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Effects of Arbuscular Mycorrhization in Sterile and Non-sterile Soils

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Abstrak: Mycorrhiza, satu perhubungan mutualistik antara fungi dan tumbuhan peringkat tinggi, telah didokumentasikan secara terperinci, tetapi fakta-fakta tentang perkembangan fungi *arbuscular mycorrhizal* dan kesan-kesannya terhadap pertumbuhan kacang tanah (*Arachis hypogea* L.) kurang diketahui. Maka, status *mycorrhizal Glomus spp.* telah dikaji di dalam pelbagai keadaan/jenis tanah: tanah yang tidak diautoklav, tanah yang diautoklav dan tanah yang diautoklav bersama mikrobiota. Keputusan menunjukkan bahawa kedua-dua *arbuscular mycorrhizal* (AM) *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and *Glomus. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske menjangkiti kacang tanah, tetapi telah memberi impak yang berbeza terhadap pertumbuhan kacang tanah, bergantung kepada kandungan biomas mikrob tanah. *G. mosseae* didapati yang paling efektif dalam memperbaiki pertumbuhan kacang tanah.

Kata kunci: *Arachis hypogea,* Mikrobiota Tanah, Kolonisasi Mycorrhiza, Fungi AM

Abstract: Mycorrhiza, a mutualistic association between fungi and higher plants, has been documented extensively, but much less is known about the development of arbuscular mycorrhizal (AM) fungi and their effects on the growth of peanuts (*Arachis hypogea* L.). Therefore, the mycorrhizal status of *Glomus spp.* was investigated in the following diverse substrate soil conditions: non-autoclaved soil, autoclaved soil and autoclaved soil plus soil microbiota. The results indicated that both the arbuscular mycorrhizae, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske were infective to peanut, but displayed a differential impact on peanut growth depending on the microbial biomass content of the substrate soils. *G. mosseae* proved to be the most effective at improving peanut growth.

Keywords: *Arachis hypogea,* Soil Microbiota, Mycorrhizal Colonisation, AM Fungi

INTRODUCTION

The relationship between the level of mycorrhizal colonisation and the chemical and physical characteristics of the soil are known to be quite variable (Newman *et al*. 1981; Motosugi *et al*. 2002). Mycorrhizal colonisation has been reported to fluctuate with soil pH (Read *et al.* 1976) and soil phosphorus content (Jeffries *et al.* 1988). Some species of arbuscular mycorrhizal (AM) fungi are adapted to acid or alkaline soils, while others occur in both soil conditions (Porter *et al.* 1987).

Although little is known about the interrelationships between AM fungi and ubiquitous soil-inhabiting microorganisms, some studies have reported that soil microbiota enhance the germination of AM fungal spores (Azcon-Aguilar *et*

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al. 1986), the level of root colonisation by AM fungi (Azcon-Aguilar & Barea 1985) and mycorrhizal plant growth (Meyer & Linderman 1986). However, there is contradictory evidence that the soil microbiota suppresses plant growth (Hetrick *et al.* 1987), mycorrhizal root colonisation (Hetrick *et al.* 1986), sporulation and mycorrhizal fungal spore germination (Ross 1980; Wilson *et al*. 1988).

In a greenhouse experiment, Middleton *et al.* (1989) studied the effects of soil sterilisation (through gamma radiation and aerated steam) and inoculation with AM fungi on the mycorrhizal colonisation, nutrition and growth of peanut plants (*Arachis hypogea* cv. Virginia Bunch). They found that a range of soil sterilisation methods influenced mycorrhizal colonisation of peanut roots. Plant dry weight and the number and weight of reproductive structures were reduced to varying extents, depending on how the respective sterilisation methods affected subsequent levels of mycorrhizal colonisation. Significantly, these reductions in growth could be overcome by inoculating the sterilised soil with AM fungal spores. Peanuts benefit from mycorrhizal association, increasing dry matter yield, phosphorus (P) uptake and stimulation of root and shoot growth as a result (Rao *et al*. 1990; Bergero *et al*. 2003).

This study assessed the mycorrhization, growth-enhancing performance and interaction of two AM fungi, *Glomus mosseae* and *Glomus fasciculatum,* with and without soil microbiota (*A. hypogea* L. var. hypogea cv. Florunner) in sterile and non-sterille soil. Sudangrass (*Sorghum sudanense*) was used for inoculum production. An important consideration for the selection of this plant as a trapping host is that it is a member of the family Graminae, which is known to have no common pathogenic root fungus with legumes and other dicotyledonous crops (Sieverding 1991). Also, its extensive root system results in greater mycorrhiza formation.

MATERIALS AND METHODS

Substrate Soil

The substrate soil was collected from 10–15 cm of the top soil of an undisturbed sand dune community near Riyadh (24° 20' North, Latitude; 46° 20' East, Longitude), Saudi Arabia. The sand dunes were fragmentally dominated by *Moricandia sinaica* (Boiss.) Boiss., *Calotropis proceara* (Ait.) Ait., *Datura innoxia* Mill*., Ricinus communis* L*., Rhazya stricta* Decne. and *Bassia* sp*.* The soil was sieved through a 2 mm mesh screen to remove debris and gravel. Four parts of the sieved soil were mixed with one part (w/v) of peat moss (Rose Garden Torf), and sterilised with solar pasteurisation, in which wet potting peat moss was covered by polyethylene sheeting and exposed to direct sunlight. The experiments were designed to compare three treatments of substrate soils, as follows.

Non-autoclaved soil substrate
The soil was not subjected to any further sterilisation or fumigation. The presence of indigenous AM fungi (not identified) was detected by pot culture using Sudangrass as the trapping host plant. The AM fungi incidence ranged from 16%–34% as assessed following the method of Phillips and Hayman (1970).

Autoclaved soil substrate
The soil was autoclaved for 1 h at 121°C under 105 Pa of pressure followed by a second 1 h autoclaving 24 h later.

Autoclaved soil substrate plus soil microbiota
The autoclaved soil was supplemented with original soil microbiota by mixing with 25 g of non-autoclaved soil in about 100 ml distilled water. The soil suspension was sieved through a series of screens, the finest with openings of 37 µm, fine enough to remove indigenous mycorrhizal spores but coarse enough to allow other soil microorganisms to pass through. The filtrate was further filtered using a Buchner funnel apparatus. The filtered suspension contained colony-forming units (CFU)/ml of 900 fungi and 5.5 X 10^5 CFU/ml bacteria, as estimated by dilution-plating of sieved suspensions onto either peptone yeast extract agar or potato dextrose agar (PDA). The PDA medium was supplemented with 100 µg/ml each of streptomycin sulphate and chloramphenicol. Colonies were counted after 7 days at 24°C.

Experimental Design
Samples of substrate soil received one of the following 3 treatments in a completely randomised fashion with 20 replicates. In the first treatment, 60 g of crude inoculum containing roots and soil of Sudangrass infected with *G. mosseae* was used as the mycorrhization inoculum for each pot. In the second, the sample was inoculated with 60 g of soil containing roots of Sudangrass infected with *G. fasciculatum*, while in the third (control treatment), 60 g of sterilised soil was added to each pot. The spore densities of the two AM fungi candidate, measured by the number of spores produced per gram of soil ($NOSG^{-1}S$), were adjusted to the level of 12 ± 1 spores g^{-1} by diluting the propagated inocula with sterilised sandy soil. The adjusted inocula were thoroughly mixed into the top 5 cm of the pot soil.

Seeds of peanut plants were pre-germinated in perlite. Each experimental pot (diameter 15 cm) was filled with 3.0 kg of appropriate substrate soil and sowed with 2 pre-germinated seeds. To exclude intra-specific competition, each pot was thinned to one plant seven days later. The pots were placed in a greenhouse that provided growing conditions of a 12 h photoperiod, (437 ± 13 µmol m^{-2} s^{-1}), temperature fluctuating within a range of 28°C–30°C and 30% relative humidity. Unless otherwise stated, Hoagland's mineral salt solution minus P (Hoagland & Arnon 1950) was added to each pot biweekly and distilled water was added whenever needed to maintain the soil at about 65% of total water holding capacity.

After the 9th week, the plants were harvested and different plant growth parameters were assessed: number of tillers per plant ($NOTP^{-1}$), shoot height per plant (SHP^{-1}), shoot fresh weight per plant ($SFWP^{-1}$), shoot dry weight per plant ($SDWP^{-1}$), root fresh weight per plant ($RFWP^{-1}$), root dry weight per plant ($RDWP^{-1}$), number of lateral branches per plant ($NOLBP^{-1}$), length of lateral branch per plant ($LOLBP^{-1}$), number of leaves per plant ($NOLP^{-1}$), leaf area per leaf (LAL^{-1}), root/shoot weight ratio (R/SWR) and rate of growth per week ($ROGW^{-1}$). Roots were collected and preserved in formalin-acetic acid-alcohol (FAA) fixative solution, cleared and stained. The progress of mycorrhization was assessed by percentage of colonisation (PC) and $NOSG^{-1}S$ according to Phillips and Hayman (1970).

Data Analysis

Differences among the treatments in the means of the various plant growth parameters, percent colonisation and the $NOSG^{-1}S$ were statistically quantified by Fisher's least significant difference (LSD) tests following analysis of variance (ANOVA).

RESULTS

The physico-chemical properties of the substrate soil indicated that it was calcareous (3782 µg Ca g^{-1} soil) and alkaline (pH = 7.9), but poor in both macronutrient and micronutrient content, especially P (6 µg g^{-1} soil). The texture of the soil was classified as sandy clay loam with reasonably good permeability.

The growth levels of mycorrhizal and nonmycorrhizal peanut plants (controls) were estimated to be about the same for the first month in all conditions of substrate soil. Subsequently, the growth rate per week of the controls started to decline. *G. fasciculatum* inoculated peanut plants were taller and more vigorous than the controls. However, *G. mosseae* inoculated peanut plants were generally even taller and more vigorous than the *G. fasciculatum* inoculated ones in all conditions of substrate soil.

The effects of AM fungi on peanut growth irrespective of substrate soil conditions were compared (treatments A1, B4, C7, A2, B6, C8; Fig. 1). The influence of AM fungi on peanut growth was first analysed without reference to the effect of the various soil treatments by statistically comparing all of the data accumulated for peanut plants grown in all three conditions of substrate soils in the presence of *G. mosseae* (treatments A1, B4, C7; Fig. 1) or *G. fasciculatum* (treatment A2, B6, C8; Fig. 1).

G. mosseae inoculated peanut plants showed significantly more growth ($P \geq 0.05$) compared with controls and *G. fasciculatum* inoculated plants, as indicated by $NOTP^{-1}$, SHP^{-1}, $SDWP^{-1}$, $NOLBP^{-1}$, $NOLP^{-1}$, LAL^{-1}, $RFWP^{-1}$, $RDWP^{-1}$, R/SWR and $ROGW^{-1}$. *G. mosseae* inoculated peanut exceeded the controls on the $LOLBP^{-1}$. *G. fasciculatum* inoculated plants showed significantly greater increase in growth ($P \geq 0.05$) compared with the controls, as measured by SHP^{-1}, $SFWP^{-1}$, $NOLBP^{-1}$, $LOLBP^{-1}$, $NOLP^{-1}$, $RFWP^{-1}$ and $ROGW^{-1}$ (Fig. 2).

The levels of mycorrhizal colonisation and sporulation of *G. mosseae* were significantly higher ($P \geq 0.05$) than those of *G. fasciculatum,* as measured by indices of percent colonisation (PC) and $NOSG^{-1}S$ (Fig. 2). The effect of different substrate soil conditions on peanut growth was also compared (A, B, C; Fig. 1). This analysis compared the influence of the substrate soil and the soil microbiota on peanut growth when the plants were grown in the autoclaved soil supplemented with the original soil microbiota, autoclaved soil or non-autoclaved soil, irrespective of AM fungal inoculation. The autoclaved soil supplemented with soil microbiota increased peanut growth significantly ($P \geq 0.05$) over both autoclaved and non-autoclaved soil, as measured by the $NOTP^{-1}$, SHP^{-1}, $NOLBP^{-1}$, $NOLP^{-1}$, LAL^{-1}, RS/WP^{-1} and $ROGW^{-1}$. Autoclaved soil supplemented with the original soil microbiota exceeded the non-autoclaved soil on $SFWP^{-1}$ $SDWP^{-1}$, $RFWP^{-1}$ and $RDWP^{-1}$. The autoclaved soil appeared to significantly stimulate peanut plant growth compared to non-autoclaved natural substrate soil, as measured by $SFWP^{-1}$, $SDWP^{-1}$, $NOLBP^{-1}$, $NOLP^{-1}$, $RFWP^{-1}$ and $ROGW^{-1}$, (A, B, C; Fig. 1 and Fig. 3). Interactions among *G. mosseae*, peanuts and conditions of the substrate soil were also examined (treatments A1, B4, C7; Fig. 1). When the interactions of *G. mosseae* versus substrate soil on peanut growth in all three soil conditions were considered, the enhancement in the growth of peanut plants inoculated with *G. mosseae* was found to be significantly higher ($P \geq 0.05$) in autoclaved soil with added-back soil microbiota compared with the other two substrate soils, as measured by $NOTP^{-1}$, SHP^{-1}, $SDWP^{-1}$, $NOLBP^{-1}$, $LOLBP^{-1}$, $NOLP^{-1}$, $RFWP^{-1}$ and $ROGW^{-1}$. The growth of peanut plants in autoclaved soil with added-back microbiota exceeded that of those grown in non-autoclaved soil in $SFWP^{-1}$, LAL^{-1}, $RDWP^{-1}$ and R/SWR. When *G. mosseae* was added to the autoclaved soil, a significant increase in peanut growth ($P \geq 0.05$) was observed compared with plants grown in non-autoclaved natural substrate soil, as indicated by SHP^{-1}, $SFWP^{-1}$, $SDWP^{-1}$ and R/SWR (treatments A1, B4, C7; Fig 1 and Fig. 4).

G. *mosseae* had significantly higher root colonisation and sporulation levels ($P \geq 0.05$) in autoclaved soil supplemented with soil microbiota compared with autoclaved and non-autoclaved soils. Nevertheless, the levels of root colonisation and sporulation in autoclaved soil were significantly higher compared with non-autoclaved soil (Fig. 4).

Interactions between *G. fasciculatum*, peanut growth and conditions of substrate soil were likewise examined (treatments A2, B6, C8; Fig. 1). When considering the interactions of *G. fasciculatum* versus conditions of substrate soil on peanut growth, there was a significantly greater increase in the growth of peanut plants inoculated with *G. fasciculatum* ($P \geq 0.05$) in the autoclaved soil mixed with original soil microbiota compared with plants grown in the autoclaved and non-autoclaved soil, as measured by the indices $NOTP^{-1}$, SHP^{-1}, $NOLBP^{-1}$, $NOLP^{-1}$, $RFWP^{-1}$ and $ROGP^{-1}$. The growth of peanut plants in autoclaved soil with added-back microbiota exceeded that of those grown in non-autoclaved soil on R/SWR. *G. fasciculatum* inoculated peanut plants in the autoclaved soil displayed a significant increase in growth compared with those grown in non-autoclaved natural soil, as indicated by $NOLP^{-1}$ and R/SWR (treatments A2, B6, C8; Fig. 1 and Fig. 5).

The levels of colonisation and sporulation of *G. fasciculatum* were significantly higher (P ≥ 0.05) in autoclaved soil supplemented with soil microbiota compared with the other two substrate soils. However, the PC and sporulation of this fungus was still significantly higher (P ≥ 0.05) in autoclaved soil than in non-autoclaved soil (Fig. 5).

In general, we conclude from these results that both *G. mosseae* and *G. fasciculatum* were infective to peanut, but that *G. mosseae* was the major sporulator with the highest PC level and was more effective in increasing peanut plant growth on several indices. The autoclaved soil amended with soil microbiota was the best substrate for both AM fungi and peanut growth.

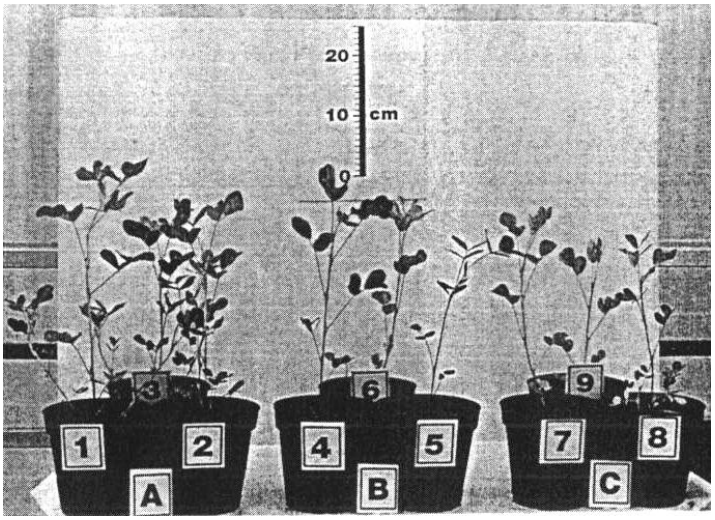

Figure 1: Comparison of vegetative growth among mycorrhizal inoculated peanut plants and controls in three soil conditions.

Notes:

A. Autoclaved substrate soil supplemented with soil microbiota
   1. *G. mosseae* inoculated peanut plants
   2. *G. fasciculatum* inoculated peanut plants
   3. Control

B. Autoclaved soil substrate
   1. *G. mosseae* inoculated peanut plants
   2. *G. fasciculatum* inoculated peanut plants
   3. Control

C. Non-autoclaved soil substrate
   1. *G. mosseae* inoculated peanut plants
   2. *G. fasciculatum* inoculated peanut plants
   3. Control

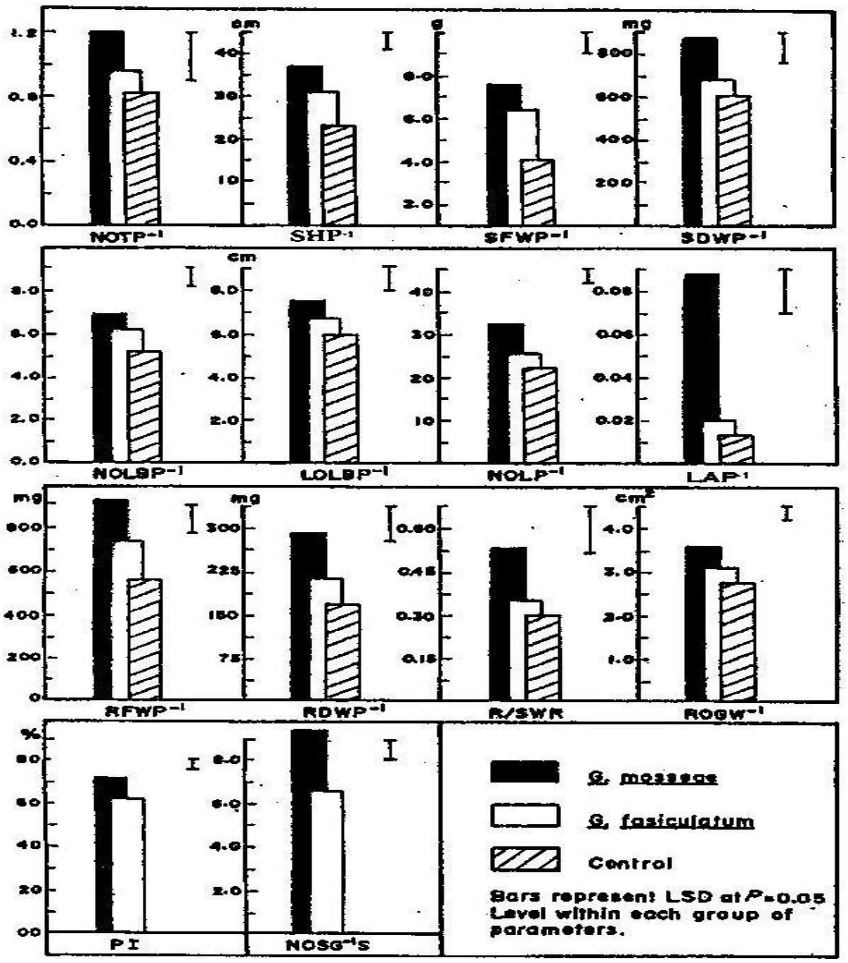

Figure 2: Influence of AM fungi on growth indices, percent of infection and number of spores per g soil.

Notes:

$NOTP^{-1}$	-number of tillers/plant	SHP^{-1}	-rate of growth/week
$SFWP^{-1}$	-shoot fresh wt./plant	$SDWP^{-1}$	-shoot dry wt./plant
$NOLBP^{-1}$	-number of lateral branches/plant	$LOLBP^{-1}$	-length of lateral branches/plant
$NOLP^{-1}$	-number of leaves/plant	LAP^{-1}	-leaf area/plant
$RFWP^{-1}$	-root fresh wt./plant	$RDWP^{-1}$	-root dry wt./plant
R/SWR	-root/shoot wt. ratio	$ROGW^{-1}$	-shoot height/plant

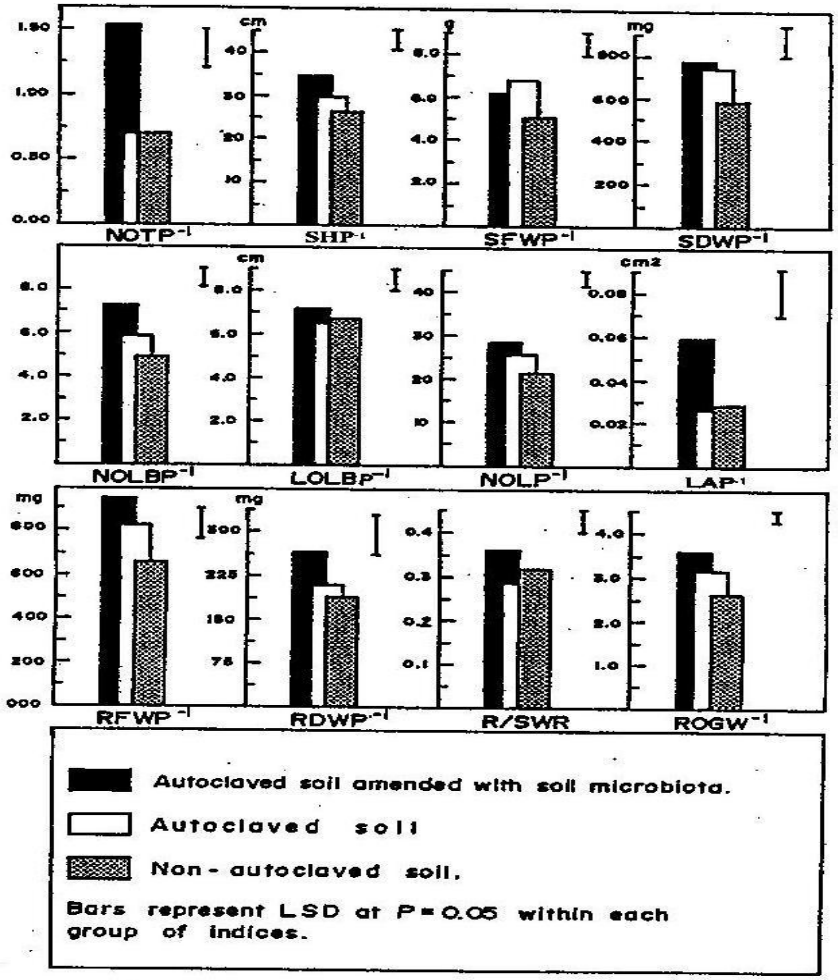

Figure 3: Influence of the conditions of substrate soil on peanut plants.

Notes:

$NOTP^{-1}$	-number of tillers/plant	SHP^{-1}	-rate of growth/week
$SFWP^{-1}$	-shoot fresh wt./plant	$SDWP^{-1}$	-shoot dry wt./plant
$NOLBP^{-1}$	-number of lateral branches/plant	$LOLBP^{-1}$	-length of lateral branches/plant
$NOLP^{-1}$	-number of leaves/plant	LAP^{-1}	-leaf area/plant
$RFWP^{-1}$	-root fresh wt./plant	$RDWP^{-1}$	-root dry wt./plant
R/SWR	-root/shoot wt. ratio	$ROGW^{-1}$	-shoot height/plant

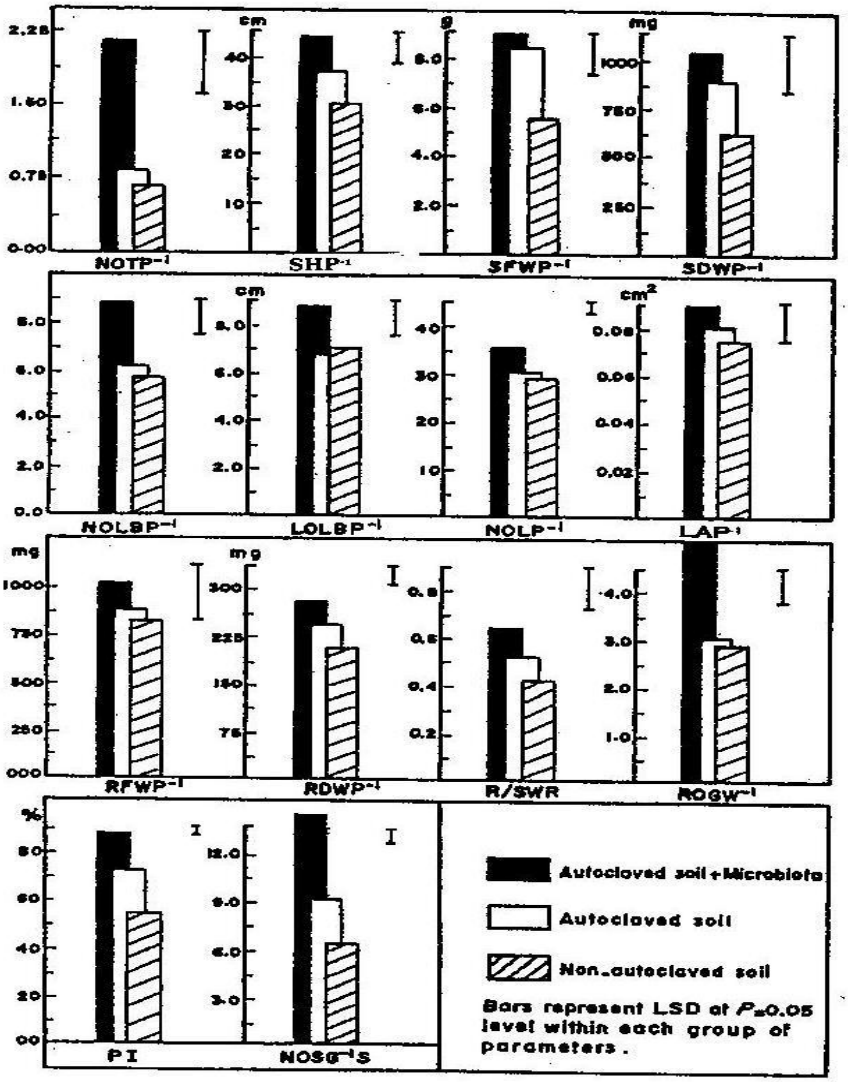

Figure 4: The interaction of *G. mosseae* vs. conditions of substrate soil on growth indices, percent of infection and number of spores per g soil.

Notes:

$NOTP^{-1}$	-number of tillers/plant	SHP^{-1}	-rate of growth/week
$SFWP^{-1}$	-shoot fresh wt./plant	$SDWP^{-1}$	-shoot dry wt./plant
$NOLBP^{-1}$	-number of lateral branches/plant	$LOLBP^{-1}$	-length of lateral branches/plant
$NOLP^{-1}$	-number of leaves/plant	LAP^{-1}	-leaf area/plant
$RFWP^{-1}$	-root fresh wt./plant	$RDWP^{-1}$	-root dry wt./plant
R/SWR	-root/shoot wt. ratio	$ROGW^{-1}$	-shoot height/plant

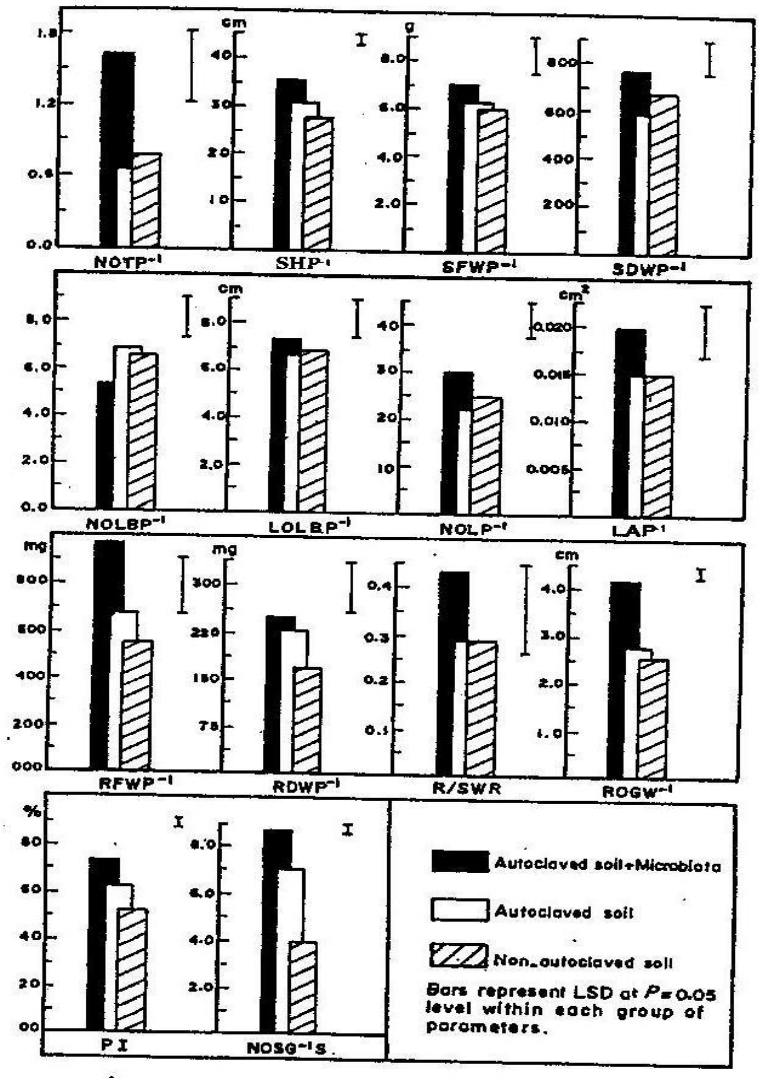

Figure 5: The interaction of *G. fasciculatum* vs. conditions of substrate soil on growth indices, percent infection and number of spores per g soil.

Notes:

$NOTP^{-1}$	-number of tillers/plant	SHP^{-1}	-rate of growth/week
$SFWP^{-1}$	-shoot fresh wt./plant	$SDWP^{-1}$	-shoot dry wt./plant
$NOLBP^{-1}$	-number of lateral branches/plant	$LOLBP^{-1}$	-length of lateral branches/plant
$NOLP^{-1}$	-number of leaves/plant	LAP^{-1}	-leaf area/plant
$RFWP^{-1}$	-root fresh wt./plant	$RDWP^{-1}$	-root dry wt./plant
R/SWR	-root/shoot wt. ratio	$ROGW^{-1}$	-shoot height/plant

DISCUSSION

The physico-chemical properties of the substrate soil indicate that it was calcareous and alkaline, low in soluble salts and organic matter content and poor in macronutrients, especially P. Nutrient deficiencies of the substrate soil used for both inoculum production and peanut growth were corrected by applying Hoagland's mineral salt solution lacking P. Nutrient solution without P usually enhances mycorrhizal colonisation of roots (Hepper *et al.* 1988) and fungus sporulation (Douds & Schenck 1990). Though the physical properties of the substrate soil were not studied in detail, its texture was likely to be good for the maintenance of adequate humidity and aeration for peanut growth.

Most legumes are symbiotic with both nodule-forming *Rhizobium* spp. and AM fungi, and the tripartite relationship of host-*Rhizobium*-AM fungi is unlike either dipartite symbiosis. When legumes are symbiotic with both *Rhizobium* spp. and AM fungi, plant growth is generally much greater than with either alone (Hoflich *et al.* 1994). The substrate soil was collected from an undisturbed sand dune community. Such virgin soil, which has never been cultivated with peanut before, was not anticipated to host any efficient strains of *Rhizobium* spp. that could develop symbiosis with peanut plants. The properties of the soil were also discouraging for the establishment of a tripartite relationship of peanut-*Rhizobium*-AM fungi as nodulation of leguminous plants is adversely influenced by the poor macronutrient content (NPK) or lack of micronutrients (for example molybdenum, boron, zinc, manganese and cobalt). Also, the formation of nodules may be influenced by the pH of the soil, salinity, antagonistic microorganisms, soil P content and the presence of fungicides (Anderson & Domsch *et al.* 1978). Whatever the reason, no rhizobial nodulation was observed in any peanut plant throughout the experiments. The lack of *Rhizobium* was advantageous as it allowed this study to examine mycorrhizal development and its dipartite effects on the growth of peanut without complications.

Visual estimates of the growing peanut plants indicated that growth after AM fungal inoculation with *G. mosseae* or *G. fasciculatum* was the same for all treatments and controls in the first month. This may be because the AM fungi either initially acted as parasites or were slow to get established. Clearly, they were initially not effective in increasing the transfer of heavy immobile elements which can not readily diffuse into the rhizospheres of the host. The growth of the control peanut plants started to decline in the second month compared with mycorrhizal plants; at the end of this period the control peanut plants were extremely stunted and grew poorly. So only after an initial lag phase did AM fungi become beneficial symbiotic microorganisms that increased the growth and plant biomass of mycorrhizal host plants. Our results are supported by evidence that this delay is mainly caused by increasing P uptake in AM fungi (Mosse 1973; Harrier & Paterson 2002).

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.* The *G. mosseae* inoculated peanut plants were generally taller and more vigorous than the *G. fasciculatum* inoculated plants. This could be attributed to the fact that *G. mosseae* is considered to be more effective in

alkaline and calcareous soils such as the substrate soil used in this experiments (with a pH of 7.90, ~ 1% $CaCo_3$ and 3782 µg Ca g^{-1} soil) than *G. fasciculatum,* which is more adapted to acidic soils (Khaliel 1990). It may be that one of the most important factors affecting the symbiotic relationship is the interaction between the AM fungus and soil.

Although the mechanisms and interactions of soil microorganisms with AM fungi are not well understood, the work reported here reinvestigated the question of whether the improved growth facilitated by mycorrhizal plants inoculated with crude inoculum (roots and soil of plant infected with an AM fungus) is due to the AM fungus alone or to the cumulative effects of the mycorrhizal fungus and the associated original soil microorganisms. Generally non-mycorrhizal and mycorrhizal peanuts grew less in non-autoclaved soils compared with autoclaved soils and amended autoclaved soil, as measured by several growth indices. There was no evidence that indigenous soil pathogens caused this suppression of peanut growth because no symptoms were detected. Furthermore, when root segments were stained for mycorrhizal colonisation, no fungal infections whatsoever could be detected in the root tissue. So there is no evidence that indigenous soil pathogens caused suppression of peanut growth. It was concluded that suppression was likely to be attributable to the competitive activity of soil microorganisms in general. Autoclaving thus likely removed these competing microorganisms, some of which may have been indigenous AM fungal species that were not as effective as the introduced species (Linderman 1992). Another explanation is that autoclaving may have increased nutrient availability.

The degree to which the mycorrhizal growth response is suppressed by the substrate soil appears to be highly dependent on the soil microbes present. For the autoclaved soil supplemented with soil microbiota, the microbial content was low (microbial extract of only 25 g of non-sterile soil was added back to each 3.0 kg autoclaved soil). As a consequence, more nutrients may ultimately be available for uptake by mycorrhizal peanut plants because less microbial inter and/or intraspecific competition would be expected in this soil. At the same time this condition seemed to also improve mycorrhizal formation, as indicated by the significantly higher PC and $NOSG^{-1}S$ in autoclaved soil amended with soil microorganisms compared with the autoclaved or non-autoclaved soil treatments. Another possibility was that the presence of indigenous AM fungi, as detected by Sudangrass host plant using pot culture technique, would undoubtedly interact with the introduced AM fungi and may influence the quantity of mycorrhizas formed as well as mycorrhizal functioning (Hepper *et al.* 1988). Also, the AM fungi in the non-sterile soil could have been attacked by mycoparasites that might play a role in limiting AM fungal populations and therefore possible further effects on plant growth. This argument could explain the low PC and mycorrhizal sporulation that induced significantly less plant growth in non-autoclaved soil when compared with the two counterpart substrate soils.

Soil microorganisms, however, enhanced the performance of *G. mosseae* over *G. fasciculatum* in the autoclaved substrate soil mixed with soil microbiota. This finding is similar to the results obtained by Azcon *et al*. (1990). They observed that soil microorganism increased the infection by *G. mosseae* and decreased the establishment of *G. fasciculate* in the roots of *Medicago*

sativa. The results also showed that addition of soil microbiota to the autoclaved soil contributed to the success of mycorrhizal sporulation (in terms of production of spores per gram of soil) and resulted in high PC of peanut plants, which would have had a positive impact on mycorrhization of peanut. This finding supports the observations of earlier works that some soil microorganism were found to enhance the sporulation of AM fungi (Ross 1980), mycorrhizal root colonisation (Azcon-Aguilar & Barea 1985) and mycorrhizal plant growth (Meyer & Linderman 1986). Although it is clear that soil microbiota is able to suppress mycorrhizal responses, the mechanism(s) responsible for this phenomenon remain a mystery (Johnson 1993). Therefore, further studies are encouraged towards understanding these interactions in order to identify favourable conditions for the development of AM fungi.

Peanut plants in non-autoclaved soil showed reduced growth despite the fact that there was no disease in these plants. This soil contains many more microbes than either of the other soil because of autoclaving and only adding back a small amount of microbiota will lead to far fewer microbes than untreated soil. It will also contain indigenous AM fungi which were removed by autoclaving or filtering in other two soil conditions. Reduced growth in non-autoclaved soil may be due to there being fewer nutrients available or to indigenous microbes that may suppress mycorrhization.

The observation that plants did even better when a few microbiota without indigenous AM fungi were present in otherwise sterile soil suggests that indigenous AM fungi are important in suppressing growth in the 9^{th} month culture. There may have been competition in this soil but clearly it did not outweigh the effect of the removal of these fungi.

The results presented in this paper have established that inoculation with two AM fungi had different effects on the growth indices of peanut plants grown in various substrate soils with the same and different microbial biomass content. Also, it documented that the introduced AM fungus *G. mosseae* adapted more successfully, behaved more effectively and performed better in mycorrhization of the peanut in alkaline calcareous soil. Peanut plants generally do least well in non-autoclaved soil but they do best in autoclaved soil with added back microbiota (lacking indigenous AM fungi). These findings establish the potential for mycorrhization with *G. mosseae* for improving the growth and production of this essential oil-producing plant. Therefore, it would be logical to select this AM fungus for peanut inoculation using autoclaved soil amended with original soil microbiota in any subsequent trials of this study.

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