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EFFECT OF BIO-INOCULANTS ON CROP GROWTH, YIELD AND SEED QUALITY ATTRIBUTES OF ALFALFA (*MEDICAGO SATIVA* L.) CV. RL-88

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SUMMARY

An experiment was carried out at the department of Seed Science and Technology (E-block) Eastern Dry Zone of College of Agriculture, UAS, GKVK, Bengaluru to find out the efficacy of bio-inoculants seed treatments on crop growth, seed yield, and quality attributes of alfalfa. The results on growth parameters *viz*., the highest plant height (87.33 cm and 84.50 cm), chlorophyll content (42.57 and 48.59 SPAD value), the total number of pods per plant (149.67 and 154.56), number of filled seeds per pod (4.11 and 5.33), seed yield (180.54 and 256.15 kg ha^{-1}) and seed test weight (3.246 and 3.340 g) recorded in seed treated with a recommended dose of fertilizer (RDF) 20:100:40 NPK kg ha-1 + *Pseudomonas fluorescens* + FYM treatment. Whereas the number of nodules per plant (4.33 and 4.44) was recorded highest in 10:100:40 NPK kg ha^{-1} + *Rhizobium meliloti* + FYM both during *kharif* and *rabi* season. Among seed, quality attributes the seed germination percentage (92.00 and 92.78 %), seedling dry weight (349.14 & 424.83 mg), Seedling Vigor Index (1001 and 1287), the electrical conductivity (0.2800 and 0.2704 dSm-1) and total dehydrogenase enzyme activity (1.078 and 1.083 OD value), recorded highest in 20:100:40 NPK kg ha-1 + *Pseudomonas fluorescens* + FYM. Whereas, the seed crude protein content (19.19 and 19.22 %) was recorded highest in seeds treated with *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens* + FYM over the other treatment combinations during *kharif* and *rabi* season respectively.

Key words: alfalfa, root nodules, seedling vigour index

Alfalfa (*Medicago sativa* L.) is popularly known as lucerne and rightly called as "Queen of Forage". In India, it occupies an area of one million hectares and provides 60 to 130 t/ha of green forage, and seed yield varies from 2.2 to 4.0 q/ha thus an attempt was made to bridge the seed yield. In Karnataka, it occupies an area of 3121.23 ha which accounts for 0.03% of the net cropped area (Elumalai Kannan, 2012). In addition, the crop also fixes 83 - 594 kg/ha of atmospheric nitrogen through the root nodules.

Botanically, alfalfa belongs to the family *Fabaceae*, perennial. It normally lives four to eight years under better agronomic cultivation practices depending on variety and climate. The plant grows to a height of up to 1 meter and has a deep root system. This makes it very resilient, especially to drought. The crop is rich in protein (13.3-26.6%), phosphorus (0.14-0.66%), calcium (0.92-2.9%), magnesium (0.11-0.64%), carotene (9.27 mg per 100 g), fiber (20-30%) and vitamin A & C (Abdul Khalak, 1989). Due to its high palatability, it serves as an alternative to concentrating feed components for dairy animals, race horses, poultry, piggery, etc.

One of the major constraints encountered in seed production is the lack of technology to carry over the seeds until the next planting season of the several factors which affect the yield & seed quality. The main factor limiting quality seed production in India is the cost of chemical fertilizers used to forage crops. Chemical fertilizers are playing a crucial role to meet the nutrient requirement of the crop. Persistent nutrient depletion is posing a greater threat to sustainable agriculture. Therefore, there is an urgent need to reduce the usage of chemical fertilizers and in turn, increase the usage of organics which is needed to check the yield and quality levels. The use of organics alone does not result in a spectacular increase in crop yields, due to their low nutrient status. This necessitates looking into alternatives that are ecologically sound and financially feasible. Such as, the use of bio-fertilizers with a reduced dose of judicious usage of fertilizer is an innovative crop management technique.

MATERIALS AND METHODS

The Field experiment is laid out in Factorial Randomized Block Design with three replication with gross plot size: 2.1 m x 2.0 m=4.20 m^2. The experimental plot soil is of red sandy clay loam with slightly acidic (pH 6.26) and the electrical conductivity was normal (0.12 m mhos/cm at 25 ?C). The nitrogen (240 kg ha^{-1}) was low, whereas the phosphorus was high (62.83 kg ha^{-1}) and the potash was medium (190.83 kg ha^{-1}). The organic carbon (0.48 %) was low with boron (2.2 m eq/100g), calcium (3.2 m eq/ 100 g), magnesium (1 m eq/100 g), and sulphur (2.150 ppm) and these were found to be slightly below the normal. During the previous season of *kharif* -2014, the site was grown with red gram crop for seed purposes with the recommended package of practices and, in the previous season of *rabi* - 2014, sunflower was grown for seed purposes with normal agronomic practices. As per the package of practices the recommended dose of fertilizer was applied *viz.,* 20 kg N, 100 kg P_2O_5, and 40 kg K_2O in the form of urea, Single super phosphate and muriate of potash respectively.

Seeds were treated with different bio-inoculants single and in consortium with the reduced dose of fertilizer to know the efficacy of *Rhizobium meliloti, Bacillus megaterium,* Vascular Arbuscular Mycorhizal Fungi, *Frateuria aurantia, Pseudomonas fluorescens* and FYM. from the test transferred to the required quantity of adhesive solution (*Gum arabica*) taken in the small container and made a slurry (40-50 ml) adhesive solution was poured over the seed taken in a container with simultaneous mixing to give a uniform black coating over the entire surface of the seed later inoculated seeds were dried uniformly at room temperature two hours before sowing.

The seeds of alfalfa were sown @ 5 kg per hectare, with the line spaced 30 cm apart and 10 cm between the plant-to-plant seeds were sown at a shallow depth of 2-3 cm. After sowing light irrigation was given to maintain the optimum moisture for uniform seed germination. The thinning operation followed after two weeks of sowing and retained a single healthy seedling per hill, to provide optimum space for an individual plant to avoid nutrient competition, gap filling was done to maintain a 100 percent plant population.

Four replications of twenty-five seeds were weighed up to two decimal places. The seeds were washed thoroughly with distilled water. The surface sterilized seeds were soaked in 25 ml of distilled water and incubated for 24 hours. Then, the steeped water from the soaked seeds was collected and electrical conductivity (EC) of the leachate was measured in the digital conductivity meter model. After subtracting the EC of the distilled water from the value obtained from the seed leachate, the actual EC due (the leachate) to electrolyte was measured and expressed in dSm^{-1} at 25 + 1 ?C (Presley, 1958).

RESULTS AND DISCUSSION

The seeds treated with *Rhizobium* and phosphate solubilizing bacteria showed good nodulation as compared to other treatments might be due to increased nitrogenase activity by *Rhizobium* and PSB produce growth hormones, *viz*., IAA, auxins, gibberellins, and vitamins which are conducive to better nodulation besides biofertilizers provide a better environment for nodule bacteria by improving physical properties of soil. Combined inoculation of *Rhizobium* and phosphate-solubilizing bacteria showed a positive effect on nodulation, indicating the synergistic association between them. It is well documented that arbuscular mycorrhizal fungi (AMF) colonization and Arbuscular Mycorrhizal (AM) fungal activity is enhanced by co-inoculation with *Rhizobium*, and other beneficial microbes resulting in better plant performance in terms of production and quality. Alfalfa has a specific relationship with *Sinorhizobium meliloti* (previously known as *Rhizobium meliloti*).

The plant growth parameters *viz*., The highest plant height (87.33 cm and 84.50 cm) and chlorophyll content (42.57 and 48.59 SPAD value) recorded in 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM, which was on par with 20:100:40 NPK kg ha^{-1} (R) + FYM whereas, the lowest for above values in order recorded in *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi+ *Frateuria aurantia* +

TABLE 1
Effect of spacing and nutrient levels on plant growth and seed yield attributes of alfalfa (Medicago sativa L.) cv. RL-88

S. No.	Treatments	Plant height (cm)		Leaf chlorophyll content (SPAD VALUE)		No. of Nodules/ plant		Total No. of pods/ plant		No. filled seeds/pld		Seed yield/ ha		Test weight of seeds	
		Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi
1.	F_1	84.87	83.17	40.59	46.40	1.00(5.74)	1.33(6.54)	137.89	142.56	3.56	4.67	145.43	199.16	3.208	3.295
2.	F_2	69.77	66.60	30.62	36.97	1.00(5.74	1.33(6.54)	74.44	80.44	3.00	3.11	80.70	109.99	2.974	3.051
3.	F_3	81.40	78.23	38.05	44.40	4.33(11.93)	4.44(12.04)	111.89	114.67	3.11	4.11	96.62	147.06	2.656	2.769
4.	F_4	79.37	76.20	35.48	41.83	2.11(7.82)	2.33(8.36)	94.67	98.89	3.22	4.11	92.86	146.51	2.660	2.785
5.	F_5	82.50	79.33	38.38	44.73	1.00(5.74)	1.33(6.54)	128.67	133.56	3.89	4.78	106.05	172.15	2.762	2.842
6.	F_6	74.17	71.00	34.45	40.80	1.00(5.74)	1.33(6.54)	85.67	89.67	3.00	3.33	84.24	114.08	2.631	2.719
7.	F_7	61.27	58.10	33.65	40.00	1.00(5.74)	1.33(6.54)	80.67	84.78	3.11	3.44	84.58	116.44	2.651	2.732
8.	F_8	78.20	78.20	34.78	41.13	1.00(5.74)	1.33(6.54)	91.67	95.67	3.67	4.22	111.70	144.01	2.677	2.773
9.	F_9	87.33	84.50	42.57	48.59	2.33(8.74)	2.78(9.50)	149.67	154.56	4.11	5.33	180.54	256.15	3.246	3.340
10.	F_{10}	68.40	64.89	29.07	34.41	3.56(10.84)	3.89(11.27)	68.56	74.67	3.00	3.00	72.28	96.17	2.877	2.929
	S.Em±	0.16	0.22	0.24	0.18	0.13	0.17	0.31	0.69	0.10	0.13	0.47	0.50	0.008	0.027
	CD (P=0.05)	0.45	0.63	0.69	0.52	0.36	0.49	0.88	1.96	0.29	0.36	1.34	1.42	0.022	0.077
	CV (%)	5.46	7.79	12.16	8.57	14.04	18.37	9.24	20.10	16.83	19.13	13.82	12.30	1.41	4.77

Note: The values mentioned in the brackets are arc-transformation
Treatments Description
F_1: 20:100:40 NPK kg ha-1 (RDF) + FYM
F_2: 10:50:20 NPK kg ha-1 (50% of RDF) + FYM
F_3: 10:100:40 NPK kg ha-1 + Rhizobium meliloti + FYM
F_4: 20:50:40 NPK kg ha-1 + Bacillus megaterium + FYM
F_5: 20:100:20 NPK kg ha-1 + VAM Fungi+ FYM
F_6: 10:50:20 NPK kg ha-1 + Bacillus megaterium + VAM Fungi+FYM
F_7: 10:50:20 NPK kg ha-1 + Frateuria aurantia + FYM
F_8: 10:50:20 NPK kg ha-1 + Rhizobium meliloti + Bacillus megaterium + VAM Fungi + Frateuria aurantia + FYM.
F_9: 20:100:40 NPK kg ha-1 + Pseudomonas fluorescens + FYM
F_{10} : Rhizobium meliloti + Bacillus megaterium + VAM Fungi +Frateuria aurantia + FYM

Pseudomonas fluorescens + FYM [(68.40 cm and 64.89 cm) and (29.07 and 34.41 SPAD value)] during *kharif* and *rabi* season (depicted in Table 1). The significant and consistent increase in plant growth parameters noticed under higher nutrient levels followed by RDF + bioinoculant treatment might be due to the increased availability of energy sources for a prolonged time from the integrated source of nutrients and due to the fixation of atmospheric nitrogen by rhizobium and release of phosphorus by addition of PSB which resulted in the luxuriant crop growth due to enhanced cell division, elongation and differentiation as compared to lower nutrient levels NPK ha^{-1}. The results uphold the findings of earlier studies made by Nagaraju and Mohankumar (2010) in soybean and Kadam *et al.* (2014) in black gram.

The effect of nutrient levels was found significant for the number of nodules per plant. Among the different nutrient levels, the treatment 10:100:40 NPK kg ha^{-1} + *Rhizobium meliloti* + FYM higher number of nodules per plant (4.33 and 4.44) followed by *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens*+ FYM (3.56 and 3.89) whereas, the lowest was recorded in 20:100:40 NPK kg ha^{-1} (RDF) + FYM (1.00 and 1.33).

The seed yield attributes were found significantly highest overall the 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM treatment exhibited around (19.44 and 22.24 %) percent increase in seed yield over the 20:100:40 NPK kg ha^{-1} (RDF) + FYM (145.43 and 199.16 kg ha^{-1}), (100 % RDF) during the *kharif* and *rabi* season. Similarly, the seed yield attributes *viz.*, total number of pods per plant (149.67 and 154.56), number of filled seeds per pod (4.11 and 5.33), seed yield (180.54 and 256.15 kg ha^{-1}) and test seed weight (3.246 and 3.340 g) during *kharif* and *rabi* season respectively (depicted in Table 1). The marked increase in seed yield per plant and hectare noticed in the higher nutrient levels might be attributed to its increased availability of essential nutrients to the plants favoring higher crop growth and flowering parameters, as seen from the findings of the present study. It might have resulted in higher production, translocation, and accumulation of photosynthates into the developing sinks (seeds) from the photosynthetic sources and it resulted in a higher number of reproductive branches, number of racemes per plant, filled pods per raceme, seed yield per plant and harvest index as compared to those grown at lower nutrient levels. Scientifically the higher nutrient higher dose combined with the bioinoculant treatment produces growth hormones *viz.,* IAA, auxins, gibberellins, and vitamins which are conducive to better nodulation besides biofertilizers provide a better environment for nodule bacteria by improving physical

TABLE 2
Effect of spacing and nutrient levels on seed quality attributes of alfalfa (Medicago sativa L.) cv. RL-88

S. No.	Treatments	Seed germination percentage		Seedling dry weight		Seedling vigor index		Electrical conductivity (dS/m)		Dehdrogenase enzyme activity (OD value)		Seed crude protein content	
		Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi
1.	F_1	90.00	91.67	346.90	453.17	850	1229	0.2948	0.2864	1.002	1.009	17.41	17.61
2.	F_2	87.33	89.11	294.89	401.23	832	1116	0.3325	0.3274	0.909	0.914	18.76	18.81
3.	F_3	88.33	90.78	320.81	431.17	896	1203	0.3201	0.3127	0.958	0.964	18.31	18.36
4.	F_4	90.67	91.56	312.90	444.83	895	1181	0.2864	0.2797	0.961	0.967	18.39	18.42
5.	F_5	89.33	90.56	292.07	408.83	842	1122	0.3064	0.2977	0.973	0.978	18.35	18.42
6.	F_6	88.33	90.33	332.11	437.12	933	1231	0.3183	0.3097	0.922	0.928	17.88	17.99
7.	F_7	88.67	90.00	332.06	436.77	935	1215	0.3169	0.3070	0.917	0.923	17.76	17.86
8.	F_8	88.67	90.67	313.28	421.17	888	1153	0.3168	0.3087	0.933	0.939	17.21	17.34
9.	F_9	92.00	92.78	349.14	424.83	1001	1287	0.2800	0.2704	1.078	1.083	16.75	17.09
10.	F_{10}	87.67	89.67	276.97	372.50	738	1062	0.3316	0.3279	0.911	0.915	19.19	19.22
	S.Em±	0.54	0.61	7.54	5.58	24	25	0.0011	0.0014	0.030	0.030	0.08	0.16
	CD (P=0.05)	2.02	2.30	28.36	21.00	91	96	0.0040	0.0051	0.115	0.112	0.31	0.60
	CV (%)	1.81	2.02	7.13	3.81	8	8	1.02	1.31	9.25	7.29	1.36	2.65

Treatments Description
F_1: 20:100:40 NPK kg ha-1 (RDF) + FYM
F_2: 10:50:20 NPK kg ha-1 (50% of RDF) + FYM
F_3: 10:100:40 NPK kg ha-1 + Rhizobium meliloti + FYM
F_4: 20:50:40 NPK kg ha-1 + Bacillus megaterium + FYM
F_5: 20:100:20 NPK kg ha-1 + VAM Fungi+ FYM
F_6: 10:50:20 NPK kg ha-1 + Bacillus megaterium + VAM Fungi+FYM
F_7: 10:50:20 NPK kg ha-1 + Frateuria aurantia + FYM
F_8: 10:50:20 NPK kg ha-1 + Rhizobium meliloti + Bacillus megaterium + VAM Fungi + Frateuria aurantia + FYM.
F_9: 20:100:40 NPK kg ha-1 + Pseudomonas fluorescens + FYM
F_{10} : Rhizobium meliloti + Bacillus megaterium + VAM Fungi +Frateuria aurantia + FYM

properties of soil. These results are in agreement with those of Bhattacharya *et al.* (2004) in *Stylosanthes*, and Kadam *et al.* (2014) in black gram.

Seed quality parameters revealed significant differences, the application of 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM recorded lowest electrical conductivity (0.2800 and 0.2704 dSm^{-1}) while highest was recorded in 10:50:20 NPK kg ha^{-1} (50 % RDF) + FYM (0.3325 and 0.3274 dSm^{-1}) respectively *kharif* and *rabi* seasons. Whereas, the 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM recorded *viz.*, seed germination (92.00 and 92.78 %) & total dehydrogenase enzyme activity (1.078 and 1.083 OD value), the values are lowest in 10:50:20 NPK kg ha^{-1} (50% of RDF) (87.33 and 89.11 %) and (0.909 and 0.914 OD value) during *kharif* and *rabi* seasons, respectively (depicted in Table 2) This may be due to the presence of a higher amount of metabolites, which helps in the resumption of embryonic growth during germination. In addition to these metabolites, the release of certain enzymes is responsible for the degradation of macromolecules into micromolecules within the seed as reported by Ajay Gupta *et al.* (2006) in urd bean.

The seedling dry weight was recorded highest in 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM (349.14 mg and 424.83 mg) compared to all other whereas, the lowest recorded in *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens* + FYM (276.97 mg and 276.97 mg) during *kharif* season, respectively The Seedling vigour Index recorded highest in 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM (1001 and 1287) compared to all other treatment combinations whereas, the lowest recorded in *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens* + FYM (738 and 1062) during *kharif* and *rabi* seasons, respectively

The seedling length was recorded highest in 20:100:40 NPK kg ha^{-1} + *Pseudomonas fluorescens* + FYM (10.88 and 13.86 cm) compared to all others whereas, the lowest recorded in *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens* + FYM (8.94 and 11.84 cm) whereas, the seed crude protein content recorded highest in *Rhizobium meliloti* + *Bacillus megaterium* + VAM Fungi + *Frateuria aurantia* + *Pseudomonas fluorescens* + FYM (19.19 and 19.22 %) compared to all other treatments combination the lowest recorded in 20:100:40 NPK kg/ha+ *Pseudomonas fluorescens* + FYM (16.75 and 17.09%) during *kharif* and *rabi* seasons, respectively. Increase in protein content due to high uptake of nitrogen and phosphorous by slow and continuous supply through bio-fertilizers.

The results of this experiment indicated that a significant increase in the seed quality parameters observed in the higher nutrient levels may be due to the increased application of essential nutrients and it has enhanced the synthesis and accumulation of photosynthates in to the sinks and increased the seed size and weight *per se* due to higher seed yield attributes and thus resulted in higher seed quality as against those grown at lower nutrient levels.

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REFERENCES

Abdul Khalak, 1989 : seed yield of lucerne (*Medicago sativa* L.) varieties at different times of sowing and levels of fertilizers. *M.Sc., (Agri.,) Thesis,* Univ. Agric. Sci., Bangalore.

Gupta, A. Vinod Kumar Sharma, Sharma, G. D. and Pankaj Chopra, 2006 : Effect of biofertilizer and phosphorus levels on yield attributes, yield and quality of urd bean (*Vigna mungo*). *Indian J. Agron*., **51**(2): 142-144.

Bhattacharya, S., Nanda, M. K. and mukherjee, A. K., 2004 : Effect of phosphorus and cutting on biomass and seed production of *Stylosanthes* cultivars. *Forage Res.,* **30**(2): 96-98.

Elumalai Kannan. 2012 : Economics of production, processing and marketing of fodder crops in Karnataka. *Research Report.,* **11** :48.

Kadam, Swati, R., Kalegore, N. K. and Patil Snehal R., 2014: Influence of phosphorus, vermin-compost and PSB on yield attributes, seed yield and quality of black gram. *Adv. Res. J. Crop Improv.,* **5** (1) : 7-10.

Nagaraju, A.P. and H.K. Mohankumar, 2010: Effect of micronutrients and bio inoculants on growth and yield of soybean (*Glycine max* L. Merrill). *Mysore J. Agric, sci.,* **44**(2): 260-265.

Presley, H. T., 1958: Relation to protoplast permeability of cotton seed viability and pre-disposition to seedling disease. *Pl. Dis. Rep.,* **42**: 52.