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Crop productivity and nutrient uptake of fenugreek (*Trigonella foenum-graecum* L.) as influenced by inorganic fertilizers and bio-inoculant (*Rhizobium*, PSB and KSB)

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Abstract
The experiment was conducted at Vegetable Research Centre, Maharajpur, Department of Horticulture, JNKVV, Jabalpur, MP. during *Rabi* 2019-2020 to find out the best combination of bio-fertilizer with different levels of nitrogen, phosphorous and potassium and to determine the microbial population in soil.The design was laid out in Randomized Block Design (RBD) with three replications. Each replication consists of 16 treatments. All the treatments were randomized separately in each replication with variety RMt-1 using inorganic fertilizer and bio-inoculants *Rhizobium*, PSB and KSB.RDF+ *Rhizobium*+PSB+KSB gave higher values of all growth attributes traits, yield contributing traits i.e.harvest index, number of nodules at 30 DAS, dry weight of plant at 60 DAS, test weight seed yield per plant (3.15), total yield (13.13 q ha^{-1}) whereas 75% of RDF+ *Rhizobium*+PSB+KSB gave maximum microbial population [7.75($56.23x10^6$), 7.68($47.86x10^6$) and 7.70($50.11x10^6$) cfu g^{-1}), available nutrient status on soil and plant and protein content (21.28%) in seed.

Keywords: Rhizobium, PSB, KSB, Bio-inoculant, Inorganic fertilizer

Introduction

India is the world's biggest seed spice grower, consumer, and exporter. Fenugreek (*Trigonella foenum-graecum* L.), also known as *methi*, is a multipurpose crop grown in northern India during the winter months. It is an annual herb of the Fabaceae family with the subfamily Papilliaceae. It is one of the country's most popular seed spices. Because of its triangular yellowish-white flowers, it was given the name *Trigonella* from the Latin language, which means "little triangle." Since the Greeks used it as a medicine, spice, and cattle feed from the beginning, it was also known as Greek hay. The origins of fenugreek are thought to be in South-Eastern Europe and West Asia.

Rajasthan, Gujarat, Madhya Pradesh, Haryana, Maharashtra, Uttar Pradesh, Punjab, Bihar, Tamil Nadu, and Andhra Pradesh are the main fenugreek producing states. In 2017-2018, the total area and yield of fenugreek were 219720 hectares and 311280 tonnes, respectively (Spice Board, 2019) [19]. Rajasthan is the top producer of fenugreek, followed by Madhya Pradesh, Gujarat, and Haryana. It is cultivated on an area of 53440 hectares in Madhya Pradesh, with a yield of 104220 tonnes (Spice Board, 2019) [19].

Nitrogen is important in the production of chlorophyll and, as an essential component of compounds such as amino acids, nucleic acids, nucleotides, enzymes, coenzymes, vitamins, and alkaloids, it contributes to plant growth. The general effect of phosphorus on plant metabolism is considered to improve symbiotic nitrogen fixation and to play a significant role in the energy conversion mechanism in the plant body. Potassium is the third most important essential plant nutrient, and it is needed for enzyme activation, protein synthesis, photosynthesis, as well as product consistency. However, due to a lack of fertiliser recommendations for different agro-climatic conditions, farmers usually add either too much or too little fertiliser in fenugreek, affecting crop production and profit margins. Similarly, the effects of various bio-fertilizers must be assessed. Shekhawat *et al*. (2012) [18] emphasised the importance of incorporating bio-fertilizers into the fertiliser network to fulfil about one-third of plant nutrient needs. Synthetic fertilisers combined with bio-fertilizers in fenugreek may be much more useful than synthetic fertilisers alone.

In India, the use of different chemical combinations in agriculture has had a severe and lasting effect on ozone, human health, and soil health. There is a need to look for alternate sources of nutrients that are both economical and environmentally sustainable, so that farmers can reduce their reliance on fertilisers while maintaining healthy soil environmental conditions.

Bio-fertilizers are environmentally efficient, less expensive, and do not rely on nonrenewable resources for production, resulting in sustainable crop production. They also contain hormones, vitamins, and other growth factors that are essential for plant growth. Fenugreek, a legume crop, responds to *Rhizobium meliloti* inoculation to meet the partial nitrogen demand. The inoculation of fenugreek with Rhizobium has been shown to increase plant and seed biomass. The available phosphorus in Indian soil is poor to average. Several strains of phosphate solubilizing bacteria (PSB) and fungi have been isolated in recent years, and they have demonstrated the ability to solubilize sparingly soluble phosphate, promote growth, and increase P uptake by plants (Whitelaw, 2000) [22]. Since there is no reserve of K-bearing minerals for manufacturing traditional K fertilisers in India, the entire consumption is imported, costing a large amount of foreign exchange. *Frateuria aurantia*, a K-solubilizing bacterium, is capable of mobilising a mixture of mica potassium into a functional shape for plants after it has been applied to crops in collaboration with other bio-fertilizers in a reasonable amount without any antagonistic effects. In this context, it is important to determine microbial solubilization of K-bearing minerals in soils, as well as their potential as K-bearing fertilisers in sustainable crop production and soil protection. As a result, the current study was designed to find out the best combination of bio-fertilizer with different levels of nitrogen, phosphorous and potassium and to determine the microbial population in soil.

Material and Methods

The experiment was carried out from November to March 2019-2020 at the Vegetable Research Centre, Maharajpur, Department of Horticulture, JNKVV, Jabalpur (MP), which is located at 23' 90" N longitude and 79' 58" E longitude, at an altitude of 411.78 metres above mean sea level. The soil of the experimental field was medium black with good drainage, uniform texture, available N 215.0, available P 2.30, available K 185.0 kg/ha and *Rhizobium* population 5.54(34×10^4), PSB population 5.47 (29×10^4) and KSB population 5.50(31×10^4) cfu g^{-1} of soil at the beginning of the experiment in 0-15 cm soil layer. During the growing season, fenugreek received one light irrigation immediately after sowing and five irrigations at various stages of development. The experiment was carried out in a three-replicate randomised block design. The treatment combinations comprised of sixteen, *viz*., control, RDF, 75% RDF, 50% RDF, 100% RDF + *Rhizobium*, 100% RDF + *Rhizobium*+ PSB, 100% RDF + *Rhizobium*+ KSB, 100% RDF +*Rhizobium*+ PSB+KSB, 75% RDF + *Rhizobium*, 75% RDF + *Rhizobium*+ PSB, 75% RDF + *Rhizobium*+ KSB, 75% RDF + *Rhizobium*+ PSB+KSB, 50% RDF + *Rhizobium*, 50% RDF + *Rhizobium*+ PSB, 50% RDF + *Rhizobium*+ KSB, 50% RDF +*Rhizobium*+ PSB+KSB. The seed rate used for sowing was 25 kg/ha, and the seed was handled with 2 g *Rhizobium meliloti* per kg of seed prior to sowing. RMt-1 seeds were dibbled at a row spacing of 30 cm. After four weeks of sowing, the plants were thinned to ensure a plant-to-plant gap of 10 cm. The prescribed N, P, and K doses of 30:30:50 kg/ha were added as a basal application just before sowing in the form of urea, single super phosphate, and muriate of potash, respectively. *Rhizobium* was used for seed treatment at a rate of 2 g/kg of seed, while PSB and KSB were applied to the soil at a rate of 3 kg/ha at the time of sowing as a par treatment in the plot. The recommended practises for cultivating high-quality crops were followed. The observations were recorded on yield and quality parameters by using standard procedure. Data were analysed as per the statistical methods given by Panse and Sukhatme (1967) [16].

All the soil and plant samples were analysed for total nitrogen content by Micro Kjeldahl’s method (AOAC, 1995) [6], phosphorus content were estimated by using colorimetric measurement (Olsen *et. al*., 1954) [15] and estimation of potassium were carried out by flame photometer (Black, 1965) [9]. To evaluate the effect of different treatment on microbial population in soil the study was done by adopting serial dilution method (David and Davidson, 2014) [11]. Protein content of the fenugreek seed was calculated by Micro Kjeldahl’s method (AOAC, 1995) [6].

Result and Discussion

Result depicted in Table 1 shows that inorganic and bio-inoculant doses as well as there combinations significantly affect growth attributes, *viz*. seed yield per plant, total yield, test weight, harvest index number of nodules per plant at 30 DAS and dry weight of plant at 60 DAS in fenugreek. The application of 100% RDF+*Rhizobium*+PSB+KSB *i.e.* T_8 gave higher values of all these growth attributes. This might be due to the combined application of inorganic fertilizer and bio-inoculant which increased nutrients availability, photosynthetic activity, chlorophyll formation, nitrogen metabolism in plants which ultimately improving plant height resulting in vigorous plant growth. Similar beneficial combined effect of inorganic and bio-inoculant on growth parameters was also recorded by Ali *et al.* (2009), Anwer *et al*. (2012), Meena *et al.*(2014) and Raiyani *et al.* (2018) [3, 5, 14, 17].

In addition, inoculation of plant with microbes increase dry matter content (Alagawadi and Gaur, 1998) [2]. Over 80%of the bacteria isolated from rhizosphere can produce IAA (Arshad and Frankenberger, 1998) [7]. This increase in dry matter production of inoculated plants may be attributed to enhanced nodulation, higher nitrogen fixation rate and general improvement of root development (Erum and Bano, 2008) [12]. *Rhizobium sp*. inoculation caused an improvement in growth and yield (Akhtar and Siddiqui, 2009) [1]

The superiority of the inorganic fertiliser and bio-inoculant combination could be contributed to an improvement in the amount of growth parameters and improved nitrogen supply during the crop's life cycle. Fenugreek is a leguminous crop that fixes atmospheric nitrogen. As a result, an improved and balanced supply of nitrogen to the plant facilitates flowering and fruiting, as well as the supply of food content and its subsequent partitioning in the sink. Phosphorus supply plays a unique role in energy storage and conversion. The crop's balanced nitrogen supply during its life cycle reduced leaf senescence and allowed it to meet the increased assimilate demand of plant sinks, resulting in a higher number of pods and test weight due to bold grain formation.

The improved supply of nutrients and their translocation resulted in considerably higher seed yield in fenugreek under *Rhizobium* inoculation along with PSB and KSB in soil application.

Nutrient status of soil and plant is depicted in Table-2 and revealed that N,P,K content in soil and plant was significantly increased by various doses and there combination of inorganic fertilizer and bio-inoculants. Maximum N,P,K content in soil and plant was recorded with treatment T_{12}(75% of RDF+*Rhizobium*+PSB+KSB). Similar beneficial combined effect of inorganic and bio-inoculant was also recorded by Fahim *et. al.* (2016) [13]. The higher content in soil and seed by combined use of inorganic and bio-inoculant might be ascribed due to balanced availability of nitrogen throughout the lifecycle of the crop (Chodhary *et al.*, 2011) [10].

Scrutiny of data in Table- 3 shows that *Rhizobum*, PSB and KSB population was significantly increased by various doses and there combination of inorganic fertilizer and bio-inoculants. Maximum *Rhizobum*, PSB and KSB population was recorded with treatment T_{12}(75% of RDF+*Rhizobium*+PSB+KSB) [7.75 log cfu(56.23×10^6 cfu g^{-1} soil), 7.68 log cfu (47.86×10^6 cfu g^{-1} soil) and 7.70 log cfu (50.11×10^6 cfu g^{-1} soil) respectively]. Whereas, it was observed lowest under control [5.28 log cfu (19.05×10^4 cfu g^{-1} soil), 5.51 log cfu (32.35×10^4 cfu g^{-1} soil) and 5.58 log cfu ((38.01×10^4 cfu g^{-1} soil)].

Soil properties, such as parent material, soil organic matter and clay content can also influence soil microbial population as soybean was sown in *kharif* season, this may be a reason for higher microbial population in soil after harvesting of fenugreek. Major improvements were found in soil microbial communities after green manure with annual legumes such as peas, resulting in a rise in bacterial numbers by 385 per cent and overall biomass by ~200 per cent opposed to wheat grown consecutively for 6 years (Biederbeck *et al*. 2005) [8]

In legume-based cropping system, chickpea field had higher bacterial population (5.43 log10 cfu g^{-1} soil) followed by oilseed-based cropping field (mustard and soybean; 5.42 log10 cfu g^{-1} soil). In cereal cropping system, maize field had higher microbial population (5.40 log10 cfu g^{-1} soil) followed by rice–wheat (5.38 log10 cfu g^{-1} soil) and lowest in vegetable-based cropping system (5.41 log10 cfu g^{-1} soil). Land management, vegetation and climate are key factors which influence the abundance of microbial communities and their diversity in the top profiles (Shrivastava *et al*. 2014) [20].

In a cereal/legume crop rotation system, Alvey *et al.* (2003) [4] have reported that different crops affected the number, species and diversity of soil microorganisms. The higher microbial community noticed in legume-based cropping system of the Indo-Gangetic Plains is attributed to the higher soil nutrients and organic carbon along with stimulated microbial activity. Rotational crop root exudates and root residues decomposed in the soil can affect nutrient availability to soil microbes.

Under quality traits protein content in seed was studied in fenugreek**.** Protein content in seed was found to be significantly increased by various treatments of inorganic fertilizer and bio-inoculants. It is apparent from the data that maximum protein content was recorded with treatment T_{12} *i.e.* 75% of RDF+*Rhizobium*+PSB+KSB (21.28%). Whereas, it was observed lowest under control (18.41%).

This result corroborate with the findings of Choudhary *et al.* (2011) [10] who applied 50% RDN through FYM + 50% RDN through VC + *Rhizobium* and Sunanda *et al.* (2014) in *kasuri methi* who supplied 75% N + Recommended dose of P and K + FYM (7.5 t ha^{-1}) + *Rhizobium* (1.5 t ha^{-1}) + *Azospirillum* (5 kg ha^{-1}) +PSB (5 kg ha^{-1}) and recorded maximum protein content in seed. This results also confirmed with Anwer *et al.* (2012) [5].

The increased protein content in seed is directly proportional to the increased N content in seed. Because of integrated nutrient management, increased N content of seed resulted in higher protein content. In addition to its function in protein production, N is an essential component of chlorophyll, which is the primary absorber of the light energy needed for photosynthesis.

Table 1: Effect of inorganic fertilizers and bio-inoculant (*Rhizobium*, PSB and KSB) on yield parameters in fenugreek

Treatment	Seed yield per plant (g)	Seed yield (qha^{-1})	Test weight (g)	Harvest index (%)	Number of nodules per plant at 30 DAS	Dry weight of plant at 60 DAS (g)
Control	1.45	6.29	9.38	35.63	24.40	1.27
RDF	2.46	10.80	10.45	44.45	26.60	1.52
75% RDF	2.33	9.65	12.74	50.12	25.33	1.45
50% RDF	1.51	7.03	11.43	38.31	25.40	1.35
T2+Rhizobium	2.55	12.07	11.81	48.69	26.67	1.33
T2+Rhizobium+PSB	2.42	10.59	12.36	45.40	34.07	1.42
T2+Rhizobium+KSB	2.66	12.57	12.11	56.60	28.80	1.57
T2+Rhizobium+PSB+KSB	3.15	13.13	14.58	58.39	35.07	1.67
T3+Rhizobium	2.33	10.06	12.43	44.42	25.27	1.41
T3+Rhizobium+PSB	2.38	10.56	12.74	40.49	26.80	1.49
T3+Rhizobium+KSB	2.38	10.17	12.70	39.50	31.20	1.37
T3+Rhizobium+PSB+KSB	2.73	12.79	14.20	56.25	34.80	1.60
T4+Rhizobium	1.55	7.40	12.51	45.10	29.13	1.45
T4+Rhizobium+PSB	1.85	8.38	12.72	45.58	31.13	1.53
T4+Rhizobium+KSB	1.77	8.09	12.71	50.25	30.93	1.46
T4+Rhizobium+PSB+KSB	2.24	9.30	13.11	41.80	30.53	1.48
C.D.	0.43	1.87	1.18	7.81	1.27	0.044
SE(m)	0.14	0.65	0.40	2.69	3.67	0.127

RDF- Recommended Dose of Fertilizer, PSB - Phosphorus Solubilising Bacteria, KSB -Potassium Solubilising Bacteria, C.D. – Critical Difference and SE(m)- Standard Error of the Mean

Table 2: Effect of inorganic fertilizers and bio-inoculant (*Rhizobium*, PSB and KSB) on available nutrient status on soil and plant after harvesting in fenugreek

Treatment	Nitrogen content in soil (kg/ha)	Phosphorus content in soil (kg/ha)	Potassium content in soil (kg/ha)	N content in plant (%)	P content in plant (%)	K content in plant (%)
Control	227.67	3.5	229.66	13.64	4.59	6.80
RDF	237.67	4.4	240.33	15.31	5.13	7.06
75% RDF	253.33	4.87	250.67	24.08	7.48	9.35
50% RDF	244.33	4.67	248.33	18.88	5.47	7.84
T2+Rhizobium	242.67	4.43	243.66	19.08	5.72	8.25
T2+Rhizobium+PSB	252	4.1	248	20.06	6.51	8.96
T2+Rhizobium+KSB	241	5.43	254.33	20.78	7.36	8.15
T2+Rhizobium+PSB+KSB	262	7.23	269	31.85	12.2	10.40
T3+Rhizobium	249.33	4.03	245	18.35	7.43	8.14
T3+Rhizobium+PSB	252.67	3.97	244	27.08	9.56	8.43
T3+Rhizobium+KSB	230	4.33	256.66	16.49	6.15	10.19
T3+Rhizobium+PSB+KSB	270	9.3	274.33	33.04	13.51	11.11
T4+Rhizobium	246.33	6.33	250.33	26.59	9.40	8.55
T4+Rhizobium+PSB	236.33	6.47	240.66	17.41	8.76	8.88
T4+Rhizobium+KSB	241	6.9	250.67	24.40	10.79	9.28
T4+Rhizobium+PSB+KSB	234.67	4.23	236	26.52	11.10	8.76
C.D.	18.03	0.659	22.03	2.36	0.56	1.73
SE(m)	6.21	0.227	7.59	0.81	0.19	0.60

Table 3: Effect of inorganic fertilizers and bio-inoculant (*Rhizobium*, PSB and KSB) on microbial population (Rhizobium, PSB and KSB) after harvesting and protein content in seed of fenugreek

Treatment	*Rhizobium* population (cfu g^{-1})	PSB population (cfu g^{-1})	KSB population (cfu g^{-1})	Protein content in seed (%)
Control	5.28(19.05×10^4)	5.51(32.35×10^4)	5.58((38.01×10^4)	18.41
RDF	6.42(26.30×10^4)	5.59(38.90×10^4)	5.62(41.68×10^4)	18.64
75% RDF	6.65(44.66×10^5)	5.71(51.28×10^4)	6.69(48.97×10^5)	18.57
50% RDF	6.64(43.65×10^5)	6.63(42.65×10^5)	6.53(33.88×10^5)	18.88
T2+Rhizobium	6.69(48.97×10^5)	6.66(45.70×10^5)	5.61(40.73×10^4)	18.83
T2+Rhizobium+PSB	7.62(41.68×10^6)	6.64(43.65×10^5)	5.79(61.65×10^4)	18.85
T2+Rhizobium+KSB	5.65(44.66×10^4)	6.58(38.01×10^5)	5.65(44.66×10^4)	19.20
T2+Rhizobium+PSB+KSB	7.64(43.65×10^6)	7.59(38.90×10^6)	7.43(26.91×10^6)	20.52
T3+Rhizobium	6.48(30.19×10^5)	6.52(33.11×10^5)	5.86(72.44×10^4)	19.47
T3+Rhizobium+PSB	6.65(44.66×10^5)	6.53(33.88×10^5)	6.66(45.70×10^5)	19.16
T3+Rhizobium+KSB	6.62(41.68×10^5)	6.58(38.01×10^5)	5.94(87.09×10^4)	19.23
T3+Rhizobium+PSB+KSB	7.75(56.23×10^6)	7.68(47.86×10^6)	7.70(50.11×10^6)	21.28
T4+Rhizobium	6.65(44.66×10^5)	6.62(41.68×10^5)	6.24(17.37×10^5)	19.86
T4+Rhizobium+PSB	5.63(42.65×10^4)	6.60(39.81×10^5)	6.48(30.19×10^5)	19.24
T4+Rhizobium+KSB	6.57(37.15×10^5)	5.71(51.28×10^4	6.56(36.30×10^5)	18.86
T4+Rhizobium+PSB+KSB	6.54(34.67×10^5)	6.52(33.11×10^5)	6.18(15.13×10^5)	19.70
C.D.	0.019	0.076	0.197	0.984
SE(m)	0.066	0.221	0.570	0.339

cfu g^{-1} – colony forming unit $gram^{-1}$

Conclusion

Thus, it can be concluded that the use of 100% RDF+*Rhizobium*+PSB+KSB found to be best in all parameters except in microbial population, available nutrient status and protein content in fenugreek.

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