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STUDY ON EFFECTIVENESS OF DUAL INOCULATION WITH *RHIZOBIUM* AND VA MYCORRHIZA ON GROWTH AND BIOMASS PRODUCTION OF FABA BEAN *(VICIA FABA* L.) GROWN IN KOTA DISTRICT OF RAJASTHAN

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Pot experiments were carried out to investigate the effect of *Rhizobium leguminosarum* and VA Mycorrhiza on the growth and biomass production of faba bean. The biofertilizers were applied in combination. The obtained results demonstrated that the dual inoculation of faba bean plants signi?cantly increased the plant biomass, nodulation, nitrogen ?xation activity in comparison with uninoculatedcontrol. On the other hand, co-inoculation signi?cantly increased the total plant protein, chlorophyll N-content and phosphorus content in plant tissue and percentage of root colonization.The study clearly shows that a combined application of biofertilizers is an essential requirement for the growth and biomass production of faba bean.

Keywords : Dual inoculation; *Rhizobium;* VA mycorrhiza; *Vicia faba.*

Introduction

Faba bean *(Vicia faba* L.) is the important grain and forage legume in all around the world. It serves as an important source of protein for humans. The nitrogen-fixing interaction between *Rhizobium,* legumes and the mycorrhizal association is the most two commonly studied symbiosis[1]. Biofertilizers areproducts containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes[2,3].

Rhizobia stimulate plant growth mainly by modifying root development, which improved macro and micronutrients and water uptake, particularly in the early stages of plant development[4]. The plant host organisms may be affected by one or more mechanisms such as nitrogen fixation, enhancing nutrient uptake, production of plant growth promoting substances, phytohormones, and organic acids, as well as protection of plant from pathogens[5-8] and the organism benefits by acquiring photosynthates from the plant.

Arbuscular mycorrhizae (AM) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection, and soil quality. The AM fungi expand their filaments in soil and plant roots. This filamentous network promote bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network[9]. (V)A Mycorrhizal symbiosis may benefit the host plant primarily by increasing the ability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae[10]. A significant increase in plant dry weight, N and P content were observed wherever the tripartite association of rhizobia, mycorrhizal fungi, and legumes were present[11].

Material and Methods

The plants of *Vicia faba* and soil were collected from 5 different fields of 3 selected localities, namely KeshavRaiPatan, Kaithoon and Borkhera of Kota district of Rajasthan. Collection, isolation, purification and authentication of rhizobium culture were done by recommended method[12]. Multiplication of rhizobial culture was done onYeastExtract Mannitol Agar medium.(V)AM inoculation was procured by sieving and decanting method[13].

The seeds were inoculated with 500 mg of mycorrhizal inoculum (approximately 250 spores), by placing 2 cm below the seed level and 0.5 ml of rhizobial culture. Experiments were conducted in black cotton soil with a mild alkaline pH in earthenware pots. Healthy seeds of faba bean (*Vicia faba* L.) were employed throughout the study. Two treatments involved in the study under sterilized soil conditions are as follows:

1. Uninoculated (Control);
2. Five dual inoculations with both (V)AM fungus and *Rhizobium* (VfRHz 1-5+(V)AM) of five different fields

Table 1. Effect of dual inoculation of *Rhizobium* and (V)A Mycorrhiza on growth, nodulation of *Vicia faba* Linn. (Values are mean ± Standard Deviation of 3 replicates)

Names of pot	Shoot Length (cm)	Root Length (cm)	Fresh Wt. of plant (gm)	DryWt. of plant (gm)	No. of Pods	Pod Wt. (gm)	No. of Nodules	Nodule Fresh Wt. (mg)	Nodule Dry Wt. (mg)
VfRhz 1	26.9±0.5507	22.8±0.2657	42.0±0.5589	12.0±0.6813	28±1.5275	17.6±0.1400	196±1.00	593±3.055	487.5+0332
VfRhz2	28.1±0.4219	23.6±0.2003	53.8±0.5850	13.2±0.2605	32±1.00	19.6±0.5577	244±2.0816	715±4.5825	598±3.5118
VfRhz3	30.7±0.1253	25.6±0.3165	54.2±0.2909	16.2±0.3121	36±1.00	22.2±0.2163	278±1.1547	834±4.5092	710±6.5064
VfRhz4	33.5±0.4550	31.2±0.2961	63.6±0.3160	17.6±0.3821	37±0.5773	22.8±0.3958	328±2.0816	986±4.0414	812±6.0277
VfRhz5	25.6±0.3579	20.5±0.1418	35.22±0.6058	11.6±0.1171	27±0.5773	15.64±0.2400	184±2.0816	562±5.5075	437±4.0414
Control	20.7±0.6557	17.2±0.3511	22.62±0.4371	05.30±0.3055	9±1.00	9.18±0.4086	ZERO	ZERO	ZERO

Table 2. Effect of dual inoculation of *Rhizobium* and (V)A Mycorrhiza on phytochemical aspects of *Vicia faba* Linn. (Values are mean ± Standard Deviation of 3 replicates)

Names of pot	Total Plant Protein (mg/g)	Total Plant Chlorophyll (mg/lt)	N-content (% dry weight)	P-content (% dry weight)	N_2ase activity
VfRhz 1	93.34±0.9465	1.04±0.0305	4.41±0.3212	0.59±0.0568	1.08±0.1193
VfRhz2	95.69±0.9624	1.17±0.0251	4.46±0.3928	0.61±0.0986	1.13±0.1311
VfRhz3	104.58±0.8818	1.34±0.04	4.44±0.4067	0.64±0.08717	1.19±0.1081
VfRhz4	110.04±0.9816	1.42±0.0173	4.58±0.4869	0.68±0.0832	1.24±0.1305
VfRhz5	87.67±0.7902	0.94±0.02	4.38±0.3523	0.49±0.0251	0.98±0.07
Control	28.66±0.8542	0.57+0416	3.39±1.3917	0.38±0.0873	ZERO

of three selected localities.

The plants were grown for 45 days with average day and night temperatures of 28°C and 20°C, respectively. Data on shoot and root length, fresh weight and dry weight of plant, number of pods, pod weight, nodule number, fresh weight and dry weight of noduleswas recorded at 45 days after inoculation. The total plant protein was estimated by Lowery *et al.* method[14]; total chlorophyll content by Arnon method[15]; nitrogen-by Microkjeldahl method[16]; total phosphorus content in plant roots and shoots by Vanado-molybdate phosphoric yellow colour method[17] and quantitative estimation of total soluble sugars was done by Dubois *et al.* method[18].

Results and Discussion

Inoculation with vesicular arbuscular mycorrhizal fungi and rhizobial isolates significantly increased shoot-root length, fresh-dry weight of plant, number of pods, pod weight, number of nodules and fresh-dry weight of nodules respectively, in comparison to the uninoculated control. This interaction was studied by conducting a pot culture experiment in sterilized conditions. The effects of dual inoculations were compared with uninoculated control plants (Table 1).

A. Plant growth and nodulation:- The most remarkable results were obtained from the seedlings inoculated with dual combination of rhizobial isolate VfRhz 4+(V)AM, whose growth and nodulation parameters like shoot length (33.5 cm), root length (31.2 cm), fresh weight of plant (63.6 gm), dry weight of plant (17.8 gm), number of pods (37), pod weight (22.8 gm), nodule number (328), nodule fresh weight (986 mg) and nodule dry weight (812 mg) were higher , respectively than that of VfRhz 3+(V)AM, VfRhz 2+(V)AM, VfRhz 1+(V)AM and VfRhz 5+(V)AM at 45 days of inoculation. The uninoculated (control) plants, grown in sterilized soils did not form any nodules and recorded the lowest values for all above parameters (Table 1).

B. Phytochemical parameters:- The dual inoculation had significant effect on phytochemical parameters like total plant protein, total plant chlorophyll, N-content, P-content and N2ase activity. Significant positive correlations were observed between colonization of (V)AM and all nitrogen fixing bacterial isolates. Among all five dual combinations, VfRHz 4+(V)AM was observed statistically most significant in total plant protein (110.04 mg/g), total plant chlorophyll (1.42 mg/g), N-content (4.58 %), P-content (0.68 %) and N_2ase activity (1.24 μ mol. C_2H_4. g nodule fresh weight^{-1} h^{-1}). The above mentioned phytochemical parameters were much lower in non-treated control faba seedlings (Table 2).

The results indicate that dual inoculation of faba bean with *Rhizobium* and (V)AM fungus results in significant increase in the growth *i.e.,* fresh and dry weight, length of the root and shoot etc. nodulation as compared to uninoculated control. Previous researches revealed that nodulation, growth, yield, and nutrient uptake of faba beans can be significantly enhanced by both *Rhizobium* and mycorrhizal inoculation[19-23]. Moreover, early findings reported that the dual inoculation with both rhizobia and mycorrhizae induced significant increase in plant dry weight, N and P content of faba bean than uninoculated control[19,24]. It is also reported in green gram[25] and cowpea[26-28] that tripartite symbiosis between host plant, *Rhizobium* and (V)AM fungi increased the growth and nodulation.

(V)AM improved the uptake of nutrients by extra radical mycorrhizal hyphae[29]. Similar effects of mycorrhizae were also reported by Mamta and Tilak[30]. They studied the effect of *Rhizobium* species and mycorrhizal fungus on nutrient of mungbean.Inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation[31].

In conclusion the results of this study indicate that the dual inoculation with (V)AM fungus and *Rhizobium* is beneficial to *Vicia faba* L. for its better growth and development with increased growth, nodulation, phytochemical parameters and hence probable increase in the N fixation by the plant as also reported by different workers in the other legumes.

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