Research Article

Effect of rhizobium, VAM and rock phosphate inoculation on growth and productivity and isolation of VAMF spores of *Pisum sativum*

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Abstract

Rhizobium, VAM and rock phosphate inoculation has a significant utilization in *Pisum sativum*. The present study was conducted to study the influence of rock phosphorus fertilization, Vesicular Arbuscular Mycorrhizae (VAM) and rhizobium inoculation on growth and yield parameters of *Pisum sativum*. The experiment was laid out in randomized complete block design during summer season at the Department of Botany University of Peshawar Pakistan. A composite soil sample was collected for analysis of soil for physical and chemical properties. Rock phosphorus fertilization, application along with AMF and rhizobium inoculation increased the growth parameters significantly. Inoculation under Phosphorus deficient soil which have pivotal role in the mycorrhization, growth and productivity. Dual inoculation, Rhizobium and AMF resulted in an increase in grain yield respectively, over no inoculation in *Pisum sativum*. Maximum growth was observed where AMF along with rhizobium and rock phosphate inoculation were applied. It is concluded that *Pisum sativum* should preferably be grown with dual inoculation of VAM along with rhizobium and rock phosphate. And vesicular arbuscular mycorrhizal spores in the rhizospheric soils of *Pisum sativum* at three different stages, vegetative, flowering and fruiting highest spores density recorded at fruiting stage and increased with the age of plant and also effected by the treatment highest in dual inoculation as compared to single inoculation.

Key words: Arbuscular mycorrhizae fungi; RP fertilizer; AMF spores; *Glomus* spp; *Clerocystis*; *Aciculospora*

Introduction

Arbuscular mycorrhizas (AM) are widespread symbiotic interactions that are commonly described as the result of co-evolution between fungi and plants where

both partners benefit from the reciprocal nutrient exchange [1]. Arbuscular mycorrhizal (AM) symbioses are formed by approximately 80% of vascular plant species in all major terrestrial biomes [2-3].

Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and generally increases plant growth to a greater extent than inoculation with only one [4]. Inoculation alone or a combination of beneficial microorganisms including AMF, rhizobia, PGPR and PSB (Phosphate Solubilizing Bacteria) have been shown to increase green gram and chickpea growth and productivity, nitrogen fixation and nutrient uptake [5-14]. The AMF inoculation, alone or in combination with rhizobial inoculation, resulted in large increases in yield, root colonization and phosphorus content of the seed and shoot [15].

The present studies were conducted to evaluate the beneficial effect of bio-fertilizers (VAM, *Rhizobium* and Rock phosphate) inoculation alone and in combination on growth, productivity and spore density of *Pisum sativum*.

Materials and methods

The present study was conducted during summer at the Department of Botany, University of Peshawar Pakistan. Seeds of Pea (*Pisum sativum*) were sown in circular earthen pots (21 cm height and 70.6cm diameter) filled with a mixture of soil which was sterilized in Laboratory of Soil and Environmental Department of Agricultural University and was mixed with sand in ratio of 2:1 (v/v). Rhizobia and AMF propagules mixed. Rhizospheric soil from wheat field having high spore number of different AMF i.e. *Glomus fasciculatum*, *G. mosseae* and *G. aggregatum* and roots of wheat and maize infected with Arbuscular mycorrhiza were used as rhizobase inoculum. The root pieces along with soil base inoculum (rhizospheric soil) were spread uniformly in layers at a depth of 3cm and 6cm before sowing. Inoculum for each pot consisted of 180g of mycorrhizal infected roots and adhering soil. *Rhizobium* seed inoculation was done by using effective *Rhizobium leguminosarum*

(*Rhizobium* spp.) obtained from Dept. of Soil Science, NARC Islamabad and applied. The seeds were coated with gum acacia and 10kg pot⁻¹ soil was used. Soil used during the experiment was collected from the University of Peshawar. The soil was air-dried, sieved (4 mm) and characteristics determined at soil laboratory of the department of Soil and Environmental Science, The University of Agriculture, Peshawar. Characteristics of the soil included soil texture as sandy loam, organic matter 6.65%, nitrogen 0.044% and phosphorus 1.13% [16]. The pH of saturated paste (7.30) was determined. Seeds were inoculated individually with *Rhizobium* and AM fungi (applied as layering on soil surface) and combination of both and different levels rock phosphate fertilizer. The plants were irrigated with tap water as and when required. The plants were collected from each replication for sampling at vegetative, flowering and fruiting after sowing. Plant height, pods plant⁻¹ and seeds pod⁻¹ data were recorded on 45th day. The following treatments were used in the study.

T1 =Control (Un inoculated)

T2 =Inoculation with VAM fungus alone

T3 =Inoculation with *Rhizobium* alone

T4 =Inoculation with *Rhizobium* + VAM (Dual inoculation)

T5 =Application with Rock phosphate alone

T6 =Inoculation with Rock phosphate +VAM (Dual inoculation)

T7 =Inoculation with *Rhizobium* + VAM + Rock phosphate

Spore density

Statistical analysis

Experimental data were statistically analyzed by subjecting the data to analysis of variance (ANOVA) technique, the means were subjected to LSD test and differences among treatments were determined at $p \leq 0.05$ using the Statistical Analysis System (SAS) [17] program [18].

Results and discussions

Plant height

The ANOVA revealed highly significant differences among the treatments for plant height at harvesting stage. Results of VAM, RHZ, RHZ+VAM, RP and VAM+RP inoculation on plant height of *Pisum sativum* (Tables 1-2 and Fig.1). It is evident from the data VAM+RP and RHZ+VAM enhanced the plant height compared to the control. Results match with [19], that Dual and triple inoculation (co-inoculation) of microbes (bioagents) has beneficial effects on plant growth and development. Although, dual inoculation had good response but triple inoculation also exhibited significant positive response on growth of *E. saligna* seedlings. Mixed VAM +*Rhizobium* +RP had more increase in height. Our results correlate with the findings of [20-21], that both rhizobial and mycorrhizal symbioses can act synergistically on promoting plant growth. Our results also agree with the findings of [22], who stated that treatments which included rock phosphate (RP) and seed treatment with phosphate solubilizing bacteria (PSB) and *Rhizobium* application RP+PSB+*Rhizobium* recorded higher values

of growth parameters including plant height, number of branches per plant, number of seeds per plant and pod length. Our results are also in-line with the findings of [23-26]. The lowest plant height was recorded in control treatment as evident from data in (Tables 1-2 and Fig.1). Our findings are further in-line with [27], who also reported significant effects for plant height VAM+RP and RHZ+VAM produced the highest plant height which was higher than control treatment (control) in *Pisum sativum*. This result was in agreement with that of [28] where it was reported that plant height of soybean was highest with 20 kg P ha⁻¹. Same observation by [29] that synergistic effects of the combined application of rhizobia and AM fungi enhance plant growth to a greater extent than singular inoculation. The plant height was increased with the RP+VAM+Rhiz. reached to maximum compared to the treatment of each of them alone as shown in Table1-2. Our finding match with [29] who studied the influence of phosphorus fertilization and *Rhizobium* inoculation on growth and yield parameters of mungbean (*Vigna radiata*).

Figure 1. Effect of VAM, VAM+RHZ and VAM+RP inoculation on pods per plant of *Pisum sativum*

Number of leaves plant⁻¹

Data regarding number of leaves plant⁻¹ revealed highly significant differences for bio-fertilizer treatments. Mean values for number of leaves plant⁻¹ at different bio-fertilizer are given in Tables (1-2). Significance of RP+VAM+Rhiz effect shows an observable effect on number of leaves plant⁻¹. Our results are in accordance with [30] who studied the effect of bio-fertilizers on the growth and yield of green gram (*Vigna radiata*). Our results are according to [31] who reported that the effect of seed inoculation with *Rhizobium* and phosphate solubilizing bacteria. Rh + PSB significantly increased plant height, number of branches, number of leaves and leaf area.

Number of pods per plant

The ANOVA for number of pods plant⁻¹ showed highly significant differences among bio-fertilizer treatments at harvesting stage. Our results are in agreement with that of [9] who reported that combined inoculation of *G. intraradices* with *P. straita* plus *Rhizobium* to pathogen inoculated plants caused greater increase in plant growth and number of pods than by inoculation of *G. intraradices* plus *Rhizobium* or *G. intraradices* plus *P. straita* alone. Same finding were reported by [32] that combined inoculation of VAM fungus, *Glomus macrocarpum* and *Bradyrhizobium japonicum* in soybean variety William 82 promoted maximum growth and yield. Our result showed that VAM produced significantly better growth attributes when combined with RP or Rhz as compared to the yield and yield attributes over rest of the bio-fertilizers and fertilizer (RP) alone. Our result are also in-line with [33] who reported the effect of seed inoculation with *Rhizobium* along with various levels of phosphorus on chickpea (cv. Karak-1) growth through significant increase in pods/ plant.

Pod length

Analysis of variance regarding pod length depicted highly significant differences among bio-fertilizer treatments at fruiting stage. Mean values at VAM, RHZ, RHZ +VAM, RP and VAM+RP+Rhiz. inoculation on number of pods plant⁻¹ of *Pisum sativum* following seven treatments are given in Tables (1-2). Maximum pod length was observed at RHZ +VAM (Tables 1-2). Our results agree with [34] who reported that dual inoculation, *Rhizobium* and VAM resulted in increase in grain yield, over no inoculation for the yield of green gram, viz., pods per plant, pod length, seeds per pod.

Number of seeds per pod

Highly significant differences were observed among treatments for number of seeds pod⁻¹ at harvesting stage. The highest numbers of seeds pod⁻¹ were found in VAM+Rhiz and VAM treatment which was higher than that of control plants shown in Tables 1-2 and Fig.1. Our results agree with [34] who concluded that dual inoculation, *Rhizobium* and VAM resulted in increase in grain yield, over no inoculation. Our results are further supported by the findings of [35] reported that the percentage increase in grain yield over control was observed to be significantly higher in plants inoculated with *Rhizobium* strains and phosphate solubilizing bacteria. Similar results were obtained by [22] who stated that treatments which included rock phosphate (RP) and seed treatment with phosphate solubilizing bacteria (PSB) and *Rhizobium* application of RP and +PSB+*Rhizobium* recorded higher values of growth as well as yield. Our results are also supported by [36] who found that dual inoculation of *rhizobium* + VAM was superior over single inoculation.

Table 1. Mean square values for plant height (PH₁), number of leaves (NL₁) at flowering stage, plant height (PH₂), number of leaves (NL₂), number of flowers at flowering stage, plant height (PH₃), number of leaves (NL₃), number of pods plant⁻¹, pod length and number of seed pod⁻¹ for different bio-fertilizer treatments

SOV	DF	PH ₁	#Lv ₁	PH ₂	#Lv ₂	#Flower	PH ₃	#Lv ₃	#Pod/plnt	Pod Lnth	# seed
Rep	4	12.1407	1.6714	3.3919	0.1714	0.38571	2.9079	8.1857	0.61429	1.93614	0.7571
Treat	6	6.0218	10.1905	43.0598	95.8476	0.79048	24.1750	11.1333	3.36190	0.86181	10.7810
Error	24	3.3887	4.4881	4.0305	5.9548	0.71905	1.0344	3.4190	1.56429	0.36264	2.8405
CV		16.03	11.98	6.82	18.41	0.71905	3.09	9.15	33.16	10.20	28.36

Table 2. Mean values for plant height (PH₁), number of leaves (NL₁) at flowering stage, plant height (PH₂), number of leaves (NL₂), number of flowers at flowering stage, plant height (PH₃), number of leaves (NL₃), number of pods plant⁻¹, pod length and number of seed pod⁻¹ for different bio-fertilizer treatments

Treat	PH ₁	#Lv ₁	PH ₂	#Lv ₂	#Flower	PH ₃	#Lv ₃	#Pod/plnt	Pod Lnth	# seed
T1	9.780b	15.600c	29.480b	18.000ab	4.6000a	31.560b	18.200c	3.0000b	5.4000b	3.2000c
T2	11.280ab	16.400bc	33.800a	19.800a	4.4000a	34.600a	21.200ab	3.4000ab	5.9000b	7.0000ab
T3	12.260a	18.800ab	27.900bc	10.600c	5.4000a	35.220a	19.600bc	4.2000ab	5.9400b	5.8000ab
T4	11.220ab	18.600ab	32.960a	15.200b	5.0000a	35.660a	22.600a	4.2000ab	6.7600a	7.8000a
T5	10.860ab	17.000abc	25.700c	8.800c	4.6000a	30.020c	19.000bc	2.6000b	5.7800b	5.2000bc
T6	11.740ab	17.800abc	28.500b	9.800c	4.8000a	31.220bc	19.800bc	4.0000ab	5.7800b	6.4000ab
T7	13.260a	19.600a	27.700bc	10.600c	5.4000a	32.420b	21.000ab	5.0000a	5.7800b	6.2000ab
Mean	11.486	17.686	29.434	13.257	4.8857	32.957	20.200	3.7714	5.9057	5.9429
LSD	2.4029	2.7653	2.6206	3.1853	1.1069	1.3276	2.4136	1.6326	0.7861	2.2000

Table 3. Pea (V₅) all values mean ± SEM of 3 determinations (replicates)

Treat	Vegetative stage*				Fruiting stage**				Flowering stage***			
Biofer	Gl1	Sc11	Ac1	Totl.#	Gl2	Sc12	Ac2	Totl.#	Gl3	Sc13	Ac3	Totl.#
Control	29.67±0.84	5±0.33	1±0.33	35.67±0.19	44.67±1.50	3.33±0.33	1±0.33	45.67±0.69	46.67±1.26	2.67±0.38	1.33±0.1	50.33±1.64
VAM	51.33±1.07	7.33±1.17	2.00±0.33	59.67±1.50	50.00±0.33	5.00±0.88	1.67±0.19	56.67±1.35	60±5.04	7.33±2.14	1.67±0.19	84±0.00
RHZ.	51.67±1.17	4.67±0.19	3.00±0.88	59.33±1.35	54.00±0.67	7.00±0.33	2.33±0.19	63.33±0.38	68.33±3.56	6.67±0.38	1.00±0.58	76.00±3.48
VAM+RHZ	53.33±0.96	8.67±1.26	2.67±0.38	64.67±2.34	59.33±1.50	7.33±1.39	2.33±0.19	69.00±1.33	71±1.45	8.33±1.58	2.67±0.19	82±3.00
RP15gm	33.00±0.67	5.33±0.69	2.33±0.19	40.67±0.51	48.67±0.38	1.67±0.96	3.67±0.19	54.00±1.15	52.00±0.33	4.00±0.33	1.67±0.51	57.67±0.19
MRP130gm	44.33±0.19	4.33±0.51	1.67±0.38	50.33±0.69	55.00±0.67	4.00±0.33	3.00±0.33	62.33±1.07	59.33±0.69	4.67±0.51	2.00±0.33	66.00±0.58
MRP260gm	50.00±0.67	4.33±0.45	1.67±0.51	55.67±0.38	57.00±1.07	5.00±1.20	2.67±0.33	64.67±0.19	59.33±0.84	4.67±0.69	2.00±0.33	66.00±0.58

Means of 3 replication mean±S

Spore density

In the present work soil was collected from different pots with plants at three different stages of growth. Three genera of endogonaceous spores were identified which were *Glomus mosseae*, *G. aggregatum* and *G. fasciculatum*, *Acaulospora melleae*, *A. rehmanii*, and *Sclerocystis clavispora*, *S. taiwanensis* species. Quantitative distribution of VAMF spores at three different stages of the host showed variations. Average number of spores counted per 100 gms of soil was different in terms of increase from plant to plant, variety to variety and stage to stage

showed in Table(3) and Figure (2.). Their number increased gradually from vegetative stage to fruiting stage. Our findings are in agreement with the [37-38] who investigated that genus *Glomus* is a predominantly distributed genus in the soil all over the world and was most efficient and produced statistically higher mycorrhizal colonization than other mycorrhizal fungi also agree with the report of [39] who stated the influence of AM fungi on growth indices revealed that *G. mosseae* was the more effective fungus in stimulating peanut growth when compared with *G. fasciculatum*.

Figure 2. Gl. (*Glomus* species), Scl. (*Sclerocystis* spp.), Ac. (*Acaulospora* spp) Effect of VAM, VAM+RHZ and VAM+RP inoculation on spores density at three growth stages. Key: (1, for vegetative stage, 2 for flowering stage and 3 for fruiting stage)

Conclusion and recommendations

An experiment was conducted at the Department of Botany, University of Peshawar Pakistan to study the effect of different bio-fertilizer treatments on growth of Pea. Seven treatments were applied including Control, VAM, Rhizobium, Rhizobium+VAM, Rock phosphate, Rock phosphate+VAM and Rock phosphate+VAM+Rhz. Rock phosphorus fertilization alone, application along with VAM and rhizobium inoculation increased the growth parameters significantly. Maximum number of pods plant⁻¹ were

observed where VAM along with rhizobium inoculation were applied. It is concluded that *Pisum sativum* should preferably be grown with dual inoculation of VAM along with rhizobium and rock phosphate. Quantitative distribution of VAMF spores at three different stages of the host showed variations, in terms of increase from plant to plant, variety to variety and stage to stage.

Authors' contributions

Conceived and designed the experiments: T Yaseen & F Hussain, Performed the experiments: T Yaseen, Analyzed the data: T Yaseen, Contributed reagents/ materials/ analysis tools: T Yaseen & M Shakeel,

Wrote the paper: T Yaseen

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