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INTERACTIVE EFFECT OF APPLIED FERTILIZERS AND SYMBIOTIC MICRO-ORGANISMS IN COWPEA CROP.

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Results of this study demonstrated that cowpea yields were higher in mycorrhizal as compared to non-mycorrhizal treatments. Phosphorus application contributed to an increase in the yield, however, increase was greater in mycorrhizal treatments at 0.0, 2.5, and 5.0 mg P Kg^{-1} soil levels and reverse happened at 10.0 and 20.0 mg P treatments. As regards phosphorus concentration in cowpea plants, it was higher in mycorrhizal treatments. Number and weight of nodules was also higher with mycorrhizal inoculation. However, probability of response to applied phosphorus fertilizer was more in non-mycorrhizal treatments.

INTRODUCTION

Crop yields were directly related to content of "minerals" in the manures applied to soil[1]. This statement is more valid today because of introduction of high fertilizer requiring crop varieties and continuous nutrient depletion of soil by intensive cropping. All other factors being constant, plant growth is function of two variables, nutrient intensity and balance, both equally important.

Deficiency of nutrients especially fertilizer elements N, P and K had led to use of organic and inorganic fertilizers. This is an expensive way of maintaining soil fertility and in certain cases may not be economical. At present need exists to investigate a method which may be less costly and practical as well.

Nature has bestowed plants' roots with myriads of soil microorganisms. These relationships are qualitatively and quantitatively dynamics, leading to interactions which may be symbiotic, pathogenic and neutralism. Two recognized beneficial associations are Rhizobia-legumes and mycorrhiza-plant roots. Former association is responsible for nitrogen supply, while latter Symbiosis is involved in Phosphorus and some micronutrients availability. Rhizobia-legume association is of immense global importance to plant succession, soil fertility, agriculture and general biological productivity. A vast, diverse, and greatly expanding literature, attest to significance vested in this plant-microbe interaction[2]. Similarly better growth response associated with improved P supply by mycorrhizal roots is well established[3]. However, very little is known about integrated effect of these microorganisms on plant growth.

Moreover, repressive or otherwise effect of applied fertilizers with respect to these symbiosis had not been fully recognized. Darbyshire[4] observed that 10-micrograms of nitrate or nitrite nitrogen per clover plant had no effect on plant but did delay nodulation by *Rhizobium trifoli.* Similarly most studies including those at Rothemsted had indicated effect of applied P fertilizer on mycorrhizal population to be negative one[5]. In contrast to above Kruckelmann[6] found few spores in plots given no fertilizer and most in plots given N.P.K. plus farmyard manure.

In order to test validity of those research views, a study was conducted with the following objectives:

1. To observe combined effect of Rhizobium and mycorrhizae on cowpea (*Vigna Unguiculata* L.) plant growth,
2. To study effect of applied fertilizers especially P on Rhizobia-mycorrhiza infection,
3. To establish critical levels of fertilizer elements for cowpea crop under prevailing conditions.

MATERIALS AND METHODS

The study was conducted in green-house of Soil Science Department, University of California, Riverside during spring 1982. Oakley, sandy loam soil which was sterilized by autoclaving twice and allowed to air out for one week was used in the experiment. Some of the characteristics of the soil were:

Ec_e	pH	Exchangeable cations				CEC
dsm^{-1}		(meg 100^{-1} g soil)				
		ca	Mg	k	Na	
1.3	7.0	2.93	0.88	1.10	0.35	5.20

Soils were added to plastic pots at rate of 4.25 kg per pot.

Main treatments in experiment were plus (+) Mycorrhiza and minus (–) Mycorrhiza. Mycorrhizal treatments were inoculated with *Glumous fasciculatus* specie by adding 5g of untreated inoculum. Inoculum was added at 5 cm depth and covered with a layer of soil. Other group or pots with minus (–) mycorrhiza were treated with 5g of autoclaved inoculum and 10 ml of filtrate per pot as background. This operation was done on April 10, 1982.

Subtreatment consisted of five levels of P fertilizer application at rate 0.0, 2.5, 5.0, 10.0 and 20.0 mg Kg^{-1} soil, as $Ca(H_2PO_4)_2$ corresponding to 0.0, 5.0, 10.0, 20.0 and 40.0 Kg P per hectare, Nitrogen at 20 mg N per Kilogram soil (40 kg N $hectare^{-1}$) as NH_4NO_3 and K at rate 150 mg K per Kilogram soil (300 Kg $hectare^{-1}$) as K_2SO_4 were also added as basal dose. Fertilizer additions were made on April 8, 1982. In all there were four replications and pots were placed on wooden benches according to completely Randomized Block design.

Two surface sterilized, Rhizobia inoculated cowpea seeds were planted on April 10, 1982, just above mycorrhizal inoculum layer and covered with 2 cms soil. Observations regarding germination were made and after plants were fully established, one plant was rogued out on April 17, 1982. Criteria for removal of plant was that remaining plants should be of equal height.

Irrigation was signalled by tensiometer reading of –50 centibars. Over-watering was avoided to provide aerated conditions for better flourishment of microorganisms, especially Rhizobium.

Eight weeks old plants were harvested and fresh weight recorded. Plants were thoroughly washed with demineralized water, dried at 60°C for 24 hours and dry weight taken. Plant roots were extracted by pouring soil and washing Rhizobial nodule with water. Nodules number and weight were recorded.

Plant leaves were ground in Wiley grinding mill and analyzed for nitrogen and phosphorus. Nitrogen was estimated by Kjeldahl digestion method titurating distillate with 0.01 N H_2SO_4. Phosphorus was analyzed colorimeterically by using Bausch-Lomb Colorimeter. Statistical computations were made according to Steel and Torrie Statistical methods[7].

RESULTS AND DISCUSSION

★The fresh weight of control (no fertilizer) (+) mycorrhiza was 7.75 per cent greater as compared to (–) mycorrhiza treaments.With phosphorus application growth increased in both treatments (+ and – mycorrhiza). However, increase was higher in treatment with mycorrhizal inoculation (fresh weight data plotted in figure I), With the exception of the highest P level of 20 mg Kg^{-1}, in which non-mycorrhizal indicated a slight increase of 0.9 g as compared to its ounterpartner mycorrhizal treatment. Reason for this decrease may be repressive effect of high P on mycorrhizal infection and ultimately on root and plant development. Mosse[3] indicated that increased level of phosphate in soil resulted in decrease of mycorrhizal infection.

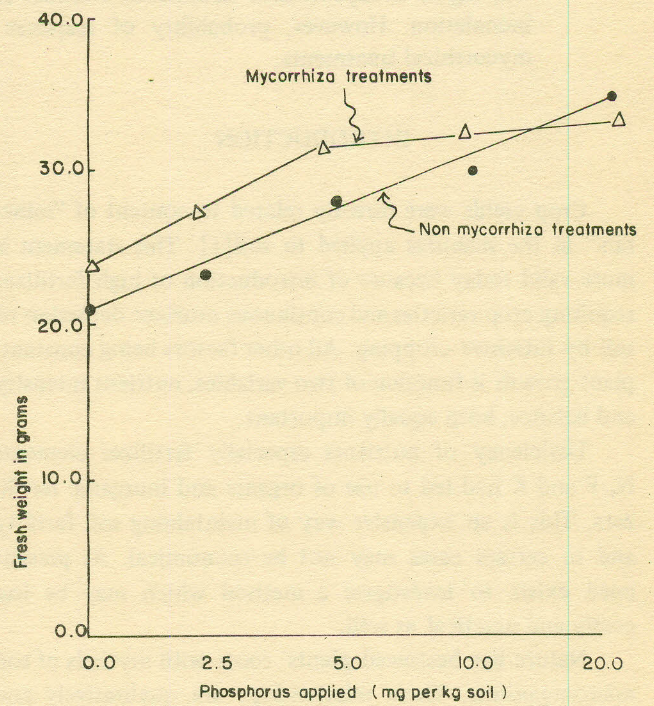

Fig. 1. Fresh weight yield of cowpea as affected by micorrhiza inoculation.

Dry matter yield data (Table 1) indicated that at lower P levels (0.0, 2.5, and 5.0 mg Kg^{-1} soil) mycorrhiza inoculation resulted in higher yield, while reverse happened at higher levels. This revealed that if no P fertilizer was applied, then mycorrhizal infection may be helpful in obtaining better growth. These findings were consistent with those of Sanders and Tinkers[8]. They obtained 2.22 and 13.05 g onion yield in control (no fertilizer) uninfected and infected mycorrhizal plants, respectively.

At present beneficial effect of mycorrhiza on plant growth is well recognized. Ectomycorrhiza are known to host on Pinaceae, Fagaceae, and Betalaceae tree species. Endomycorrhiza consisting of genus Glomus, Endogone, Aeaulospora and Sclerocystis are capable of infectig a wide

★ All data discussed is average of four replications.

Table -1. Dry matter yield of cowpea crop as affected by applied phosphorus and mycorrhiaal inoculation (Weight in grams)

Treatment (P – Fertilizer Applied)	(+) Mycorrhiza	(–) Mycorrhiza
0.0 mg P kg^{-1} soil	2.90 ab*	2.25 a
2.5 mg P kg^{-1} soil	4.0 ab	3.40 ab
5.0 mg P kg^{-1} soil	5.35 c	4.83 bc
10.0 mg P kg¯1 soil	5.24 c	5.43 bc
20.0 mg P kg^{-1} soil	6.12 c	6.28 c

***The treatments with same letter were non-significant at 5% level of significance by Duncan's Multiple Range Test.**

varieties of crop plants with the exception of Cruciferaceae, Chenopodaceae, Fumeraceae, and Polygonaceae plant families. An interesting feature of *Glomus fasciculatus* Mycorrhiza under study in this experiment was that it had also been observed in certain citrus tree species in Southern California in additon to normal crop plants.

The question arises that why yield in mycorrhizal treatments at same level of P (0, 2.5, 5.0 mg) was higher than non-mycorrhizal treatments. The phosphorus concentration in cowpea plant (Table 2) at these levels was 0.75, 1.64, 1.67 and 0.68, 1.08, 1.30 mg g^{-1} in case of (+) mycorrhiza and (–) mycorrhiza treatments, respectively. This increased P concentration in plant was probably one of the reasons for increased yield in (+) mycorrhiza treatments. Mosse[3] had also observed increase in P percent with resultant yield increase. Sanders and Tin Kers[9] measured phosphorus in flow into infected and uninfected onions and found them to differ by a factor of four. A secondary reason for growth increase may be beneficial effect of mycorrhiza on Fe, Zn, and K uptake. At 10 and 20 mg P levels, P uptake in (+) and (–) mycorrhiza was non-significant, indicating P uptake was independent of mycorrhiza at this stage. One reason for this behavior may be attributed to ineffectiveness of mycorrhiza at these levels. These findings are similar to Hayman[5], who at Greatfield IV Rothemsted observed that vesicular arbuscular infection was dominant at low to intermediate than at high P levels.

Another reason may be that plants absorb P from labile pool (applied fertilizer) rather than slow mycorrhizal source, which involves solubilization/mineralization of P before it is taken up.

Vigorous plant growth is attributed to increased P availability, but mechanism is not well documented. Increased availability may be due to increased root growth with more absorbing area, increase in active period of uptake, increase in maximum uptake per unit surface, shortening of transport path from soil to root, ability to utilize other forms of P not easily available and storage of PO_4^{-3} in sheath were possible known mechanisms, may be combined effect of these, responsible for increased P concentration in cowpea plants.

As regards N concentration in cowpea (Table 2) it was higher in non-mycorrhizal treatments except in 10 mg P treatments. Interactions between Rhizobia and mycorrhiza are still poorly understood, yet they are found to exist in nature. In this experiment one possible explanation for decreased N concentration may be competitive (competition for nutrients, energy source and space) interaction between two organisms. Other reasons for this difference

Table-2. Nitrogen and phosphorus concentration of cowpea plant

Treatments	(+) Mycorrhiza		(-) Mycorrhiza	
(P-Fertilizer Applied)	Nirtogen %	Phosphorus %	Nitrogen %	Phosphorus %
0.0 mg P kg^{-1} soil	1.72 a*	0.75 c	2.09 a	0.68 c
2.5 mg P kg^{-1} soil	1.74 a	1.64 cd	1.77 a	1.08 c
5.0 mg P kg^{-1} soil	1.87 a	1.67 cd	1.88 a	1.30 c
10.0 mg P kg 1 soil	2.45 an	2.15 cd	2.03 a	2.18 cd
20.0 mg P kg^{-1} soil	2.87 b	3.25 d	3.01 b	3.10 d

***The treatments with the same letter were non-significant at 5% level of significance by Duncan's Multiple Range Test. The letter a and b stands for nitrogen, while ca and d allocated to phosphorus.**

may be mechanism of N uptake. Corrodus[10], found that mycorrhiza readily absorb NH_4 but not NO_3.

Number and weight of nodules (Table 3) indicated that (+) mycorrhiza treatments should be more effective in N fixation, anomaly can be ascribed to the fact that some of the nodules were ineffective in nitrogen fixation. However, Safir and Boyer[11] demonstrated that soybean-rhizobia system could be improved by its association with mycorrhiza.

ratio.

One interesting feature in study was effect of mycorrhizal inoculation on probability response curve. In mycorrhizal treatments probability values were 0.77, 0.56, 0.42 as compared to 1.0, 0.68, 0.47 in non-mycorrhizal treatments at 0.0, 2.5 and 5.0 mg P levels. This indicated that there is less likelihood of response in mycorrhizal treatments to applied P fertilizer, which demonstrated beneficial role of mycorrhiza in P nutrition of plants. The

Table-3. Nodulation (numbers and weights) in cowpea crop

Treatments	(+) Mycorrhiza		(–) Mycorrhiza	
(P-Fertilizer Applied)	Number of nodules	Weight of nodules (gram)	Number of nodules	Weight of nodules (gram)
0.0 mg P kg^{-1} soil	23	0.17	11	0.10
2.5 mg P kg^{-1} soil	21	0.78	21	0.60
5.0 mg P kg^{-1} soil	42	1.78	31	1.40
10.0 mg P kg^{-1} soil	50	2.02	43	1.40
20.0 mg P kg^{-1} soil	33	2.48	25	2.50

In this experiment probability of response is indicated in Figure II. Probability is higher at lowest P levels and decreased with phosphorus additions, being 0.36 at 20 mgms (–) mycorrhizal treatment. It meant that there is still 36 per cent probability of response and fertilizer application decision lie with the farmer which he has to make keeping in view fertilizer cost and cowpea yield benefit

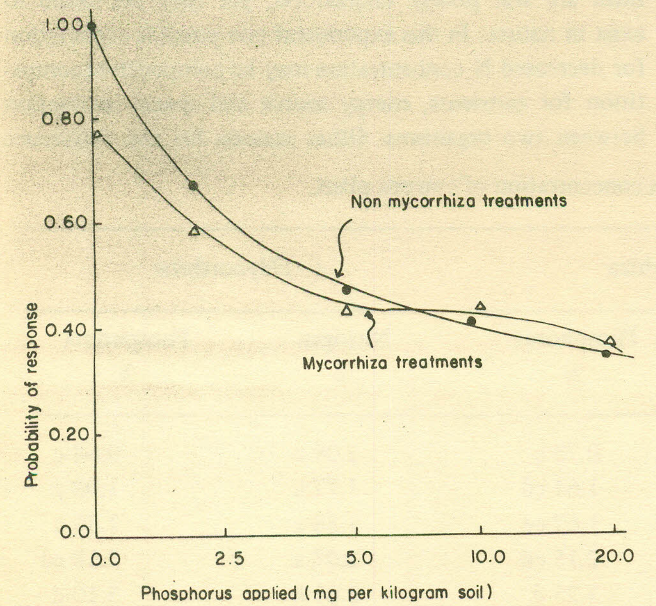

Fig. 2. Probability of response in relation to phosphorus fertilization and micorrhizal inoculation.

study led to the following conclusions:

Crop yields were higher in control (+) mycorrhiza as cmpared to (–) mycorrhiza. The phosphorus concentration in cowpea plants was almost higher in mycorrhizal treatments.

Finally it is to be added that Rhizobia-legume and mycorrhiza-plant root symbiosis are of vital importance to agriculture. Due to their great significance in crop production, more effective investigations, in this direction should be conducted.

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