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Growth response of groundnut to VAM fungus and *Rhizobium* inoculation

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ABSTRACT

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Influence of VAM fungus and *Rhizobium* inoculation on groundnut growth response in relation to growth, nodulation, phosphorus content and phosphatase activity was studied in pot culture studies. The treatments consisted of inoculation with VAM fungus (*Glomus mosseae*) only, *Rhizobium* only, dual inoculation with VAM fungus and *Rhizobium* and control under sterilized soil conditions. Seeds of groundnut were seeded in 6" or 9" pots containing different inoculua, respectively. Concentration of VAM used as inoculum was 250 spores per pot, whereas *Rhizobium* was 10^6 Cells. Data were counted at the 15, 25, and 45th day after inoculation. Dual inoculation resulted in significant increase in growth, number of nodules, mycorrhizal colonization than inoculation with VAM fungus*Rhizobium* alone. The amount of phosphorus and activity of acid and alkaline phosphatases increased significantly with dual inoculation than with individual inoculations. The significance of the results is discussed.

Key words : *Arachis hypogaea*, *Rhizobium*, Vesicular-arbuscular mycorrhiza, plant growth response.

INTRODUCTION

Vesicular Arbuscular Mycorrhizal (VAM) fungi form a symbiotic relationship with the host by colonizing the roots. Associative effects of VAM with rhizobia have been well documented [(20)]. Legumes are generally nodulated by rhizobia but many legumes grow poorly and failed to nodulate even with the rhizobial inoculation in autoclaved soil unless they were inoculated with mycorrhiza also [(4)]. The tripartite symbiosis between leguminous plants, *Rhizobium* species and VAM fungi has been the subject of intensive research in recent years [(17,18)]. A synergistic beneficial effect of dual inoculation with VAM fungi and *Rhizobium* in growth and nutrition in legumes has been demonstrated by many workers [(5,6,7,10,14,18)]. Generally VAM fungi are known to improve phosphate nutrition, which in turn enhances plant growth and N2 fixation. Leopald and Hofner [(13)] reported the combined inoculation of clovers with *Rhizobium* strains and Tunsia isolate of *Glomus etunicatum* and application of rock phosphate gave greatest shoot dry matter, yield increase and has variable effects on numbers of large nodules formed.

However, few attempts have been made to estimate the effect of *Rhizobium* on the development of VAM fungi [(8)] and conversely that of VAM fungi on nodulation [(9)] and in turn effect of this combined inoculation on phosphorus nutrition. Hence the present study was undertaken to investigate the effect of individual and dual inoculations on plant growth, nodulation, VAM colonization and phosphate metabolism in groundnut, on which no such attempts were made, though being an economically important oil yielding crop plant.

MATERIALS AND METHODS

Rhizobium inoculum preparation

The *Rhizobium* species used in the present study was isolated from groundnut root nodules and maintained on Yeast Extract Mannitol Agar, as described by Ahmad *et al*. [(1)].

VAM fungus inoculum preparation

Sand : soil (1 : 1) mixture containing spores and infected root segments of maize, infected with *Glomus mosseae* (*Nicolson and Gerdeman*) and grown for 90 days, served as the mycorrhizal inoculum. 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 (10^6 cells).

Experiments were conducted in red loamy soil with a pH of 7.6 in 9" or 6" earthenware pots. Bold and sound seeds of groundnut (*Arachis hypogaea* L.). Var TMV2 were employed throughout the study. Four treatments involved in the study under sterilized soil conditions are as follows:1.Uninoculated control (T1) ; 2.Inoculation with VAM fungus alone (T2); 3.Inoculation with *Rhizobium* alone (T3); 4.Dual inoculation

with both VAM fungus and *Rhizobium* (T4)

The plants were grown for 45 days with average day and night temperatures of 37℃ and 33℃, respectively. The plant roots were periodically examined for the progress of infection in about 9 plants at each sampling period. Samples were collected at random from three pots and the percentage of mycorrhizal infection of the plant roots was estimated according to the method of Philips and Hayman [16].

Data on nodule number and fresh weights of the plants was recorded at 15, 25, 45 days after inoculation. The total phosphorus content in plant roots and shoots was estimated by Vanado- molybdate phosphoric yellow colour method [12]. The soluble acid and alkaline phosphatase activities were determined according to the method of Gianinazzi-pearson and Gianinazzi [11], with some modifications.

RESULTS

VAM colonization

Significant increase in the percent of root colonization, from 15 days through 45 days, was observed in the groundnut plants inoculated with VAM fungus and *Rhizobium* together, as compared to VAM fungus alone. In VAM fungus inoculated plants the per cent of colonization by VAM fungus was 70% at 45 days which increased to 81% by dual inoculation and at 25 days the per cent of colonization in VAM fungus inoculated plants was 49.97% which was increased to 68.87% by dual inoculation (Fig.1). The number of arbuscules, vesicles and spores increased significantly by dual inoculation with VAM fungus and *Rhizobium* (Figs.2&3). At 45 days a large number of spores of VAM fungus were detected in soils inoculated with VAM fungus and *Rhizobium* together, as compared to VAM fungus alone (Fig.4).

Nodulation

The uninoculated (control) and VAM fungus alone inoculated nodulated plants, grown in sterilized soils did not form any nodules. The number of nodules produced by *Rhizobium* alone inoculation was 21, which was increased to 33 by dual inoculation.

Plant Growth

Dual inoculation with VAM fungus and *Rhizobium* improved the growth response compared to single inoculations or the uninoculated control. The fresh and dry weights of the non-nodulating plants (uninoculated and VAM fungus inoculated) were smaller than those of nodulating plants (VAM+*Rhizobium* and *Rhizobium* inoculated). Dual inoculation with VAM fungus and *Rhizobium* greatly increased the fresh and dry weights of root and shoot systems. Total fresh and dry weight at 45 days was greater in dual inoculated plants. Plants inoculated with VAM fungus and *Rhizobium* grew significantly taller as compared to the VAM fungus alone or *Rhizobium* alone inoculated plants (Table 1 and 2)

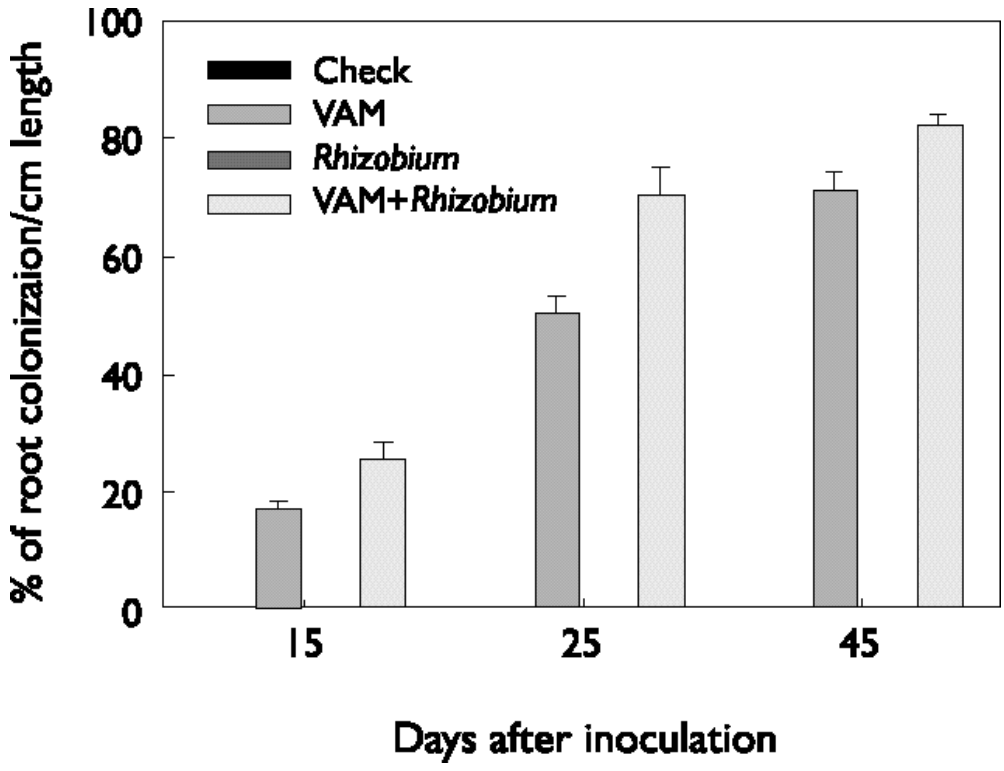

Fig. 1. Effect of VAM fungus and *Rhizobium* on root colonization of groundnut by VAM at different stages of growth.

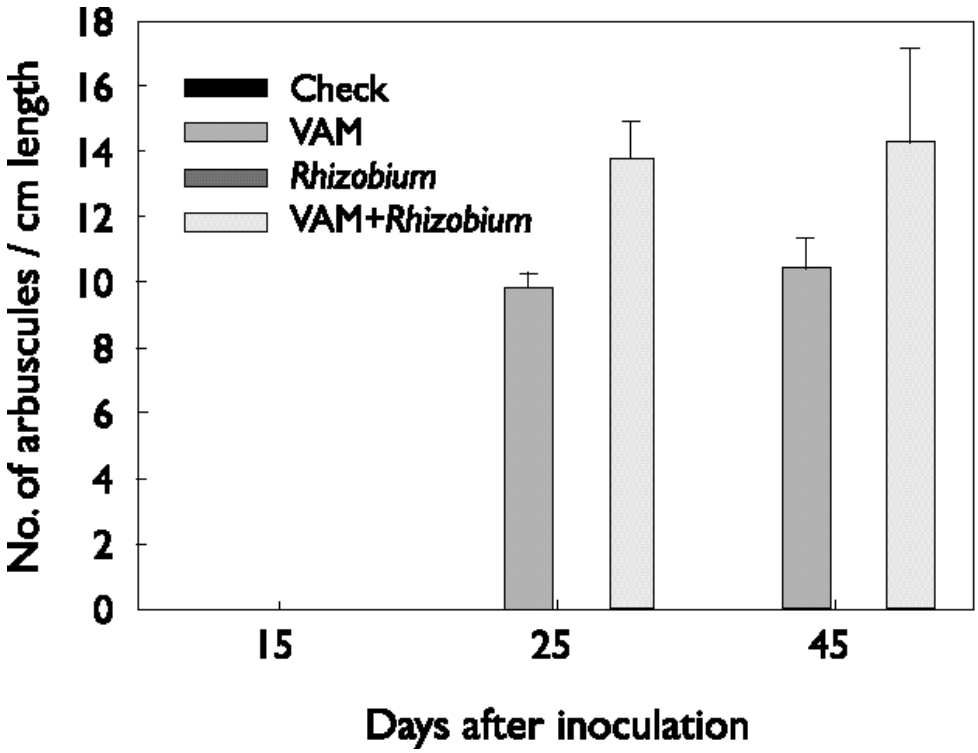

Fig. 2. Effect of VAM fungus and *Rhizobium* on formation of arbuscules in groundnut roots at different stages of growth.

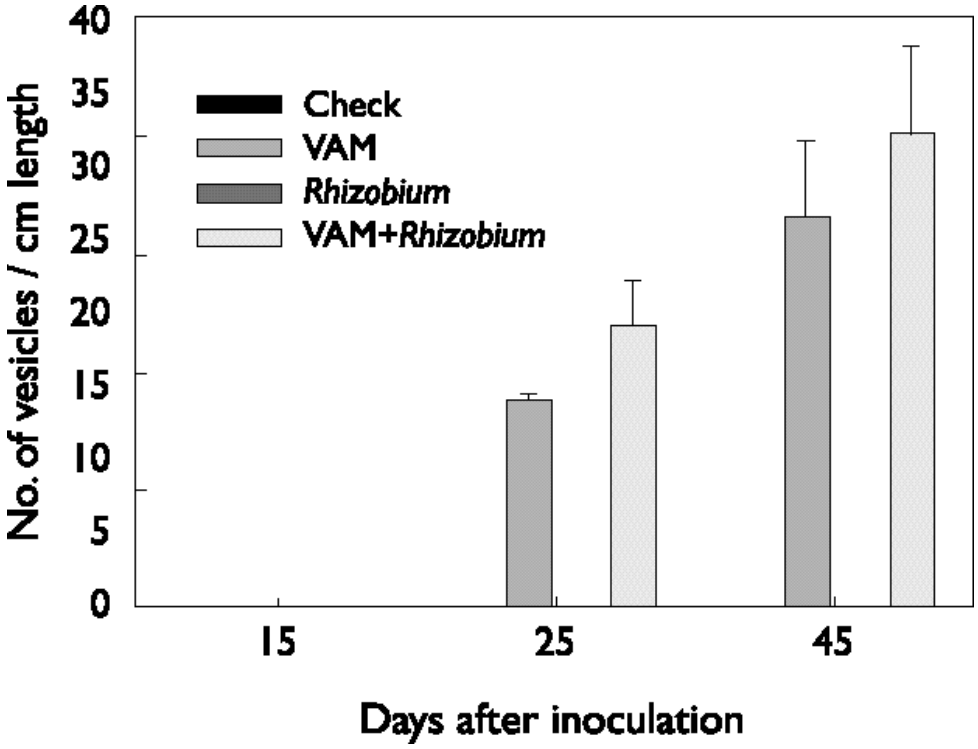

Fig. 3. Effect of VAM fungus and *Rhizobium* on formation of vesicles in groundnut roots at different stages of growth.

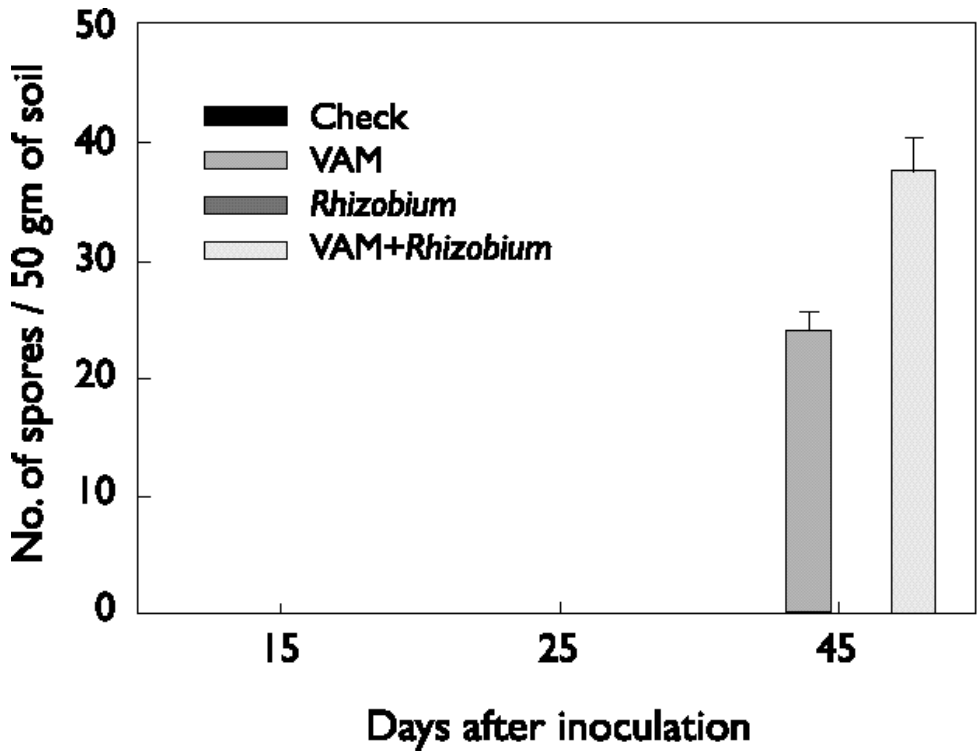

Fig. 4. Effect of VAM fungus and *Rhizobium* on spore production in pot soil at different stages of growth.

Table 1. Effect of VAM fungus and *Rhizobium* on fresh and dry weight (mg) of groundnut at different stages of growth

Treatment	Weight of root (mg)			Weight of shoot (mg)		
	15 days [1]	25 days	45 days	15 days	25 days	45 days
CK	631.33 ± 2.404 [2] (64.67 ± 2.906)[3]	811.00 ± 9.019 (262.67 ± 6.334)	1243.33 ± 7.513 (503.67 ± 7.881)	1446.33 ± 3.48 (253.33 ± 3.528)	2574.33 ± 14.530 (462.67 ± 6.065)	4513.00 ± 3.786 (1161.33 ± 8.838)
VAM	774.0 ± 5.292 (83.33 ± 2.028)	909.67 ± 11.837 (294.67 ± 3.283)	1771.66 ± 6.489 (670.66 ± 7.881)	2234.00 ± 8.281 (296.00 ± 2.309)	3054.00 ± 18.028 (600.00 ± 2.309)	4866.67 ± 37.419 (1255.33 ± 7.513)
Rhizobium	746.67 ± 3.528 (85.33 ± 2.028)	971.67 ± 8.007 (312.00 ± 2.08)	1885.33 ± 5.824 (712.67 ± 4.364)	2289.66 ± 6.692 (298.00 ± 4.164)	3093.67 ± 12.72 (641.00 ± 6.659)	5233.67 ± 3.18 (1464.33 ± 8.991)
VAM + *Rhizobium*	784.00 ± 3.464 (89.33 ± 2.404)	1174.66 ± 8.667 (387.33 ± 1.732)	2342.00 ± 9.292 (802.66 ± 5.365)	2349.33 ± 5.207 (337.67 ± 3.180)	3284.00 ± 8.327 (672.67 ± 10.975)	6233.00 ± 6.657 (1498.67 ± 6.888)

[1.] 15, 25 and 45 days after inoculation.
[2.] ± indicates Standard Error (S.E.).
[3.] Figures in Parenthesis indicate the dry weights

Table 2. Effect of VAM fungus and *Rhizobium* on length (cm) of groundnut plant at different stages of growth

Treatment	Length of root (cm)			Length of shoot (cm)		
	15 days [1]	25 days	45 days	15 days	25 days	45 days
CK	9.17 ± 0.763	14.90 ± 0.17	18.77 ± 0.2	12.00 ± 0.86	27.36 ± 0.78	35.63 ± 0.91
VAM	13.06 ± 0.6	15.93 ± 0.2	24.50 ± 0.40	18.17 ± 0.77	33.80 ± 1.35	42.8 ± 0.72
Rhizobium	13.76 ± 0.25	16.4 ± 0.2	26.47 ± 0.96	18.26 ± 0.75	36.90 ± 0.95	45.47 ± 0.15
VAM+*Rhizobium*	13.93 ± 0.3	16.80 ± 0.2	28.90 ± 1.1	19.67 ± 0.47	39.37 ± 0.32	49.50 ± 0.28

[1.] 15, 25 and 45 days after inoculation.
[2.] ± indicates Standard Error (S.E.).

P-Content

Total quantity of phosphorus in plants increased by dual inoculation with VAM fungus and *Rhizobium* and also with VAM fungus alone. The plants with dual inoculation accumulated a larger amount of phosphorus in both root and shoot systems. This increase was more pronounced by dual inoculation at 45 days (Figs.5&6).

Phosphatases

The activity of alkaline phosphatase was maximum in plants inoculated with VAM fungus and *Rhizobium* together or VAM fungus alone and minimum in the Rhizobium alone or uninoculated control plant roots at 25 days as compared to 15 and 45 days. Slight increase in the acid phosphatase activity was also observed in VAM fungus alone or VAM fungus and *Rhizobium* inoculated plant roots as compared to uninoculated ones (Figs.7&8).

Dual inoculation of VAM fungus with *Rhizobium* had a significant positive effect on most of the growth responses. The coinoculation significantly increased the number of nodules, VAM fungus colonization, plant biomass, length, phosphorus content and phosphatase activities. This response was better due to dual inoculation than single inoculation with either VAM fungus or *Rhizobium* alone.

DISCUSSION

The results indicate that dual inoculation of groundnut with *Rhizobium* and VAM fungus results in significant increase in the mycorrhizal colonization, number of nodules, growth i.e., fresh and dry weight, length of the root and shoot, as compared to single inoculation or the uninoculated control. This is in agreement with the reports of Thiagarajan and Ahmad [19] in Cowpea (*Vigna unguiculata*. L.). The increase in plant growth may be attributed to increased uptake of phosphorus by VAM fungus, which increase the activity of root nodule bacteria as also reported by Mytton and Livesey [15]. It is evident from our study that the maximum number of arbuscules, vesicles and spores in dual inoculated plants may be due to the fact that both VAM fungus and *Rhizobium* are active in root cortical cells. That means the presence of one symbiont may be influencing the activity of the other as also suggested by Vejsadova *et al*. [21] in Soybean.

Mycorrhizal and dual inoculated plants recorded higher concentrations of phosphorus in their root and shoot systems. The increase in the phosphorus content with dual inoculation may be due to VAM hyphae network which is substantiated by the observed increase in P content of plants inoculated with VAM fungus and *Rhizobium* compared to plants inoculated with single organism suggests the possible synergistic beneficial effects on growth and nutrition of groundnut plants. Similar observations were recorded by Ames *et al*. [2]; Thiagarajan *et al*. [18] in different plant species. Major part of the beneficial effects of VAM fungus is

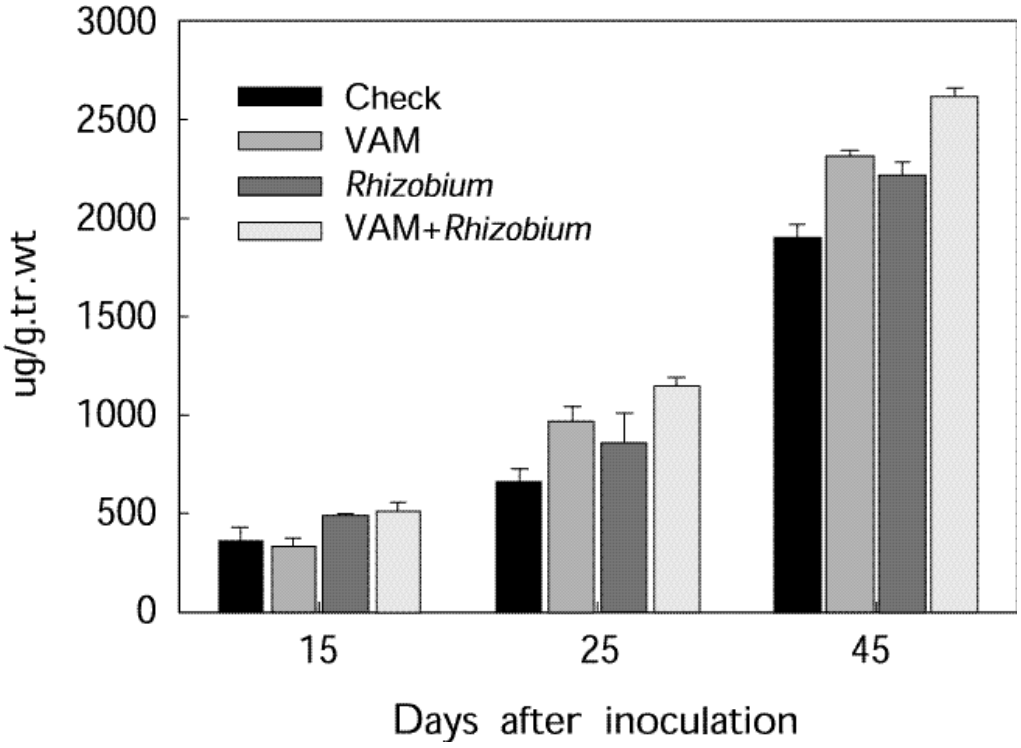

Fig. 5. Effect of VAM fungus and *Rhizobium* on total phosphorus of roots of groundnut at different stages of growth.

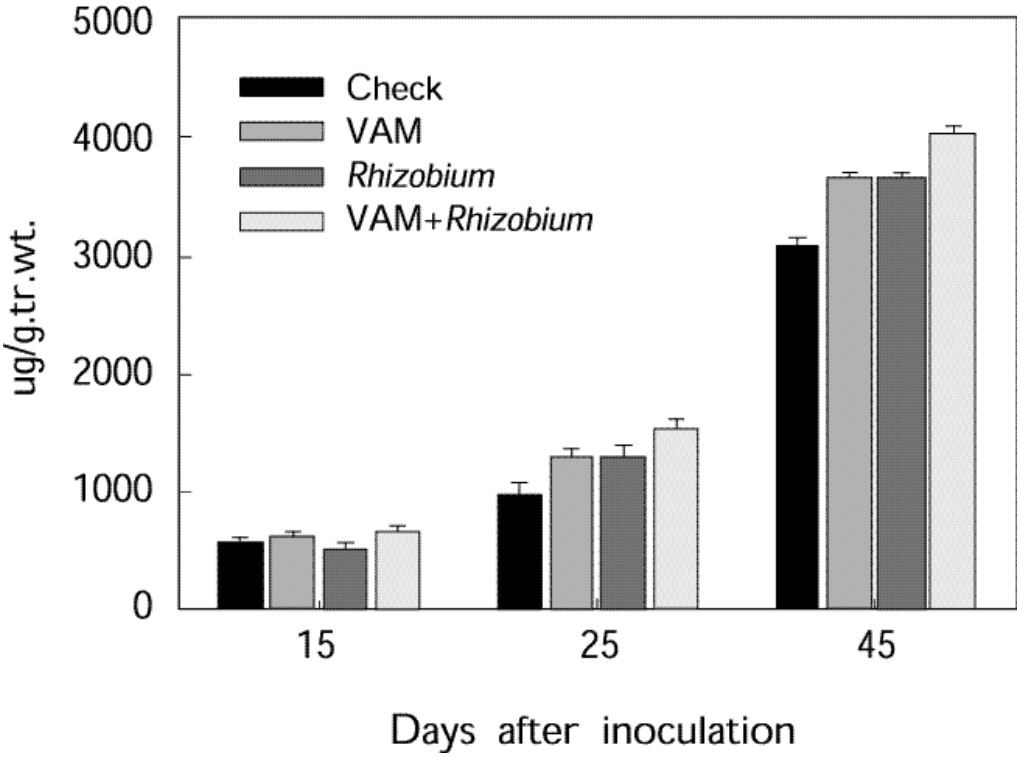

Fig. 6. Effect of VAM fungus and *Rhizobium* on total phosphorus of shoot of groundnut at different stages of growth.

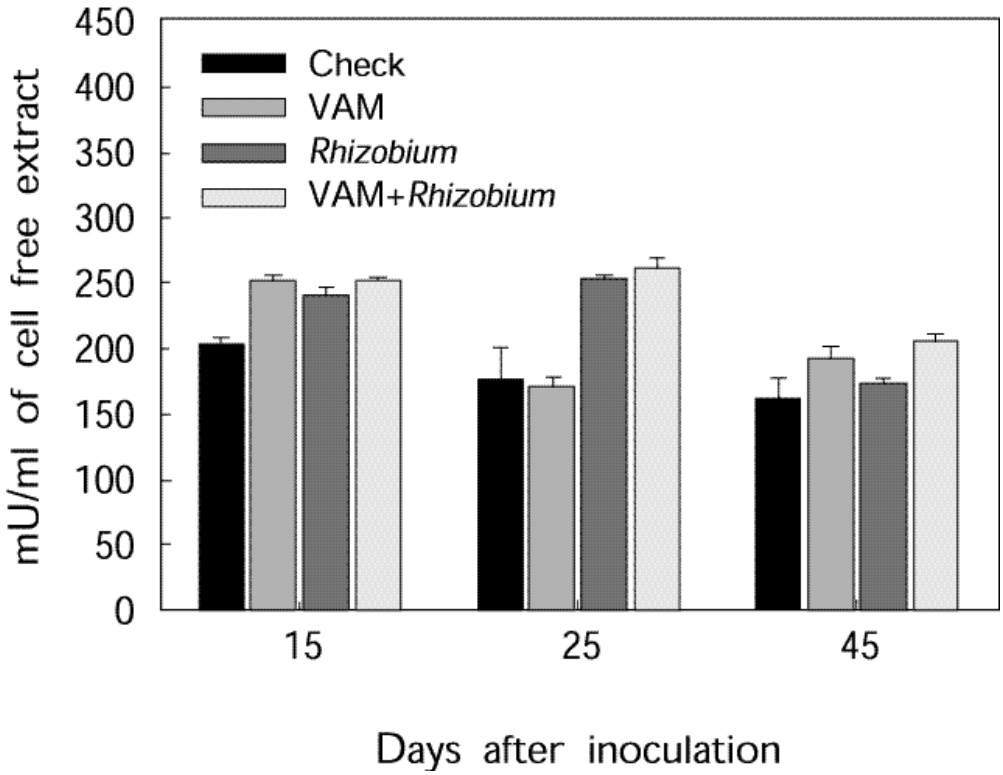

Fig. 7. Effect of VAM fungus and *Rhizobium* on acid phosphatase activity of roots of groundnut at different stages of growth.

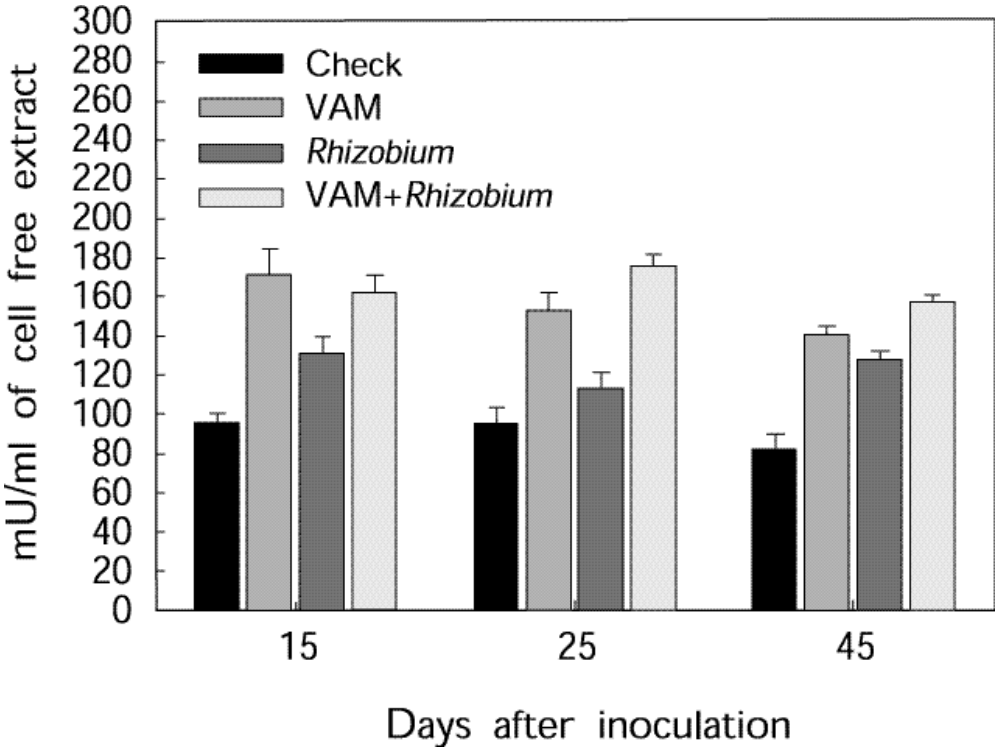

Fig. 8. Effect of VAM fungus and *Rhizobium* on alkaline phosphatase activity of roots of groundnut at different stages of growth.

attributed to its role in phosphorus uptake and translocation through the involvement of phosphatases in the transport of phosphorus. This conclusion could be derived from the reports that mycorrhizal onion roots have recorded greater specific alkaline phosphatase activity than uninoculated controls [(11)] and mycorrhizal Trigonella roots have recorded greater acid and alkaline phosphatase activities than in non-mycorrhizal roots [(3)].

It is evident from the results that dual inoculation of *Rhizobium* and VAM fungus may enable to improve the phosphorus nutrition, VAM colonization and nodulation in peanut and hence the improved plant growth. It is quite likely that inoculation with both VAM fungus and *Rhizobium* (dual) instead of individual inoculations could be beneficial to better growth and yield of the plant. There is need to have an idea of the physiology of the VAM fungus infected plants for understanding probable reasons for improved vigour and growth of the plant which ultimately lead to better yield. Such studies are underway. In the tropics most farmers try to grow legumes without much capital input in their farming system, by using dual inoculation, to achieve a better yield than single inoculation [(10)].

In conclusion the results of this study indicate that the dual inoculation with VAM fungus and *Rhizobium* is beneficial to groundnut for its better growth and development with increased P uptake, nodulation 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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摘　要

Devi, M. C [1,2]., and Reddy, M. N [1]. 2001. 內生菌根菌 (VAM) 與根瘤菌對落花生生育的影響. 植病會刊 10:71-78. ([1.] 印度 Department of Microbiology, Sri Padmavati Mahila Visvavidyalayam, Tirupati- 517 502; [2.] 聯絡作者，電子郵件:mopuri_nr@yahoo.com；傳真:08574-50417)

落花生接種內生菌根菌 (Vascular Arbuscular Mycorrhizal Fungus, VAM) 與根瘤細菌 (*Rhizobium*) 後對植物的生長、根瘤型成、磷含量及磷活性的影響研究以盆栽試驗測定之。處理包括單獨接種內生菌根菌 (*Glomus mosseae*) (每盆約 250 spores)、根瘤細菌 (*Rhizobium* sp.) (每盆約106 cells)、同時接種內生菌根菌與根瘤細菌、及均無接種者。所有實驗均在滅菌土壤 (pH7.6) 中進行，花生種子播種於含有接種源的六吋或九吋瓦缽中。實驗結果顯示，同時接種內生菌根菌與根瘤細菌的處理，其植株的生長、根瘤數目、內生菌根菌纏據情形，均比單獨接種的處理顯著為佳。而且，內生菌根菌＋根瘤菌處理的植株體內的磷含量，以及酸性與鹼性磷酸酵素 (acid, alkaline phosphatases) 的活性亦增加，且顯著較單獨接種者與對照處理為高。本文同時討論試驗結果的顯著性。

關鍵詞：落花生、根瘤細菌、內生菌根菌、植物生長反應