-promoting substances (Sharma et al., 2019).

Straw yield : Straw yield (kg/ha) was significantly higher under zero tillage (4839) as compared to conventional tillage (3931) with an increase of 23% (Table 4). The increase in straw yield was probably due to better vegetative growth of faba bean under ZT. Among different biofertilizer treatments, the highest straw yield (kg/ha) was obtained in *Rhizobium spp.* + PSB + VAM (4966) while the lowest straw yield was recorded in control (3976)

 TABLE 4

 Effect of tillage and biofertilizers on protein content (%) and protein yield (kg/ha) of faba bean

Treatments	Straw yield (kg/ha)	Protein content (%)	Protein yield (kg/ha)	
Tillage method				
T ₁ : Conventional Tillage	3931	25.81	691	
T_{2} : Zero Tillage	4839	26.74	881	
SĒm ±	51	0.22	13	
CD (p≤ 0.05)	147	0.63	37	
Biofertilizers inoculations				
B ₁ : Control (No inoculation)	3,976	25.31	657	
B ₂ : <i>Rhizobium</i> spp.	4,195	26.69	765	
\mathbf{B}_{3} : PSB	3,988	25.40	676	
B ₄ : VAM	4,026	25.68	716	
B_{5} : <i>Rhizobium</i> spp. + PSB	4,371	26.88	805	
B_6 : <i>Rhizobium</i> spp. + VAM	4,914	27.12	908	
B_7 : PSB + VAM	4,646	25.85	826	
B_{s} : <i>Rhizobium</i> spp. + PSB + VAM	4,966	27.29	935	
SËm±	101	0.43	25	
CD (p≤ 0.05)	294	1.26	73	

*Significant at p≤ 0.05; NS- Non-significant at p>0.05; PSB-Phosphorus Solubilizing Bacteria; VAM- Vesicular Arbuscular Mycorrhizae.

thus achieving a significant increase of 990 kg/ha. The increase in straw yield with *Rhizobium*, PSB and VAM might be due to more availability of nutrients by

Treatments	N uptake (kg/ha)		P uptake (kg/ha)		K uptake (kg/ha)	
	Straw	Seed	Straw	Seed	Straw	Seed
Tillage method						
T ₁ : Conventional Tillage	28.24	110.65	4.27	5.36	98.02	31.10
T ₂ : Zero Tillage	38.88	140.95	5.71	7.06	121.54	39.89
SĒm±	0.65	2.02	0.12	0.12	1.94	0.72
CD (p≤ 0.05)	1.90	5.87	0.35	0.36	5.64	2.09
Biofertilizers inoculations						
B ₁ : Control (No inoculation)	25.97	105.17	3.72	4.68	97.06	30.00
B ₂ : <i>Rhizobium</i> spp.	31.36	122.48	3.93	5.32	103.22	33.68
B ₃ : PSB	27.48	108.27	4.41	5.50	98.77	31.69
B ₄ ² : VAM	28.48	114.66	4.00	5.48	100.23	32.18
B_{ξ}^{\dagger} : <i>Rhizobium</i> spp. + PSB	35.99	128.77	5.46	6.60	109.82	35.48
B _c : <i>Rhizobium</i> spp. + VAM	42.08	145.37	5.76	6.92	124.13	40.06
B_7 : PSB + VAM	33.85	132.15	6.06	7.34	118.04	38.74
B_{o} : <i>Rhizobium</i> spp. + PSB + VAM	43.29	149.55	6.58	7.89	127.00	42.16
SËm±	1.31	4.04	0.25	0.25	3.89	1.44
CD (p≤ 0.05)	3.80	11.73	0.72	0.72	11.29	4.18

 TABLE 3

 Effect of tillage and biofertilizers on NPK uptake by straw and seed yield (kg/ha)

*Significant at p≤ 0.05; NS- Non-significant at p>0.05; PSB- Phosphorus Solubilizing Bacteria; VAM- Vesicular Arbuscular Mycorrhizae.

increased microbial activities in soil (Sharma et al., 2019).

Protein content and yield : The data in table revealed that protein content (%) in zero tillage (26.74) was significantly improved by 0.93% over conventional tillage (25.81). Maximum protein content was recorded in *Rhizobium spp.* + PSB + VAM (27.29) which was statistically at par with all the Rhizobiumcontaining treatments. The protein yield (kg/ha) under zero tillage (881) was significantly higher by 190 (kg/ ha) over conventional tillage (691). Rhizobium spp. + PSB + VAM (935 kg/ha) resulted in significantly superior protein yield over rest of the treatments except Rhizobium spp. + VAM (908 kg/ha) which was significantly higher over control (657 kg/ha). The increase in protein content may be attributed to nitrogen fixed in root nodules and efficiently translocated to seeds.

CONCLUSION

Based on the findings of this study, it may be ascertained that zero tillage application is significantly better over conventional tillage in improving dry matter production, NPK content & uptake, straw yield, protein content and protein yield of faba bean (Vicia faba L.). Similarly, faba bean seed treatment with co-inoculation of Rhizobium spp. + PSB + VAM has a more profound effect in improving these parameters than the single inoculation or no-inoculation of the biofertilizers. Thus, it may be recommended from the study that sowing under zero-tillage accompanying seed treatment with combined biofertilizer inoculation of *Rhizobium spp*. + PSB + VAM should be followed to achieve better dry matter production, NPK content & uptake, straw yield, protein content and protein yield of faba bean (Vicia faba L.).

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