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Mycorrhizal Development and Effects on Growth of the Peanut (*Arachis hypogaea* L.)

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Ph.D May, 1997

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DEDICATION

To the memory of the spirit of my dear father (Haj / Wad Elkhider). *The great self-dependent man, who taught me practically to restrain anger and pardon people; For God loves and rewards those who do good.*

With love and gratitude to my mother and family

EXAMINERS

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Contents in Brief

Contents in detail

ABSTRACT

The association between the growth of peanut (*Arachis hypogaea* L.) and arbuscular mycorrhizal (AM) fungi of the genus was investigated by measurements;

- mycorrhizal status of *Glomus spp* in diverse substrate soil conditions.
- mycorrhizal dependency and nutrient uptake.
- potential for mycorrhizal biocontrol of a bacterial pathogen.
- mycorrhizal response to salinity stress.
- effect of fungicides on *Glomus mosseae* mycorrhizal association.

Generally these investigations indicated that both the AM fungi *Glomus mosseae* and *Glomus fasciculatum* were infective to peanuts, but displayed a differential effectiveness depending on the soil microbial biomass content in the soil. *Glomus mosseae* gave the best overall results in improving peanut growth and therefore it was selected for peanut mycorrhization in further experiments. There appeared to be a threshold phosphorus requirement level for nonmycorrhizal peanuts, below which relative mycorrhizal dependency of the peanut was inclined to be significantly pronounced. *Glomus mosseae* protected peanut seedlings against the pathogenic bacterium *Erwinia carotovora*, it suppressed the pathogen population, improved the nutritional status of the plant, decreased the susceptibility of peanut seedlings to the bacterial soft rot disease and significantly alleviated disease effects. The fungus also demonstrated an ability to reduce NaCl salt stress syndrome. *Glomus mosseae*/peanut association in soils treated with relatively high dosages of Aspor and Plantvax fungicides was seriously affected and did not improve peanut growth substantially and appears to result in the loss of mycorrhizal benefits. This study indicates that *Glomus mosseae* may be a potential component to improve peanut production in low-input sustainable agrosystems.

DECLARATION

I hereby declare that this thesis has been composed by myself and except where otherwise acknowledged the work contained herein is my own.

Candidate signature.....

Candidate name : Khalafalla A. Elkhider

Date....27.09.1997....

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ABBREVIATIONS USED

AM	Arbuscular mycorrhiza(l)
AR	Annular
ANOVA	Analysis of variance
CFU	colony forming units
Conc.	concentrated
concn	concentration
cv.	cultivar
d	day(s)
dSm^{-1}	decisiemens per meter
DTPA	diethylenetriaminepentaacetic acid
DW	dry weight
DWP^{-1}	dry weight per plant
EC	electrical conductivity
$EC_{s.i.w}$	electrical conductivity of saline irrigation water
EC_{soil}	electrical conductivity of soil solution
FW	fresh weight
FWP^{-1}	fresh weight per plant
g	gram(s)
h	hour(s)
ha	hectare(s)
INVAM	international culture collection of VA mycorrhizal fungi
kg	kilogram(s)
log	logarithm
LOLB	length of lateral branch
LSD	least significant difference
LTD	log-transformed data

M	mycorrhizal
M.C.	moisture content
meq.	milliequivalent
mg	milligram(s)
min.	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mmohs	millimohs
N	normal
nm	nanometre(s)
NM	nonmycorrhizal
No.	number
$NOLB^{-1}$	number of leaves per branch
$NOSG^{-1}S$	number of spores per gram soil
$NOTP^{-1}$	number of tillers per plant
O. M.	organic matter
P	probability
Pa	Pascal
pH	hydrogen ion minus log concentration
PI	percent infection
ppm	part per million
R/SWR	root/shoot weight ratio
$RDWP^{-1}$	root dry weight per plant
$RFWP^{-1}$	root fresh weight per plant
RMD	relative mycorrhizal dependency
$ROGW^{-1}$	rate of growth per week

rpm	revolution per minute
s	second(s)
S.D.	standard deviation
$SDWP^{-1}$	shoot dry weight per plant
S.E.M.	standard error of mean
S. G.	specific gravity
$SFWP^{-1}$	shoot fresh weight per plant
SHP^{-1}	shoot height per plant
SMP	salinity-stressed mycorrhizal peanuts
SNMP	salinity-stressed nonmycorrhizal peanuts
SW	saturated weight
TEA	triethanolamine
TWHC	total water holding capacity
VAM	vesicular-arbuscular mycorrhiza(l)
var.	variety
vs.	versus
wk	week(s)
µg	microgram(s)
µm	micrometer(s)
µmol m^{-2} s^{-1}	micromole of photons per square meter per second
°C	degree Celsius
<	smaller than
>	greater than
≤	equal or smaller than
≥	equal or greater than
≈	approximately

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General introduction

1
Literature Review

1.1
Introduction

1.1.1
History and taxonomic relationships

The evolutionary history of mycorrhizal associations is very old. Mycorrhizas have been identified in the fossil records of the earliest land plants. Therefore, it has been suggested that they evolved simultaneously with the development of land plants dating back to about 370 million years (Harley and Smith,1983; Mosse,1986). Based on paleobiological evidence, Pirozynski and Malloch (1975) suggested that the evolution of mycorrhizal associations may have been a critical step in the invasion of land by plants and their subsequent survival and expansion in terrestrial habitats. The first land plants encountered a harsh environment due to the poorly developed soils that would have been available (containing no organic matter to hold nutrients and water). In addition, plant roots and root hairs had not yet evolved at the time of the first land colonisation and the role of mycorrhizas in the evolution of land plants may have been specifically to enhance nutrient uptake (Allen, 1991). The first known report of mycorrhizal associations was made in 1842 when, according to Rayner (1927), Schleiden observed the presence of thread-like structures and slimy contents in several cells of the cortex of orchid roots. In 1847, Reissek exerted efforts to reveal the origin and nature of the thread-like structures present in the root cells of angiosperms, especially Orchidaceae. He concluded that these structures were, undoubtedly, fungi. This work is considered to be the first accurate step towards understanding the association between root cells and fungal mycelia

now known as a mycorrhiza. These observations remained unappreciated and debatable among scientists, until 1879 when deBary recognised their significance and gave the term "symbiosis" to mutually beneficial associations of dissimilar organisms living together. Now defined as "mutualism" (Killham,1994). Since that time, mycorrhizas have been considered to be typical examples of symbiosis. Kamienski (1881) described associations between Monotropa and fungal hyphae and mentioned that a complete fungal layer was formed around the roots. He also pointed out that absorbency and ion uptake from the soil must pass through this fungal layer. Frank (1885) was the first to give the name "mycorrhiza" to the symbiotic association between roots and fungal hyphae. He believed that the fungi performed the function of root hairs and could translocate water, salts and soluble organic materials to the plants.

In 1887, Frank proposed that mycorrhizas could be classified into ectotrophic and endotrophic forms. Following Frank's proposal, the terms ectotrophic and endotrophic came into general botanical use. Stahl (1900), in the beginning of this century, recognised two groups of plants: mycorrhizal and non-mycorrhizal plants. He divided the mycorrhizal plant group into obligately mycorrhizal and facultatively mycorrhizal plants. Obligately mycorrhizal plants are always depend on fungal colonisation of the root to obtain their nutrients while facultatively mycorrhizal plants may either be colonised or remain free of mycorrhizal fungi depending on the characteristics of the substratum. Gerdemann (1974) divided mycorrhizas into three major groups according to the morphological and the anatomical nature of the association. These groups are:

- Ectomycorrhizas (sheathing).

They have a sheath or a mantle of fungal mycelium surrounding the short root system and intercellular penetration by hyphae in the root cortical tissue,

resulting in an internal network of hyphae known as the "Hartig net".

• Ectendomycorrhizas (intermediate forms).

They are characterised by the formation of both intercellular and intracellular hyphae in root tissues. The sheath may be thin and reduced or even absent.

• Endomycorrhizas (non-sheathing).

They do not form a mycelial sheath but form intracellular hyphae within the root tissues that are connected to a network of external hyphae.

The fungal associates of both ectomycorrhizas and ectendomycorrhizas typically are Basidiomycetes (Agaricales). A few are members of the Ascomycetes, Fungi Imperfecti or Zygomycetes. These are the main mycorrhiza formers of trees in temperate zones. Endomycorrhizas are further subdivided into two groups based on the presence or absence of septation in the fungal hyphae. Those produced by septate fungi belong to the Basidiomycetes (Aphyllophorales) and are found in the Orchidaceae, Ericales, and a few other plant groups. Those formed by aseptate fungi in the Zygomycotina are known as vesicular-arbuscular mycorrhizal (VAM) fungi, recently reclassified by Morton and Benny (1990) as arbuscular mycorrhizal (AM) fungi of the Order Glomales. VAM associations produced by Glomalean fungi consist of three components: host root cells, hyphae which grow inter-and interacellularly through the root cortex and external mycelia in the soil. The name given to these fungi is derived from two characteristic structures formed within the cortical cells:

• Vesicles, which are intercortical swellings of internal hyphae that serve as a storage frequently containing lipid droplets (see Appendix E - Plate 2).

• Arbuscules, which arise on the intercellular hyphae as lateral branches which penetrate the cells (usually) of the inner cortex and branch repeatedly to form a branch-like structure within the cell lumen. The finely branched hyphae are

closely surrounded by the plasmalemma of the cell; due to the large surface contact arbuscules are most intensive connection between the VAM fungus and the host plant. Arbuscules are the organs through which VAM fungi provide their hosts with inorganic nutrients in exchange for organic carbon. It seems that the name Arbuscular mycorrhizas (AM) is gradually replacing VAM in the scientific literature (Brundrett *et al.*,1996).

Most members of many families of angiosperms and gymnosperms, together with ferns, lycopods and bryophytes develop AM fungi in their roots (Harley and Smith,1983). Exceptions are members of the families of Brassicaceae (Cruciferae), Caryophyllaceae, Chenopodiaceae (Sieverding, 1991), and families which form other types of mycorrhizas such as tree families in temperate regions, for example Pinaceae, Fagaceae etc. (see above). Morphological and structural features of the spores and their subtending hyphae are the principal taxonomic criteria for AM fungal species. No true sexual structures are known to exist. At present six genera are known whose members formed an arbuscular mutualistic symbiosis with terrestrial plants. These are ***Gigaspora, Scutellospora, Acaulospora, Entrophospora, Glomus,*** and ***Sclerocystis*** (detailed descriptions of the genera are given in Morton, (1988) ; Walker and Trappe (1993) ; Schenck and Perez (1988) ; Brundrett *et al.*, (1996). Arbuscular mycorrhizas occur in more plant species, families and orders than all other types of mycorrhizal fungi combined. They do not tend to be host specific. For example, ***Glomus mosseae*** has been shown to colonise roots of twenty different plant species belonging to twelve different families (Mosse, 1973a).

Arbuscular mycorrhizas are widespread; they occur in the arctic, temperate, and tropical regions of the world (Hayman, 1978). The soil-based mycelium of these fungi is widely believed to be the largest component of the total fungal biomass in the soil. Arbuscular mycorrhizas are of particular importance in the tropics, where soil tends to be positively charged and thus

retains phosphate so tightly that this nutrient is available only in very limited supplies for plant growth. Although much of the basic knowledge was already well established nearly a century ago, meaningful research on AM plant interactions commenced only from about 1960, when it became possible to produce these infections under controlled conditions. This long delay into AM research was mainly attributed to the fact that these fungi are obligate symbionts that cannot readily be cultured axenically, and must be grown on roots of living host plants (Mosse,1986). This drawback means that much fungal experimental material has to be obtained by labour intensive retrieval from soil and roots of field or greenhouse grown plants. The continuation of this review will be concentrated mainly on literature relevant to AM fungi.

1.1.2
Soils and mycorrhizal associations

Since mycorrhizas are formed by interactions between plants and fungi, they must be studied as a dynamic system and not as individual organisms. Because edaphic properties clearly influence both host plants and AM fungi (see Fig. 1.0), studies of mycorrhizal systems need to consider soils as well as plants and fungi (Johnson and Pfleger, 1992). The soil is an important partner in mycorrhizal associations. It is not only a nutrient-providing medium for plant growth, but also a complex, living fragile medium that must be protected to ensure its long-term productivity (Reganold *et al.*, 1990). Arbuscular mycorrhizas form a fundamental link between the biotic and abiotic portions of the system (O'Neill *et al.*, 1991) and play a crucial role in facilitating both microbial and plant functions by transporting mineral nutrients to the host and C-compounds to the soil and its biota (Reid, 1990).

The textural and structural characteristics of the soil habitat, undoubtedly, influence soil microorganisms including mycorrhizas. Skinner and Bowen (1974) reported that soil texture and the compaction rate of the soil are the

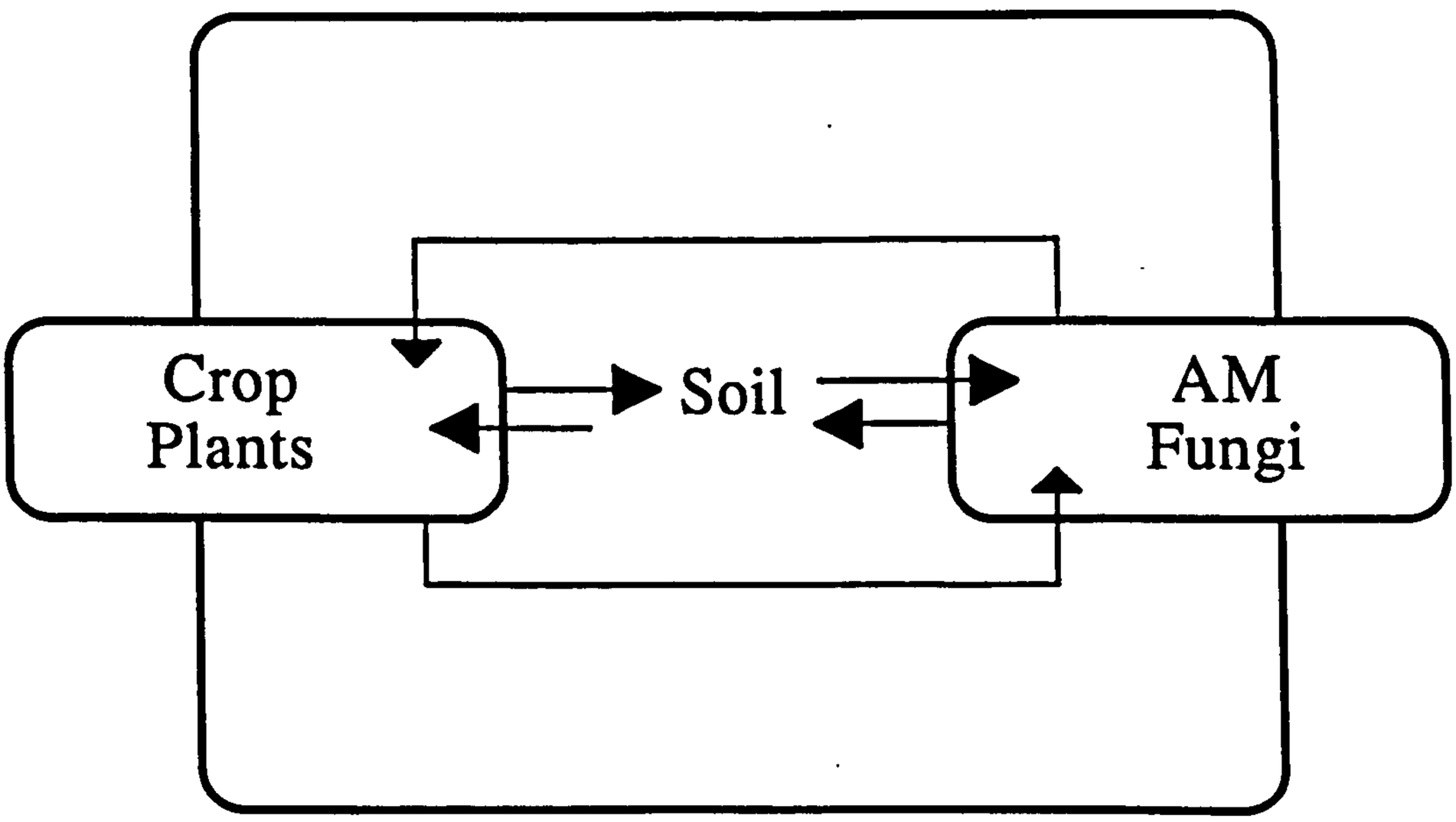

Figure 1.0
Schematic pathways which shows the interaction between plant, soil and AM fungi (redrawn from Johnson and Pfleger, 1992)

mediating factors that influence the growth of seedling root systems as well as mycorrhizal formation and effectiveness. They added that mycelial growth running into the soil can differ greatly depending on the characteristics of the soil. Differences in the degree of infection between soils can be highly significant. Intensity of infection has been shown to depend on soil pH. Graw (1979) found that strains of AM fungi reacted differently to soil pH. Stahl and Christensen (1991) reported physiological variations among isolates of *Glomus mosseae*. It seems that these physiological variations are based on long-term adaptation to edaphic factors at least in part, to soil pH. Some AM fungi do not readily adapt to soils of different pH. The high pH of alkaline soils is often associated with arid environments. In such alkaline soils, *Glomus mosseae* was found to perform better than *Glomus fasciculatum*. For example Khaliel (1990) found that *Glomus mosseae* and *Glomus fasciculatum* had quite a different effect on growth of Sudangrass (*Sorghum sudanense*) in alkaline soil (pH 8.0) in a greenhouse experiment with 83 % colonisation by *Glomus mosseae* while that of *Glomus fasciculatum* was only 11%.

In agricultural soils, that are managed conventionally, organic matter content decreases with time. This influences the microbiological processes in the soils (Whipps,1990) and the naturally balanced composition of organisms including mycorrhizas. Mosse (1986) reported that mycorrhizal plants that were given farm yard manure (organic matter) formed more arbuscules than those given equivalent amounts of mineral fertilisers. She reasoned that, the slow nutrient release from organic matter may avoid a rapid rise in internal plant nutrient concentrations and so depress mycorrhizal development.

Soil erosion decreases the mycorrhizal biomass and fertility because of its adverse impact on the soil bio-physico-chemical properties (Day *et al.*, 1987; Habte, 1989). It has been suggested mycorrhization may enhance rehabilitation of eroded sites. Aziz and Habte (1989 ; 1990) reported that selected AM fungi, along with starter fertilisation can improve revegetation and

afforestation programmes of eroded soils. Arbuscular mycorrhizas are important factors in soil stabilisation, directly, by producing large amounts of hyphae that serve to bind soil particles together (Thomas *et al.*, 1986 ; Miller and Jastrow, 1990) and indirectly, by increasing nutrient uptake they improve plant cover and root proliferation. It is believed that mycorrhizas contribute substantially to soil conservation through their role in soil aggregation. Perhaps the greatest potential for the utilisation of AM fungi in sustainable agriculture lies in the improvement of soil stability and erosion control (Bethlenfalvay, 1992). Miller and Jastrow (1990) proposed three related processes which contribute to soil aggregate formation:

- Growth of external AM fungal hyphae into the soil matrix to create the skeletal structure that holds primary soil particles together via physical entanglement.
- Creation by roots and external hyphae of the conditions that are conducive to the formation of macroaggregates.
- Enmeshment of microaggregates and smaller macroaggregates by external hyphae and roots to create the macroaggregate structure.

For soil mycorrhization, surface-sterilised AM fungal spores are frequently used as inoculum (Meyer and Linderman, 1986 ; Modjo and Hendrix, 1986) although complete destruction of contaminating parasitic microorganisms that live in the spore wall layer (Lee and Koske, 1994) is seldom possible. The most widely reported crude inoculum propagules are a mixture of spores, colonised roots, hyphae and soil from pot cultures grown in sterilised soil. More commonly, soil sievings are used to introduce similar quantities of other soil microbiota. Generally, the mycorrhizal inoculum must be infective in the soil for which it is introduced. It should produce the desired growth response or stress tolerance for the host plant under growing conditions. In addition, it must be concentrated, pathogen free with a shelf life that allows processing, distribution and use without a significant loss of

propagule numbers or potential (Jarstfer and Sylvia, 1992).

Most studies designed to assess the benefit of mycorrhizas to crop plants have been conducted in sterilised soils (Menge, 1983) and fail to consider the effect of the soil microflora on mycorrhizal symbiosis.

1.2 Mycorrhizal nutrition

Arbuscular fungi have been known for many years to stimulate nutrient uptake and growth of many herbaceous as well as woody plants and to increase the yield of several crops in laboratory, greenhouse and field trials especially by increasing the uptake of poorly mobile elements from soil (O'Keefe and Sylvia, 1991). Some plants fail to grow in the absence of AM fungi, while other show P-deficiency symptoms when grown in P-deficient nonmycorrhizal soil. Gerdemann (1964) found that mycorrhizal maize plants inoculated with *Glomus* species grew well, but nonmycorrhizal plants grew poorly and showed P-deficiency symptoms. He attributed the increased growth of the host to mycorrhizal colonisation. Similarly, it has been found that the growth of *Leptospermum scoparium* ceased and *Metrosideros umbellata* was very poor in soil with available P (3 mg P g^{-1} soil) unless the plants were inoculated with AM fungi or given additional P (Hall, 1977).

In greenhouse experiments, Powell (1979) found that inoculation with the mycorrhizal fungus *Glomus tenuis* increased the growth of ryegrass and white clover up to 48 % and 91 %, respectively. Khaliel and Elkhider (1987a) reported growth response of tomato (*Lycoperiscum esculentum*) inoculated with *Glomus mosseae* in low P soil. Mycorrhizal tomatoes had a greater dry weight and higher percentage of survival. Number of nodes, lateral branches and leaves per plant were almost doubled in mycorrhizal transplants.

The efficiency and level of colonisation depend on P level in soils. Saif

and Khan (1977) found that mycorrhizal barley plants absorbed more P from soil, had markedly increased dry weight, and yielded four times that of nonmycorrhizal plants, but the differences between mycorrhizal and nonmycorrhizal plants were almost eliminated by P fertiliser application. Supporting these results is the work by Asimi *et al.* (1979) who found that both mycorrhizal and nonmycorrhizal soybean (*Glycine max*) responded to fertilisation but increased additions of P could eliminate the mycorrhizal growth effect. Ross (1971) subjected soybean plants to different phosphate levels and found that the mycorrhizal plant had greater N, P, Ca, and Cu concentrations in their foliage. Phosphorus concentration of mycorrhizal plants at the lowest soil P level (74 kg P ha^{-1}) was greater than in the nonmycorrhizal plants at the highest soil P level (176 kg P ha^{-1}). He concluded that the roots of nonmycorrhizal soybeans are not efficient as P-absorbing organs, and mycorrhizas may aid plants in functions other than P uptake. The inoculation of barley with two AM fungi, *Glomus constrictum* and *Glomus fasciculatum,* increased total uptake of P, Cu, Zn and increased yield of grain and straw (Jensen, 1982). Plants inoculated with *Gigaspora margarita* did not differ in growth or P, Cu and Zn uptake from control plants. Nonmycorrhizal citrus seedlings have been reported to show Cu-deficiency symptoms compared with mycorrhizal (Timmer and Leyden, 1980).

The mechanism of absorption and translocation of P and other nutrients by mycorrhizal fungi is not well understood. Several hypotheses have been proposed by Safir and Nelson (1981) to explain the nutritional improvement noted in mycorrhizal plants. These hypotheses are;

- the mycorrhizal plant roots may be more efficient nutrient absorbers,
- the mycorrhizal root systems may be able to tap sources of nutrients that are not available or are less available to nonmycorrhizal roots,
- the soil network of mycorrhizal hyphae is able to absorb nutrients from a large

soil volume and translocate nutrients to the colonised roots, and

• the mycorrhizal root segments remain functional as nutrient absorbers for longer periods of time than do nonmycorrhizal root segments.

In essence, these hypotheses suggest that the absorptive surface area of the roots in mycorrhizal plants (mycorrhizosphere) is increased and the range of the area of absorption is increased by mycorrhizal hyphae to area beyond the nutrient depletion zone of nonmycorrhizal roots.

The beneficial effect may be temperature dependent in the field. Hetrick *et al.* (1984) found that the winter wheat plants did not become colonised until May and suggested that this delay may be due to the low temperature of the field soil because these plants became mycorrhizal within 10 days under greenhouse conditions. Raju *et al.* (1990) grew sorghum plants in growth chambers at 20, 25, and 30 °C in a low P soil (3.65 µg P g^{-1}soil) inoculated with *Glomus fasciculatum, Glomus intraradices* and *Glomus macrocarpum*. They found *Glomus fasciculatum* enhanced shoot growth at 20 and 25 °C, and mineral uptake at 30 °C. *Glomus macrocarpum* enhanced shoot P, K and Zn at all temperatures, and Fe at 25 and 30 °C.

1.3 Mycorrhizal dependency

Some plant species require either a mycorrhizal fungal association or large addition of P and perhaps some other nutrients for normal growth. These are the obligately mycorrhizal species. Gerdemann (1975) defined plant mycorrhizal dependency as the "degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of soil fertility". Menge *et al.* (1982) calculated mycorrhizal dependency by expressing the dry weight of a mycorrhizal plant as a percentage of the dry

weight of a nonmycorrhizal plant at a given level of soil fertility.

Evidence for mycorrhizal dependency of plant species is vital for potential successful manipulation of AM fungi-dependent plants in low-input ecosystems. Mehraveran (1977) studied mycorrhizal dependency of six citrus cultivars for *Glomus fasciculatum* mycorrhization at three P levels. He found that all citrus cultivars grew well and showed little response to P additions when they were mycorrhizal, and all of them showed growth increases in response to P additions when they were nonmycorrhizal. Peach trees, inoculated with *Glomus etunicatum* and *Gigaspora margarita*, were highly dependent on mycorrhizas for their growth and P uptake (Strobel *et al.*, 1982). These authors found that the application of high rates of NPK fertiliser did not completely substitute for mycorrhization in peach because NPK fertiliser did not improve foliar Fe, Cu, and Zn status to the same degree as AM fungi did. Kleinschmidt and Gerdemann (1972) investigated the effect of soil sterilisation and AM inoculation on six citrus species. They found that nonmycorrhizal seedlings grown in fumigated or heat-treated soils were stunted or grew poorly while mycorrhizal seedlings grew well and had greater dry weights, and higher P contents. They concluded that citrus plants were highly dependent on mycorrhizas; and the stunting, poor growth, and chlorosis of citrus plants in fumigated soil or in heat-treated soil resulted from the death of the mycorrhizal fungi rather than from toxicity. Plenchette *et al.* (1983) studied the mycorrhizal dependency of 20 plant species grown in fumigated and non fumigated soil under field conditions. They divided these plants into three groups based on their growth response to mycorrhizal colonisation in both soils. These groups were:

- Plants that became mycorrhizal, and grew well and had more growth in the non-fumigated soil but were stunted in the fumigated soil because they lacked mycorrhizas.

- Plants in nonfumigated soil were mycorrhizal and in fumigated soil were nonmycorrhizal but were not stunted because available P was high enough for normal growth without mycorrhizas.
- Plants that grew better in fumigated than nonfumigated because of soil borne pathogens; these plants had no mycorrhizas in the nonfumigated soil.

Mycorrhizal dependency varies among plant species or varieties by several factors. The morphology of plant roots affects mycorrhizal dependency in several plant species. Baylis (1970, 1972, 1975) suggested that plant genera without root hairs have a greater dependency on mycorrhizas or supplemented P for growth in P-deficient soils than plants with finely branched root systems and copious root hairs. Mosse and Hayman (1980) reported that plants with fine roots and large numbers of long root hairs, e. g. many grasses, were less dependent on mycorrhizas than plant with short, fleshy roots that lacked root hairs. They also suggested that plants with coarse roots and few root hairs were dependent on AM fungi colonisation because they can not absorb enough P, whereas plants with fine roots and root hairs were less dependent on mycorrhizal colonisation for this purpose. They also reported that slow growing species may also be less dependent on mycorrhizas. The host plant species or varieties are also influential factors. Azcon and Ocampo (1981) studied the dependency of thirteen wheat cultivars for mycorrhization by *Glomus mosseae*. They found that the mycorrhizal dependency varied with different cultivars. The experiments of Hetrick *et al.* (1984) supported these results; they found that eight cultivars varied in their response to *Glomus mosseae* or *Glomus epigaeum* in both field and greenhouse experiments.

Similarly, certain mycorrhizal fungal species or isolates have been reported to be more efficient at meeting the needs of mycorrhizal dependent plants. Pope *et al.* (1983) studied the mycorrhizal dependency of four hardwood tree species for six species of AM fungi in a low P soil. They found

that mycorrhizal dependency varied with the plant species and the associating mycorrhizal fungal species.

1.4 Mycorrhizas in biocontrol

The literature is controversial on the direct role that AM fungi may play in the biocontrol of plant diseases, but their interactions with bacterial or fungal associates in the mycorrhizosphere have not yet been fully investigated (Linderman, 1992). Research indicates that AM fungi can reduce the detrimental influences of root pathogens. For example, sporangia and zoospores production by the root pathogen *Phytophthora cinnamomi* was reduced in the presence of rhizosphere leachates from AM plants, when compared with leachates from non-AM plants (Meyer and Linderman, 1986). Baradas and Halos (1980) reported that isolates of *Glomus* and *Gigaspora* spp conferred biocontrol of *Fusarium solani* f. sp *phaseoli* on cowpea (*Vigna unguiculata*). They thought that it was probable that these AM fungal isolates and the associated microflora acted as competitors or provided mechanical and chemical barriers against the establishment of *Fusarium solani* on cowpea. Also, Garcia-Garrido and Ocampo (1988) reported that *Glomus mosseae* provides protection against *Erwinia carotovora* infection of tomatoes.

Nonetheless, the influence of AM fungi on the microbiology of the rhizosphere is inconclusive and still in debate. The possibility that bacteria might inhibit AM fungi and reduce their effectiveness has been reported by Krishna *et al.* (1982), who observed that *Streptomyces cinnamomeous* reduced the sporulation and colonisation of *Glomus fasciculatum* on finger millet. Also, Ross (1972) found that mycorrhizal soybean plants were almost always affected by *Phytophthora* root rot, whereas nonmycorrhizal plants were not. Similarly, there

are several other studies which indicate that AM fungi have no positive impact on fungal diseases. Examples are the work of Davis (1980), who examined the interaction of ***Glomus mosseae*** with ***Thielaviopsis basicola*** that causes root rot of citrus, and that of Hall and Finch (1974), who worked with avocado (***Persea americana*** Mill) and ***Phytophthora cinnamomi*** root rot.

Dehne (1982) summarised the possible mechanisms of interactions between AM fungi and plant pathogens in two general statements, as follows:

- Mycorrhizal fungi may be able to retard pathogen development in the root system. This influence is restricted to the site of mycorrhiza establishment.
- Mycorrhizal fungi may cause increased disease incidence systemically, especially in the nonmycorrhizal part of the plant. So far as is presently known, this can be attributed to better nutrition, enhanced plant growth, which may physiologically stimulate pathogens in mycorrhizal plants. With increased concentrations of assimilates those plants can then serve as better nutrient sources for plant parasitic organisms.

The symbiotic host/fungus relationship is characterised by the formation of arbuscules. These specific, haustoria-like structures of the endophyte are successively degraded. This process was interpreted as digestion of the fungus by the host (Mosse, 1973a). For this degradation to occur, the fungal cell wall of the fungus has to be attacked by the host. Therefore, roots colonised by a mycorrhizal fungus exhibit high chitinolytic activities. These enzymes have the potential to act against fungal pathogens (Dehne *et al.*, 1978).

Should subsequent experimental evidence support the hypothesis that AM fungi and their mycorrhizosphere microbes contribute to suppression of root diseases, then plant mycorrhization is likely to play a vital role in sustainable agriculture.

1.5
Mycorrhizal response to environmental stresses

As mentioned previously, AM fungi are known to occur in a broad range of environments worldwide. Individuals of AM fungi may be present in a diversity of habitats. For example, ***Glomus fasciculatum*** has been reported in Jojoba [***Simmondsia chinensis*** (Link) Schnied] in semi-arid environments in Sudan (Khaliel and Elkhider, 1987b), ***Glomus mosseae*** in forests in New Zealand (Johnson, 1977) and ***Glomus constrictum*** in leguminous cropland in Korea (Kim and Kim, 1992). Presumably, AM fungal species with such a wide distribution, must possess competent adaptation. Stahl *et al.* (1990) summarised three mechanisms that are likely to enable an AM fungal species to exhibit broad geographic adaptation. These are:

- Phenotypic plasticity.
- Physiological differentiation.
- Or some combination of these.

Much of the functional diversity of AM fungi occurs at the isolate level rather than species level (Morton and Bentivenga, 1994). Consequently habitat information is important knowledge for comparing the results of experiments or the selection of isolates for practical use (Brundrett *et al.*,1996). Evidence is accumulating that edaphic and climatic conditions are most influential in shaping AM fungi fitness to stresses. For example, Stahl *et al.* (1990) tested ***Glomus mosseae*** isolates from three dissimilar environments under uniform experimental conditions to determine whether the isolates had any physiological variations. Large variations were observed in root colonisation, spore production, and growth response of yellow sweet clover (***Meliotus officinalis*** Lam) to the different isolates. Seemingly, these AM fungal isolates had acquired diverse physiological phenomena because each isolate was ecotyped to meet its own habitat environmental stresses.

High and low soil nutrients can stress AM systems especially P. The performance of several AM fungi in soils with low P, was compared and found that plant yield was high when the inoculum used was indigenous to the soil in which the plants were grown (Lambert *et al.*,1980b). Their results suggested that indigenous strains of AM fungi may possess an adaptation to low nutrient stress and that the performance and persistence of strains otherwise more efficient in nutrient uptake may be limited by their lack of adaptation. At the other extreme, increasing P supply frequently decreases the AM infection (Ross, 1971). For example, *Glomus intraradices* has been shown to depress growth of citrus in high P soils (Peng *et al.*,1993). However, some AM fungi living in soils with high P level were able to adapt to high P stress and may be regarded as P-tolerant (Sylvia and Williams, 1992).

The capacity of AM fungi to tolerate heavy metal stress has been revealed by Gildon and Tinker (1981) who isolated a strain of *Glomus mosseae* that was capable of tolerating 100 mg Zn kg^{-1} soil. This isolate also demonstrated tolerance to cadmium (Cd) and protected the host plant. These results may indicate that mycorrhization, particularly with tolerant AM fungal isolates, could protect host plants against the effects of heavy metal stresses and thus might be very vital in reclamation and revegetation programmes of mining and polluted soil sites. Nonetheless, in a subsequent study Gildon and Tinker (1983) demonstrated that *Glomus mosseae* colonisation of onion plant could be reduced, or even eliminated, by high concentration of zinc (Zn), copper (Cu), nickel (Ni) or cadmium (Cd).

A wide range of mycorrhizal fungi has been shown to tolerate extremely high osmotic stress. Field studies addressing the impact of high osmolarity in soils on AM fungi are scarce. Some mycorrhizal halophytes survive in soils having electrical conductivities of saturation extracts in excess of 10 dSm^{-1} (Syliva and Williams, 1992). Pond *et al.*(1984) observed improved growth of tomato in salinised soil by AM fungi adapted to saline soils throughout southern

and central California and Nevada. They found mycorrhizal plant roots and spores in a range of habitats and levels of salinity up to 185 dSm^{-1}. Similarly, Hirrell and Gerdemann (1980) observed improved growth of onion and bell pepper in saline soils by infection with two fungi (*Glomus fasciculatum* and *Gigaspora margarita*). The response growth was attributed to improved P nutrition. The results of these studies may indicate that salinity appears to have little effect on AM fungi formation. However, salinity may adversely influence AM fungi formation in plants unadapted to salt stress. For example, Guttay (1976) reported a significant reduction in AM infection of sugar maples (*Acer saccharum* Marsh.) with increased soil salinity subjected to deicing salts.

It would not be expected that AM fungi have a major role in alleviating plant stress brought on by flooding because AM fungi are aerobic and are themselves likely to be affected by flooding. The presence of AM fungi in wet lands suggests that they are ecologically significant, but their function is not well understood (Wetzel and van der Valk, 1996). Possibly, AM fungi might improve the drought resistance of plants (Puppi and Bars, 1990). Ellis *et al.* (1985) found that AM wheat plants which had undergone three drought stress periods in a greenhouse trial, had twice the biomass and grain yield as non-AM wheat plants subjected to the same stress. However, some reports indicate that drought resistance is unaffected or decreased by mycorrhizas (Graham *et al.*, 1987 ; Simpson and Daft, 1990a). Most experiments of this type with AM fungi have been conducted in controlled greenhouse or growth chamber environments. Recently, Sylvia *et al.*(1993) conducted field trials to test the effect of AM fungi in water-stressed corn (*Zea mays* L.). They found a proportional increasing response of corn to inoculation with the AM fungus *Glomus etunicatum* with increasing water stress. Jarstfer and Sylvia (1992) summarised the effects of AM fungi colonisation on water stress as follows:

- Greater hydraulic conductivity.
- Lower transpiration rates per unit leaf area.

- Extraction of water from soil at lower water potential.
- More rapid recovery from water stress.

Still, the response of AM fungi to environmental stresses requires further extensive and intensive research before any conclusive narration can be given.

1.6 Mycorrhizal response to agrochemicals

It is rather too difficult to draw simple generalisations from the literature because of chemical variability in pesticide formulations and differing experimental conditions. Agrochemicals include fumigants, herbicides, fungicides and insecticides etc.

Under ideal conditions, fumigants containing methyl bromide-chloropicrin combinations are lethal to all soil organisms. Methyl bromide is detrimental to AM fungi, but the degree to which it eradicates AM fungi appears to be determined by the efficiency of the fumigation, and seems related to soil type, moisture level, temperature, and method of application (Menge, 1982). Peanut root colonisation by AM fungi was affected by sterilisation of soil with vertafume (methyl bromide + chloropicrin), while Ditrapex (DD + methyl isothiocyanate) caused no significant reduction (Middleton *et al.*, 1989). In tropical sunny climates, soil solarisation could be another possible alternative to fumigation, as it is generally effective in reducing soil pathogens (Katan, 1987) but has less detrimental effect on AM fungi (Afek *et al.*, 1991).

Herbicides are formulated to eliminate crop competitive weeds and not fungi. Therefore, it might not be anticipated that they would have any adverse effects on AM fungi. Nonetheless, the rate of herbicide application is an important factor mediating their effect on mycorrhizas. For example, at low application rates carbamate herbicide did not reduce root colonisation of alfalfa (*Medicago sativa* L.), or wheat by *Glomus mosseae*. At higher application rates,

however, phenmedipham herbicide reduced metabolic activity of *Glomus mosseae* (Ocampo and Barea, 1985).

Fungicides include many compounds differing greatly in their mode of action and effects on AM fungi, and conflicting reports are common. For example, reports of the effects of dicarboximides on AM fungi range from detrimental, to neutral, to beneficial (Johnson and Pfleger, 1992). Boatman *et al.* (1978) studied the effects of two systemic fungicides on AM fungi with the view to using them in the study of mycorrhizal P uptake. He found that the formation of mycorrhiza in clover roots was prevented by soil drenches of benomyl and thiophanate-methyl and the spread of established infections was halted. In addition to that phosphate uptake of inoculated onions and strawberry was reduced. Hale and Sanders (1982) also treated the roots of red clover plants with a drench of benomyl at a time when mycorrhizal infection was already well established. Benomyl halted further infection and reduced P inflow. The systemic fungicides, as a group, appear more damaging to mycorrhizal symbiosis than nonsystemic fungicides. They can affect spore germination and infection, as well as growth of AM fungi within the root. Since translocation is primarily upward, systemic fungicides would be most damaging to mycorrhizal fungi when applied as soil drenches. Results published by Jalali and Domsch (1975) provide evidence that systemic fungicides affect mycorrhizal growth inside the root where as several systemic fungicides, which were applied as foliar sprays, significantly reduced AM fungi development. Fungicides will also affect soil and rhizosphere populations of microorganisms. These microorganisms may interact with spore germination or infection by AM fungi. Fungicides may reduce numbers of mycorrhizal hyperparasites or predators (Atilano and Van Gundy, 1979; Daniels and Menge, 1980) and may ultimately increase mycorrhizal infection. Application rate is important in determining the effects that a fungicide may have on AM fungi. Nemec (1980) found that at high application rates, captan fungicide reduced mycorrhizal

colonisation of sour orange by *Glomus etunicatum*, but at lower rates it did not. However, Sreenivasa and Bagyaraj (1989) observed that when captan was applied at the recommended dosage, it reduced colonisation of rhodegrass (*Cloris gayana* Kunth) by *Glomus fasciculatum*; but when applied at half the recommended dosage, captan actually stimulated colonisation and spore production.

Many insecticides have been used so far to study their effects on AM fungi. Most of these insecticides were found deleterious e.g. Parvathi *et al.* (1985) reported that, in a pot culture experiment, three insecticides (carbaryl, endosulfan and parathion) all adversely affected colonisation and sporulation of *Glomus mosseae* in peanuts. Similarly, carbofuran insecticide was shown to reduce mycorrhizal colonisation in field and greenhouse-grown peanuts (Backman and Clark,1977). However, at half the recommended rate it increased root colonisation and spore production by *Glomus fasciculatum.* At this application level it also considerably suppressed contaminant fungi and nematodes in the pot culture (Sreenivasa and Bagyaraj, 1989). Again the dosage seem to be an essential factor with insecticides. For example, plant growth and AM fungi formation were not affected by 0.5 kg ha^{-1} levels of both endosulfan and quinalphos, but single or twice repeated application of these insecticides, at 5 or 10 kg ha^{-1} levels, exerted toxicity to the plant growth and AM fungi colonisation (Veeraswamy *et al*, 1993)

Should AM fungi mycorrhization be manipulated as a crop production tool in future, then more research is needed to assess the effect of agrochemicals on AM fungi and their activities. Generally, two mechanisms have been proposed to account for beneficial effects of pesticides on mycorrhizas:

- First, root colonisation may be stimulated if the pesticide alters the host plant's physiology so that the amount of soluble sugars allocated to root exudates is

increased (Schwab *et al.*, 1982 ; Jabaji-Hare and Kendrick, 1985).

• Second, mycorrhizas may be stimulated if a pesticide reduces populations of organisms antagonistic to AM fungi (Hetrick and Wilson,1991).

1.7 Peanuts and mycorrhizas

Peanut was grown as an agricultural crop as early as 950 B.C. The origin of the peanut is unknown, although it is assumed that peanut originated from Brazil or Peru, and was carried to Africa by early explorers and missionaries (Higgins, 1951). The main peanut growing belt, that circles the world, lies within tropical zones [Lats. 40°N and 40°S; annual precipitation 550-1200 mm]. It includes the southern states of the USA, Northern India, Sudan, Saudi Arabia and China etc. There are several types of wild and cultivated kinds, the peanut (groundnut, earth-nut, monkey nut, goober, pinda, pinder, Manilla nut) that we know is the fruit or pod of *Arachis hypogaea* L. of the Leguminosae family. The flower is borne above the ground and after it withers, the stalk elongates, bends down, and forces the ovary underground. The seed matures below the surface. The plant favours light sandy soil (Woodroof, 1973). Peanuts have a high food value which is well known all over the world. They provide rich ingredients to the diet in the form of lipids, proteins, carbohydrates, amino acids, valuable salts and vitamins. The peanut is also known to be an oil-yielding cash crop on large scale. The crushed seeds, an essential by-product known as cakes, are used as concentrates for domestic animals.

Leguminous woody perennials were reported to develop AM fungi associations e. g. *Acacia senegal* (Colonna *et al.*,1991). Also, other leguminous plant crops are usually infected with AM fungi under normal arable conditions (Jones, 1924). There is increasing evidence to suggest that this infection may

be of great importance to P nutrition of such legumes even where P fertilisers are used (Hale and Sanders, 1982). Waidyanatha (1980) found that without mycorrhizas growth of ***Pueraria phaseoloides, Centrosema pubescens, Calopogonium mucunoides, Desmodium ovalifolium,*** and ***Stylosanthes guianensis*** legume plants in sterilised soil failed, or was stunted. Mycorrhizal inoculation, coupled with P fertilisation, increases P concentration in many legume plants. Mahadi and Atabani (1992) found that the legumes (soybeans ***Glycine max*** L. Merrill ; lablab bean ***Lablab purpureus*** L. Sweet) inoculated with the AM fungus ***Glomus mosseae*** had more tissue P content than that with triple superphosphate fertilisation. In a pot experiment, Weber *et al.* (1992) found that inoculation with AM fungi improved growth of chickpea (***Cicer arietinum*** L.) and doubled P uptake at low and intermediate levels of triple superphosphate in sterilised low-P calcareous soil.

The first known mycorrhizal incidence reported in peanut was made by Butler in 1939. Later, AM associations in peanuts with ***Gigaspora gigantea*** and ***Glomus macrocarpum*** (Porter and Beute, 1972), ***Glomus fasciculatum*** (Krishna and Bagyaraj, 1982) and ***Glomus mosseae*** (Parvathi *et al.*, 1985) were reported. Baylis (1970) suggested that peanut, being a plant with no root hairs is liable to gain more from AM associations. Other studies provide evidence that AM fungi may indeed enhance the growth of Tamnut, Pronto, Starr, Florunner and McRan cultivars of peanuts (Krishna and Williams, 1987). Florunner cultivar was the most responsive to AM colonisation. They found that ***Glomus deserticola*** and ***Glomus intraradices*** alone and in combination with ***Bradyrhizobium*** were the most effective treatments. Peanuts infected with ***Glomus mosseae*** increased the yield of fruits, plant size and chemical content of shoots, roots and seeds (Daft and EL-Giahmi, 1976). Ishac *et al.* (1987) reported that the morphological characteristics, such as fresh and dry weight and yield of peanut plants were increased by both mycorrhizal inoculation and by phosphate fertilisation. Mycorrhizal peanuts without added phosphate had approximately the same

height, root length and number of leaves, tillers and fruits as nonmycorrhizal peanuts with added superphosphate. They also concluded that all mycorrhizal peanut plants grown with added phosphate had higher degree of nodulation as well as more dry matter, nitrogen and P contents than nonfertilised peanuts. Moreover, a more than two fold increase in the level of mycorrhizal colonisation was observed with phosphate fertilisation. Neck *et al.* (1987) studied the effect of P on growth of mycorrhizal peanut and observed that colonisation was greatest in soil with 6.25 μg ml^{-1} available P.

2
OBJECTIVES OF THE STUDY

Saudi Arabia and Sudan are part of the hot desert belt extending from Sahara in Africa to the Thar desert in the Indo-Pakistan subcontinent. The over all climate of Saudi Arabia falls within arid and semi-arid climates (Al-Jaloud, 1994). In tropical arid and semi-arid environments, low water content because of declining water tables, sand movements, saline soil conditions, deficiency of nutrients and organic matter, high evaporation rates and other factors contribute to make these environments harsh and unfavourable for plant growth. Any factor that improves nutrient and water uptake by plants or allows continued plant growth under these conditions should, undoubtedly, contribute to successful growth of agricultural crops in arid and semi-arid soils (Allen and Boosalis, 1983). A potential challenge would be to endeavour to establish a favourable root rhizosphere as alternative tactics for enhancing peanut growth under harsh unfavourable conditions. This might be achieved through arbuscular mycorrhizal colonisation.

The potential of arbuscular mycorrhizal fungi to enhance peanut growth is well recognised, though not well exploited mainly due to insufficient knowledge of most of the complex symbiotic functions of peanut/AM fungi

associations. No greenhouse or field work on mycorrhizal development in the peanut has been conducted in the Sudan and Saudi Arabia. The aim of this study was to investigate and screen the development and effects of newly introduced arbuscular mycorrhizal fungi on peanut growth without interaction of rhizobial strains in soil of Saudi Arabia. The investigations were structured to study some biologically-based factors influencing the development of AM fungi in the peanut. The objectives that will be described in this thesis therefore are:

- To compare the AM fungi colonisation of peanut in diverse conditions of substrate soil.
- To determine the mycorrhizal dependency and impact of mycorrhization on nutrient uptake and growth of peanut.
- To assess the potential for mycorrhizal biocontrol of *Erwinia carotovora* pathogen.
- To study the response and development of mycorrhizal peanut under simulated salinity stress.
- To evaluate the effects of two fungicides on development of the mycorrhizal peanut.

MATERIALS AND METHODS

1
General

1.1
Preparation of inoculum

Mycorrhizal inoculum was propagated according to the procedure devised by the international culture collection of VAM fungi (INVAM), [INVAM, Florida, 32611, USA]:-

- Starter inocula of *Glomus mosseae* (Nicol. sensu Gerd.) Gerd. & Trappe [Isolate # 361] and *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe emend. Walker & Koske from University of Florida, Department of Plant Pathology - Gainesville, Florida, USA, were used.
- Autoclaved soil was formulated from clay and sand in a ratio of 1:1 (v/v). The sand optimises aeration and niches for sporulation, reduces clay P level and prevents compaction in pots after repeated watering.
- Plastic pots (15 cm in diameter) were sterilised in 10 % clorox solution for 25 min. Rinsed thoroughly and dried.
- 1-2 cm sterilised coarse sand (80 % of the grains passing through a 2 mm sieve) was added to the bottom of the pot and then filled with autoclaved soil. The starter inocula were mixed in the top 3 cm of the soil and the pots were hand seeded with Sudangrass [*Sorghum sudanense* (Piper) Staph], then covered with sterile coarse sand (the top and bottom layers of sand reduces and often eliminates ingress of contaminating fungi or egress of AM fungi to

adjacent pots).

• Pot cultures were grown in a greenhouse.

• After 4 months, the pots were left to air-dry without disturbance until the host plants were completely wilted and dried. Under these conditions, drying is slow and allows the fungal symbionts to adapt gradually to changes in water potential. Rapid drying of pot contents after harvest can result in accelerated mortality.

• After drying, plants were cut off at soil level and the root ball broken up into 4-5 parts and placed in plastic bags with loose soil (chopping of roots and through mixing of the root/soil mixture were delayed until the inoculum was to be used).

• Inocula were stored at 4 °C for subsequent use.

1.2
Growing conditions

All inoculum and experimental pot cultures were grown in a greenhouse that provided growing conditions of 12 h photoperiod, (437±13 µmol m^{-2} s^{-1}) and temperature fluctuating within a range of 28-30 °C and 30 % relative humidity. Unless otherwise stated each pot was given a biweekly treatment with Hoagland's mineral salt solution minus P (Hoagland & Arnon, 1950 ; see Appendix A - 4, for details) and watered with distilled water whenever it was needed to maintain the soil at about 65 % of total water holding capacity.

1.3
Substrate soil

1.3.1
Collection and formulation

The substrate soil was collected from the top soil 10 -15 cm of an undisturbed sand dune community near Riyadh (24°20' North, Latitude ; 46°40' East, Longitude), Saudi Arabia. The sand dunes are fragmentally dominated by ***Moricandia sinaica, Calotropis procera, Datura innoxia, Ricinus communis, Rhazya stricta*** and ***Bassia sp.*** Soil was sieved through a 2 mm mesh screen to remove debris and stones. Four parts of the sieved soil were mixed with one part (w/v) of peat moss [Rose Garden Torf]. Peat moss was sterilised with solar pasteurisation, where wet potting peat moss was covered by polyethylene sheeting and exposed for direct sunlight. Five replicate samples were analysed for physical and chemical properties.

1.3.2
Physical Analysis

Some physical characteristics of the soil were ascertained as follows:-

1.3.2.1
Determination of moisture content

Moisture content was determined according to the method of Association of Official Agricultural Chemist [A.O.A.C.], (1965). Known weights of the soil were taken and oven dried at 105 °C for 24 h.

1.3.2.2
Determination of total water holding capacity

Total water holding capacity (TWHC) [capillary capacity] is defined as the water

retained against the gravitational pull. It was determined according to Pramer and Schmidt (1965) as follows:

Special boxes made of brass with perforated bases and rectangular in shape were used for this purpose. A filter paper was placed in each box to cover all the perforations. The boxes were then weighed, the filter papers were wetted with water, reweighed and filled with oven-dried soil which has passed a one mm sieve. The soil samples were packed as uniform as possible, the boxes were tapped on the bench after each soil addition and then placed in a flat bottomed dish containing water and left overnight. The surface of water in the dish was touching the bottom of the boxes all the time.

The next day the boxes were rapidly dried out on the outside and weighed. The boxes were then left for 24 h in an oven to dry at 105 °C and reweighed. The result was expressed as a percentage of the soil dry weight using the following formula:

$$\text{TWHC } (\%) = \big((b - a') - (c - a)\big)\big((c - a)\big)^{-1} \times 100 \qquad (1)$$

a = Weight of box + dry filter paper.

a' = Weight of box + wet filter paper.

b = Weight of box + wet filter paper + saturated soil.

c = Weight of box + dry filter paper + oven dry soil.

1.3.2.3 Determination of permeability

Permeability may be defined as a rate at which a fluid passes through a porous medium of unit area under unit gradient. The apparatus used consists of a big bottle (20 litres) acting as a reservoir for water. It was closed with a rubber stopper through which passed three glass tubes. A short tube was used for filling the bottle. The second tube acted as an air inlet tube. The third tube was

connected with a supply tube which delivered water to a number of percolation tubes held on a stand. A 200 g of air-dried soil were poured in each percolation tube and kept above a layer of sand (10 ml) and glass wool. Below each percolation tube there was a graduated cylinder to receive the percolating water. The lower end of the inlet tube determines the height of water above the soil column. The water was kept constant above the samples in the percolation tubes. The permeability of the soil was obtained by using the following formula derived from Darcy's law as described in Black *et al.* (1965):

$$P = (QL)(AH)^{-1} \quad (2)$$

P = Permeability coefficient.

Q = Flow = [Volume per unit time].

L = Length of the soil column.

A = Cross sectional area of the soil column.

H = Difference in head between the two ends of the column.

1.3.2.4
Determination of particle size distribution (Soil mechanical analysis)

The analysis was conducted by the sieve method described by Daubenmire (1974). A weighed amount of air-dried soil was shaken through progressively smaller-meshed sieves. The amount retained by each sieve was determined and expressed as percentage of the original weight of the sample.

1.3.3
Chemical Analysis

1.3.3.1
Determination of soil available macroelements (NPK)

1.3.3.1.1
Determination of nitrogen

Total nitrogen was estimated as per the method of Bremner (1965).

Reagents:

- Two normal potassium chloride (2*N* KCl).
- Magnesium oxide (MgO).
- Divarda alloy.
- Boric acid.
- Mixed indicator.

Procedure:

Twenty g of soil were extracted with 100 ml 2*N* KCl, shaken for 30 min., then filtered. A 25 ml aliquot of the extract was put in 500 ml macrokejedahl flask, then 100 ml distilled water were added to the extract, and in addition 1 g MgO + 0.5 g divarda alloy. Ammonia liberated, by steam distillation of the aliquot of the extract with divarda alloy, was received in a conical flask containing 4 % boric acid with a mixed indicator (see Appendix A - 6, for details). Boric acid and mixed indicator were mixed in a ratio of 100 : 1 respectively, on volumetric basis. The distillate was then titrated against a very weak (0.005*N*) HCl acid.

1.3.3.1.2
Determination of phosphorus

Phosphorus was determined by a colorimetric method devised by Olsen *et al.* (1954).

Reagent A:

- 12 g ammonium molybdate.
- 0.2908 g antimony potassium tartrate dissolved in 100 ml distilled water.
- Sulphuric acid (5*N* H_2SO_4).

Reagent B:

- Ascorbic acid (0.5 g dissolved in 100 ml of reagent A).
- 0.5 M $NaHCO_3$ at pH 8.5 as extracting solution.

Procedure:

Five g of air-dried soil were extracted with 10 ml 0.5 M $NaHCO_3$, shaken for 30 min. and then filtered. Five ml of the extract was then placed in a 25 ml volumetric flask. One drop of paranitrophenol indicator was added. The colour changed to yellow. To neutralise the liquid, few drops of 5*N* HCl were added. Four ml of reagent B were then added, to develop the "phosphorus blue colour".

Standard curve:

Standard solutions ranged from 0.01 to 1 µg ml^{-1} phosphate from potassium dihydrogen phosphate (KH_2PO_4) were prepared. The solutions were run in a Spectronic 20 spectrophotometer, using 880 nm wave length and amount of P present determined from the standard curve.

1.3.3.1.3
Determination of potassium

Potassium is present in soils in small amounts either in exchangeable or soluble forms. The available potassium is extracted from neutral and acidic soils by cold diluted sulphuric acid while from alkaline and calcareous soils by ammonium acetate.

Procedure:

Five g of soil were weighed in a conical flask, then 100 ml of 1*N* ammonium acetate were added. The solution was shaken for 5 min. and then filtered. The

leachate was analysed using Perkin-Elmer A. A. Spectrophotometer, model 305 to determine the amount of K present.

1.3.3.2
Determination of soil available microelements

The available microelements viz. zinc (Zn); iron (Fe); manganese (Mn) and copper (Cu) were determined by the method devised by Lindsay and Norvell (1978), using diethylenetriaminepentaacetic acid (DTPA), [see Appendix A - 2, for details] as the extracting solution.

Procedure:

Ten g of air-dried soil were extracted with 20 ml of DTPA, shaken for 2 h, then filtered through Whatman no. 40 filter paper. The determination of elements was carried out using Perkin-Elmer A. A. Spectrophotometer, model 305.

1.3.3.3
Determination of soil exchangeable elements

The exchangeable Ca, Mg, and Na were extracted by leaching 20 g of sieved air-dried soil with 500 ml of 1*N* ammonium acetate (CH_3COONH_4) at pH 7.0 (Davids, 1960). Determination was conducted using Perkin-Elmer A. A. spectrophotometer, model 305.

1.3.3.4
Determination of soil calcium carbonate

The percentage of $CaCO_3$ was determined by acid neutralisation as used by the United States Salinity Laboratory Staff, (USSLS) (Richard, 1954).

Reagents:

- Hydrochloric acid (HCl).
- Sodium Hydroxide (NaOH).
- Paranitrophenol indicator.

Procedure:

One g of air-dried soil was accurately weighed into 250 ml conical flask. Then 30 ml of HCl was added. The flask was shaken for sufficient time to finalise the reaction. Then 3-4 drops of paranitrophenol indicator were added, then NaOH was used for titration.

1.3.3.5
Determination of electrical conductivity and concentration of soluble salts

The conductivity of a dilute soil solution depends mainly on ions present and an approximate value for the amount of total soluble salts. The conductivity cell was dipped into 10 ml of distilled water and the variable resistance was adjusted on the dial to obtain Null Point. Sample suspensions were prepared from the different replications. Specific conductivity was read directly from dial. Concentration of the soluble salts in the sample suspension was obtained by multiplying the above readings by the cell constant.

1.3.3.6
Determination of hydrogen-ion concentration

The hydrogen-ion concentration was determined by preparing a 20 % soil solution, i. e; a ratio of 1 : 5 for soil and distilled water respectively. Then, the solution was mechanically shaken for 30 min. and the hydrogen-ion concentration measured using a pH-meter.

1.3.3.7
Determination of organic matter

One g oven-dried soil was weighed in a clean porcelain crucible. The crucibles containing the samples were heated at 400 °C and 500 °C for 7 h and 5 h, respectively. Then, reweighed to determine the organic matter content.

1.4
Plant Tissue Analysis

1.4.1
Washing of the samples

The plant samples were washed thoroughly twice or thrice with ordinary tap water in order to remove the dirt that was adhering to the plants. Next they were washed thoroughly with distilled water.

1.4.2
Drying of the samples

The samples were loosely packed in aluminium foil and dried in an oven at 60 °C for 48 h. In this process most of the water was lost from the sample, together with some volatile organic matter.

1.4.3
Grinding of the samples

Each dried plant sample was ground into a fine powder in a Wiley Mill. This mixed the samples well, so that an over-all analysis of the shoot system for each individual plant could be carried out.

1.4.4
Chemical analysis

Analysis of the plant samples was conducted by the methods described in Jackson (1958). The analysis methods were divided into:

1.4.4.1
Determination of total nitrogen

One g of the ground powder was transferred to a clean dry 800 ml Kjeldahl flask. Then 10 g potassium sulphate and 3 tablets of Kjeldahl catalyst were added. Then, 30 ml of sulphuric and salicilic acid mixture (mixed in a ratio of 25: 1 v/w, respectively) were added slowly. The mixture was left for about 4 h, so that the acid could act well on the organic matter. At the end of this period, the mixture turned black in colour, indicating that it was ready for the digestion process.

Digestion:

The flask containing the mixture was placed in a Kjeldahl apparatus and heated for 2 h. During the digestion the organic nitrogen in the sample was converted to soluble inorganic nitrogen, in the form of ammonium sulphate and some nitrates. At the end of the digestion, the digested samples were left to cool to room temperature. Then 300 ml distilled water were added with continuous shaking, in order to mix all the salts remaining at the bottom of the flask. Then 80 ml of 75 % sodium hydroxide solution and 2 g zinc powder were added. The flask was then immediately connected to the Kjeldahl distillation apparatus. The distillate was absorbed by 50 ml of 4 % boric acid. The distillation was stopped when about 300 ml of the distillate was collected in the flask. Titration of the distillate was made against 0.2*N* sulphuric acid, using bromocrysol green indicator.

Calculations:

$$N\% = (0.2 \times \text{burette reading})(\text{wt sample})^{-1} \times 100 \quad (3)$$

Burette reading = the volume of 0.2*N* H_2SO_4 needed to neutralise the distillate

1.4.4.2
Determination of K, Na, P, Ca, Mg, Fe, Cu, Mn and Zn.

Before the determination of the above elements by standard analytical methods, the samples were subjected to digestion by a tri-acid mixture. Here the samples underwent oxidation to simple salts by mixing nitric acid [annular (AR)] 63.01 %, perchloric acid (AR) 72 % and sulphuric acid (AR) 98.07 % in a ratio of 5 : 2 : 1 v/v/v, respectively (the tri-acid mixture that was used for this process is a very strong oxidising agent).

Digestion:

Dried ground plant sample of 1.0 g was transferred to a clean dry beaker. Thirty ml of tri-acid mixture were added. The beaker was covered and left overnight. After leaving the samples over-night, in contact with the acid mixture, it acquired a reddish-brown tint, indicating that the samples had undergone oxidation by the acid mixture. The mixture was heated on a hot plate till the volume of the solution came down to about 10 ml. Then the clear solution was transferred to a 100 ml volumetric flask and made up to the 100 ml mark with distilled water. The determination of K, Na, P, Ca, Mg, Mn, Fe, Cu and Zn was conducted with this solution.

1.4.4.3
Determination of P

Five ml of the solution were pipetted into a 50 ml volumetric flask. Then 10 ml

of vanadate-molybdate reagent (see Appendix A - 7, for details), were added, shaken and the solution made up to 50 ml with distilled water. The solution acquired a pale yellow tint. Phosphorus was determined colorimetrically using spectrophotometer with 455 nm wave length.

Calculations:

$$P\% = \frac{(4 \times \text{corrected absorbance})}{(\text{standard absorbance} \times \text{wt sample} \times 10)^{-1}} \times 100. \quad (4)$$

Standard absorbance = absorbance by phosphate standard (= 0.132).

2 ml of 100 ppm phosphate standard was diluted to 50 ml.

1.4.4.4
Determination of K and Na

Potassium and sodium were determined flame-photometrically.

Calculations:

$$K\% = (\text{corrected ppm})(\text{wt sample} \times 10)^{-1} \times 100 \quad (5)$$

$$Na\% = (\text{corrected ppm})(\text{wt sample} \times 100)^{-1} \times 100 \quad (6)$$

Dilution factors were taken into account for the calculations.

1.4.4.5
Determination of Fe, Cu, Mn, Zn, Ca, and Mg

The atomic absorption spectrophotometer was calibrated by preparing stock solutions of 1000 mg litre^{-1} for Fe, Cu, Mn. Zn, Ca and Mg by dissolving their weighed nitrate salts, (ultra grade), into 5% (v/v) nitric acid (S. G. 1.42).

Multielement standards of (0.5, 1.0 & 1.5 ppm) for each element of Fe, Cu, Mn, Zn & Mg and (1.0, 5.0 & 10.0 ppm) for Ca were prepared by appropriate mixing from their stock solutions. All dilutions were made with deionised distilled water. Then these elements were determined by direct reading from the atomic absorption spectrophotometer taking into consideration the dilution factors.

Calculations:

The calculations of Ca and Mg were made according to the following formula:

$$\text{Ca or Mg \%} = (\text{corrected ppm})(\text{wt sample})^{-1} \text{ X } 100 \quad (7)$$

Calculations of the micronutrients (Fe, Cu, Mn, and Zn), were worked out using the following formula:

$$\text{Micronutrients } (\mu g\ g^{-1}) = (\text{corrected ppm X } 100)(\text{wt sample})^{-1} \quad (8)$$

1.5 Growth measurements

1.5.1 Leaf area measurement

Leaf area was calculated by means of a Xerox copying technique. A standard curve was made by making photocopies of known areas drawn on graph paper. Then a Xerox copy was obtained. The area shapes (shadow copy) were cut out, weighed and the area plotted vs. their shape weights. The detached leaves were fixed on a paper, then the photocopy was made. Leaf shapes (shadow copy) were cut out, weighed and areas read directly from the standard curve.

1.6 Mycorrhizal Assessment

1.6.1 Fixing and preservation of roots

Roots were carefully teased apart from adhering soil and washed gently to remove soil particles. Fine roots were placed in plastic vials containing formalin-aceto-alcohol (FAA) fixative solution (see Appendix A - 3, for details) for at least 1 d.

1.6.2 Clearing and staining roots

Root staining was carried out according to Phillips and Hayman (1970), as follows:

FAA- preserved roots were cut into 1 cm long segments. Root segments were cleared in a 10 % KOH solution and heated to ≈ 90 °C for 1 h using a double water bath. (Pigmented roots were first placed in 1 % hydrogen peroxide for 3-5 min., depending on how pigmented the roots were). Cleared roots were then rinsed in distilled water, followed by a rinse in a weak acid solution (see Appendix A - 8, for details). Root segments were then placed in 0.05 % trypan blue in lactophenol (see Appendix A - 5, for details) and heated at about 90 ~ 100 °C until they started to boil. Roots were cooled and then placed on slides in clear lactophenol for microscopic examination.

1.6.3 Percent colonisation

Twenty segments of stained roots of *Arachis hypogaea* L. were mounted in clear lactophenol, covered and examined microscopically for the presence of AM fungi. The characteristic features searched for were vesicles, arbuscules and

coiling hyphae. When these were detected in a segment, infection was recorded to determine percent colonisation (PC) on each of 5 slides (5x20 roots). Each replicate therefore comprised 100 root segments.

1.6.4 Spore collection

Spore collection was determined by the wet sieving and decanting method devised by Gerdemann and Nicolson (1963) with some modifications. Twenty five g of soil were placed in a 750 ml and mixed thoroughly by vigorous stirring. The suspension was allowed to settle before gently decanting through a series of screens, culminating with a fine opening of 37 µm. The spores retained on the fine sieve were washed into Petri dishes. Spores were then counted and number of spores per g soil ($NOSG^{-1}S$) calculated.

CHAPTER 1

Mycorrhization of the Peanut in Sterile and Non-sterile Soil.

1
Mycorrhization of the Peanut in Sterile and Non-sterile Soil

1.1 INTRODUCTION

Mycorrhization of field crops with selected strains of arbuscular mycorrhizal (AM) fungi is highly effective in increasing host growth in field soils although results are variable according to conditions. Different species of AM fungi, when inoculated to the same host in the same soil under the same environmental conditions may give different growth responses. Mosse (1972) examined seven species of AM fungi colonising onion seedlings in one soil. These fungi exhibited significant differences in their effect on host growth.

The relationship between the level of mycorrhizal colonisation and the soil chemical and physical characteristics are also quite variable (Newman *et al.*, 1981). Mycorrhizal colonisations were reported to fluctuate with soil pH (Read *et al.*, 1976) and soil phosphorus content (Jeffries *et al.*,1988). Some species of AM fungi are adapted to acid or alkaline soils, while others occur in both acid and alkaline soils (Porter *et al.*, 1987; Robson and Abbott, 1989).

Although little is known about the interrelationships between AM fungi and ubiquitous soil-inhabiting microorganisms (Azcon, 1989), some workers have reported that soil microbiota were found to enhance germination of AM fungal spores (Azcon-Aguilar *et al.*, 1986), level of root colonisation by AM fungi (Azcon-Aguilar and Barea, 1985), and mycorrhizal plant growth (Meyer and Linderman, 1986). However, there is the contradictory evidence that the soil microbiota suppresses plant growth (Hetrick *et al.*, 1986, 1987), mycorrhizal root colonisation (Krishna *et al.*, 1982 ; Hetrick *et al.*, 1986), sporulation of mycorrhizas (Ross, 1980) and mycorrhizal fungal spore germination (Tommerup, 1985 ; 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 mycorrhizal colonisation, nutrition and growth of peanut plants (cv. Virginia Bunch). They found that a range of soil sterilisation methods influenced mycorrhizal colonisation of peanut roots to varying degrees. Production of plant dry weight and number and weight of reproductive structures were reduced to varying extents, depending on the effect that the respective sterilisation methods had on subsequent levels of mycorrhizal colonisation. Significantly, these growth reductions could be overcome by inoculation of the sterilised soil with AM fungal spores.

That peanuts benefit from mycorrhizal association and significant increases in dry matter yield, phosphorus uptake and stimulation of root and shoot growth has been shown by Rao *et al.*,1990. This study intends to assess mycorrhization, performance and interactions of two AM fungi namely ***Glomus mosseae*** and ***Glomus fasciculatum*** without and with the soil microbiota in sterile and non-sterile soil with ***Arachis hypogaea*** L. [var. *hypogaea* cv. Florunner]. Sudangrass was used for inoculum production. An important consideration for the selection of this plant as a trapping host is that this plant is a member of the family Graminae which is known to have no common pathogenic root fungi with legumes and other dicotyledonous crops (Sieverding,1991). Also, its extensive root system results in more mycorrhiza formation (Simpson and Daft, 1996).

1.2 METHODS

1.2.1 Substrate Soil

The substrate soil was collected, formulated and analysed as described in methods and materials section [1.3]. The experiments utilised three treatments

of substrate soil:-

1.2.1.1
Non-autoclaved soil

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

1.2.1.2
Autoclaved soil

The soil was autoclaved for 1 h at 121 °C under pressure of 10^5 Pa followed by a second 1 h autoclaving 24 h later.

1.2.1.3
Autoclaved soil plus soil microbiota

The autoclaved soil was amended with original soil microbiota to each pot by mixing 25 g of non-autoclaved soil in about 100 ml distilled water. The soil suspension was decanted 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 Buchnner funnel apparatus. The filtered suspension contained colony-forming units (CFU) ml^{-1} of 900 fungi and 5.5 X 10^5 CFU ml^{-1} bacteria, as estimated by dilution-plating sieved suspensions onto either peptone yeast extract agar or potato dextrose agar (see Appendix B - 2 & 3, for details). The potato dextrose agar medium was amended with 100 µg ml^{-1} each of streptomycin sulphate and chloramphenicol. Colonies were counted after 7 d at 24 °C.

1.2.2
Experimental Design

Each treatment of substrate soil received the following three treatments in a completely randomised design. Each treatment contained 20 replicates. In the

first treatment, 60 g of crude inoculum (propagated as described in Materials and Methods;1.1) containing roots and soil of Sudangrass infected with *Glomus mosseae*, was used as the mycorrhization inoculum for each pot. The second was inoculated with 60 g of soil containing roots and soil of Sudangrass infected with *Glomus fasciculatum* to each pot, while in the third (control treatment), 60 g of sterilised soil were added to each pot. The spore density ($NOSG^{-1}S$) of the two candidate AM fungi were adjusted to the level of 12 ± 1 spores g^{-1} by diluting the propagated inocula with sterilised sandy soil. The adjusted inocula were completely and thoroughly mixed into the top 5 cm of the soil of the pots.

Seeds of *Arachis hypogaea* L. were pregerminated in perlite. Each experimental pot was hand seeded with two pregerminated seeds into 15 cm (diameter) plastic pot filled with 3.0 kg of appropriate substrate soil. To exclude intraspecific competition, seven days later, each pot was thinned to one plant. The pots were placed in the greenhouse (see Materials and Methods; 1.2).

After 9 wk, the plants were harvested and the growth indices [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 ($ROGW^{-1}$)], measured. Roots were collected and preserved in formalin-aceto-alcohol (FAA) fixative solution, cleared and stained (see Materials and Methods; 1.6.1 and 1.6.2). The progress of mycorrhization was assessed by % colonisation (PC) [see Materials and Methods; 1.6.3] and number of spores produced g^{-1} soil ($NOSG^{-1}S$) [see Materials and Methods; 1.6.4].

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1.2.3
Data Analysis

The differences in the means of growth i
number of spores per g soil between treatm
Fisher's least significant difference (LSD) te
(ANOVA).

1.3
RESULTS

The physico-chemical properties of the subs
indicated that the substrate soil was calca
alkaline (pH = 7.9), but poor in both macro
especially phosphorus (6 µg P g^{-1} soil). The
sandy clay loam with reasonably good perm

The growth levels of mycorrhizal and
were estimated to be about the same in t
substrate soil. Then the growth rate per wee
Glomus fasciculatum-inoculated peanuts wer
controls but *Glomus mosseae*-inoculated pea
more vigorous than *Glomus fasciculatum*-ino
substrate soil.

1.3.1
Effects of AM fungi on peanut growth irres
(Treatments A1, B4, C7, A2, B6, C8 Plate ; 1.3.1

The influence of AM fungi on peanut g
reference to the effect of the various soil tre
the data accumulated for peanuts grown in t

Table 1.3.1

Physical properties of the substrate soil.

Porperties	Moisture Content M.C.	Total Water Holding Capacity TWHC	Water Permeability W.P	Particle Size Distribution			
				Coarse Sand	Fine Sand	Silt	Clay
	(%)*	(%)*	(cm/s)	(%)	(%)	(%)	(%)
Means ± S.E.	3.29 ± 0.09	20.00 ± 1.15	1.86X10-3 ± 0.59X10-3	27.70 ± 7.20	48.70 ± 2.12	3.30 ± 0.86	20.20 ± 5.11

Texture Class	Sandy Clay Loam

Means are readings of five replicates ± standard errors (S.E.).

* On dry weight basis.

Table 1.3.2
Chemical properties of the substrate soil.

Chemical Properties							
Properties	Available Macro-Elements			Available Micro-Elements			
	Nitrogen	Phosphorus	Potassium	Iron	Zinc	Manganese	Copper
	(µg/g soil)			(µg/g soil)			
Means ±	40.47 ±	6.00 ±	83.40 ±	10.68 ±	0.22 ±	1.35 ±	0.28 ±
S.E.	3.38	0.14	1.21	4.39	0.07	0.30	0.05

(Continuation)							
Exchangeable Elements			Calcium Carbonate	Electrical Conductivity	Soluble Salts	pH	Organic Matter
Sodium	Calcium	Magnesium					
(µg/g soil)			(%)	(mmho/cm)	(µg/ml)		(%)
58.08 ±	3,782.20 ±	76.5 ±	0.88 ±	0.094 ±	280 ±	7.90 ±	1.00 ±
3.68	30.91	3.47	0.03	0.02	7.00	0.16	0.01

- Means are readings of five replicates ± standards errors (S.E.)
- ¶ mmoh/cm = dS/m
- P was extracted by 0.5 M $NaHCO_3$
- Fe; Zn; Mn and Cu were extracted by DTPA
- Exhangeable elements were extracted using 1 normal ammonium acetate [1N HN_3COONH_4]
- Determination of elements were conducted by Spectronic 20 for P and Perkin-Elmer A.A. Spectrophotometer, model 305 for other elements

the presence of *Glomus mosseae* (Treatments A1, B4, C7; Plate 1.2.1) with *Glomus fasciculatum*-inoculated peanuts (Treatments A2, B6, C8; Plate 1.2.1)

Glomus mosseae-inoculated peanuts showed a significantly greater growth ($P \leq 0.05$) compared with the controls and *Glomus fasciculatum*-inoculated peanuts as indicated by growth indices of number of tillers per plants, shoot height per plant, shoot dry weight per plant, number of lateral branches per plant, number of leaves per plant, leaf area per leaf, root fresh weight per plant, root dry weight per plant, root/shoot dry weight ratio and rate of growth per week. *Glomus mosseae*-inoculated peanuts exceeded the controls on the additional index of length of lateral branch per plant. *Glomus fasciculatum*-inoculated peanuts showed significantly greater growth increase ($P \leq 0.05$) compared with the controls as measured by indices of shoot height per plant, shoot fresh weight per plant, number of lateral branches per plant, length of lateral branch per plant, number of leaves per plant, root fresh weight per plant and rate of growth per week (Table 1.3.3).

The levels of mycorrhizal colonisation and sporulation of *Glomus mosseae* were significantly higher ($P \leq 0.05$) compared with *Glomus fasciculatum* fungus as measured by indices of percent colonisation (PC) and number of spores per g soil ($NOSG^{-1}S$) [Table 1.3.3] .

1.3.2

Effect of conditions of substrate soil on peanut growth (A, B, C; Plate 1.3.1)

This analysis compared the influence of the substrate soil and the soil microbiota on peanut growth when grown in the autoclaved soil amended with the original soil microbiota, autoclaved soil and non-autoclaved soil regardless of AM fungal inoculation effects; The autoclaved soil amended with soil microbiota increased peanut growth significantly ($P \leq 0.05$) over autoclaved soil and non-autoclaved soil as measured by the indices number of tillers per plant,

Table 1.3.3
Influence of AM fungi on growth indices and % colonisation and sporulation.

Growth Indices ¶		NOT/P (cm)	SH/P (cm)	SFW/P (g)	SDW/P (mg)	NOLB/P (No.)	LOLB/P (cm)	NOL/P (No.)
Treatment	*G.mosseae*	2.0 a* ± (0.14)	37.2 a ± (1.43)	7.7 a ± (0.378)	848 a ± (60.586)	7.0 a ± (0.291)	7.5 a ± (0.334)	32 a ± (1.165)
	G.fasciculatum	1.0 b ± (0.129)	31.1 b ± (1.30)	6.4 a ± (0.306)	680 b ± (48.51)	6.0 b ± (0.311)	6.9 a ± (0.327)	26 b ± (1.151)
	Control	1.0 b ± (0.96)	23.5 c ± (1.43)	4.1 a ± (0.382)	611 b ± (40.363)	5.0 c ± (0.325)	5.9 b ± (0.421)	22 c ± (1.235)
LSD Values		0.3	4.0	1.6	141.0	1.0	1.0	3.0

Growth Indices ¶		LA/L (cm2)	RFW/P (mg)	RDW/P (mg)	R/SDWR Ratio	ROG/W (cm)	PC (%)	NOS/gSoil (No.)
Treatment	*G.mosseae*	1.84 a* ± (0.013)	925 a ± (48.386)	294 a ± (28.427)	0.35 a ± (0.094)	3.6 a ± (0.129)	73.0 a ± (1.186)	10.0a ± (0.0662)
	G.fasciculatum	1.5 b ± (2.186E-3)	737 b ± (54.413)	215 b ± (19.054)	0.30 b ± (0.044)	3.2 b ± (0.93)	62.0 b ± (1.922)	7.0 b ± (0.324)
	Control	1.5 b ± (2.037E-3)	560 c ± (41.712)	166 b ± (16.725)	0.27 b ± (0.037)	2.8 c ± (0.89)	0.0 c	0.0 c
LSD Values		0.21	135.0	61.4	0.05	0.27	4.3	1.0

NOT/P: Number of tillers per plant; SH/P: Shoot height per plant
SFW/P:Shoot fresh weight per plant; SDW/P: Shoot dry weight per plant
RFW/P: Root fresh weight per plant ; RDW/P: Root dry weight per plant
NOL/P: Number of leaves per plant.
LOLB/P : Length of lateral branch per plant; NOLB/P: number of lateral branches per plant
LA/L: Leaf area per leaf; R/SDWR: Root/shoot dry weight ratio; ROG/W: Rate of growth per week
PC: Percent colonisation ; NOS/g Soil: Number of spores per gram soil

¶ Means ± standard errors.
* Numbers followed by different letters in a given column are significantly different according to Fisher's LSD through ANOVA test.

shoot height per plant, number of lateral branches per plant, number of leaves per plant, leaf area per leaf, root/shoot dry weight ratio and rate of growth per week. Autoclaved soil amended with the original soil microbiota exceeded the non-autoclaved soil on additional indices of shoot fresh weight per plant, shoot dry weight per plant and root fresh weight per plant, and root dry weight per plant. The autoclaved soil appeared to produce significant stimulation in growth of peanuts compared with peanuts grown in non-autoclaved natural substrate soil as measured by the indices, shoot fresh weight per plant, shoot dry weight per plant, number of lateral branches per plant, number of leaves per plant, root fresh weight per plant and rate of growth per week, (treatments A, B, C; Plate 1.3.1 and Table 1.3.4).

Table 1.3.4
Influence of substrate soils on peanut growth.

Growth Indices ¶		NOT/P (cm)	SH/P (cm)	SFW/P (g)	SDW/P (mg)	NOLB/P (No.)	LOLB/P (cm)
substrate	Autoclaved Soil + Micro-organisms	**2.0 a*** ± (0.124)	**35.2 a** ± (1.437)	**6.25 a** ± (0.406)	**797.6 a** ± (50.811)	**7.0 a** ± (0.296)	**7.2 a** ± (0.331)
substrate	Autoclaved Soil	**1.0 b** ± (0.111)	**30.0 b** ± (1.609)	**6.83 a** ± (0.396)	**746.2 a** ± (51.52)	**6.0 b** ± (0.318)	**6.5 a** ± (0.387)
substrate	Non-autoclaved Soil	**1.0 b** ± (0.106)	**28.6 b** ±(1.479)	**5.10 b** ± (0.376)	**594.8 b** ± (50.461)	**5.0 c** ± (0.284)	**6.7 a** ± (0.301)
LSD Values ¥		**0.3**	**4.0**	**1.1**	**141.0**	**1.0**	**1.0**

Growth indices ¶		NOL/P (No.)	LA/L (cm2)	RFW/P (mg)	RDW/P (mg)	R/SDWR Ratio	ROG/W (cm)
substrate	Autoclaved Soil + Micro-organisms	**30.0 a** ± (1.223)	**1.6 a*** ± (0.011)	**946 a** ± (50.352)	**268.3 a** ± (28.789)	**0.35 a** ± (0.034)	**3.6 a** ± (0.129)
substrate	Autoclaved Soil	**27.0 b** ±(1.388)	**1.3 b** ± (6.186E-3)	**811.0a** ± (54.32)	**214.2 ab** ± (21.64)	**0.29 b** ± (0.049)	**3.2 b** ± (0.93)
substrate	Non-autoclaved Soil	**24.0 c** ± (1.123)	**1.3 b** ± (6.186E-3)	**663.8b** ± (49.995)	**192.0 b** ± (15.566)	**0.32 b** ± (0.093)	**2.8 c** ± (0.89)
LSD Values		**3.0**	**0.21**	**135.0**	**61.4**	**0.05**	**0.27**

NOT/P: Number of tillers per plant; SHP-1: Shoot height per plant
SFW/P:Shoot fresh weight per plant; SDWP-1: Shoot dry weight per plant
RFW/P: Root fresh weight per plant ; RDW/P: Root dry weight per plant
NOL/P: Number of leaves per plant.
LOLB/P : Length of lateral branch per plant; NOLB/P: number of lateral branches per plant
LA/L: Leaf area per leaf; R/SDWR: Root/shoot dry weight ratio; ROG/W: Rate of growth per wk

¶ Means ± standard errors.
* Numbers followed by different letters in a given column are significantly different according to Fisher's LSD through ANOVA test.

1.3.3
Interactions between Glomus mosseae, peanuts and conditions of substrate soil (Treatments A1, B4, C7, Plate 1.3.1)

Considering the interactions of *Glomus mosseae* versus substrate soil on peanut growth when added to the autoclaved soil, non-autoclaved soil and autoclaved soil with added back soil microbiota, it was found that the performance of peanuts with *Glomus mosseae* was significantly higher ($P = 0.05$) in autoclaved soil with added back soil microbiota compared with the other two substrate soils as measured by growth indices number of tillers per plant, shoot height per plant, shoot dry weight per plant, number of lateral branches per plant, length of lateral branch per plant, number of leaves per plant, root fresh weight per plant and rate of growth per week. Growth of peanuts on autoclaved soil with added back microbiota exceeded that on non-autoclaved soil on the additional indices of shoot fresh weight per plant, leaf area per leaf, root dry weight per plant and root/ shoot dry weight ratio. When *Glomus mosseae* was added to the autoclaved soil, it gave a significant growth increase ($P = 0.05$) in peanuts compared with peanuts grown in non-autoclaved natural substrate soil as indicated by shoot height per plant, shoot fresh weight per plant, shoot dry weight per plant and root/shoot dry weight ratio (A1, B4, C7; Plate 1.3.1 ; Table 1.3.5).

Glomus mosseae had significantly higher root colonisation and sporulation levels ($P = 0.05$) in autoclaved soil amended with soil microbiota compared with autoclaved and non-autoclaved soils. Nevertheless, the level of root colonisation and sporulation in autoclaved soil were significantly higher compared with non-autoclaved soil (Table 1.3.5).

Table 1.3.5
The interaction of *G.mosseae* vs. conditions of substrate soil on growth indices and % colonisation and sporulation.

Growth Indices ¶		NOT/P (cm)	SH/P (cm)	SFW/P (g)	SDW/P (mg)	NOLB/P (No.)	LOLB/P (cm)	NOL/P (No.)
Substrate	Autoclaved Soil + Micro-organisims	3.0 a* ± (0.167)	44.2 a ± (1.54)	9.0 a ± (0.423)	1042 a ± (18.56)	9.0a ± (0.418)	8.8 a ± (0.347)	36 a ± (1.664)
	Autoclaved Soil	1.0 b ± (0.2)	37.2 b ± (2.682)	8.4 a ± (0.608)	887.6 b ± (12.47)	6.0 b ± (0.296)	6.7 b ± (0.657)	31 b ± (1.278)
	Non-autoclaved Soil	1.0 b ± (0.209)	30.4 c ± (2.581)	5.5 b ± (0.647)	614.5 c ± (11.04)	6.0 b ± (0.476)	7.0 b ± (0.405)	30 b ± (1.905)
LSD Values ¥		0.3	4.0	1.6	141.0	1.0	1.0	3.0

Growth indices ¶		LA/L (cm2)	RFW/P (mg)	RDW/P (mg)	R/SDWR Ratio	ROG/W (cm)	PC (%)	NOS/g Soil (No.)
Substrate	Autoclaved Soil + Micro-organisims	1.9 a* ± (0.011)	1053 a ± (19.42)	280 a ± (13.626)	0.27 a ± (0.096)	5.0 a ± (0.039)	95 a ± (0.204)	14 a ± (0.204)
	Autoclaved Soil	1.8 ab ± (6.443E-3)	879 b ± (21.74)	244 ab ± (18.231)	0.28 a ± (0.054)	3.0 b ± (0.027)	73 b ± (0.542)	9 b ± (0.131)
	Non-autoclaved Soil	1.7 b ± (1.630E-3)	841 b ± (21.724)	205 b ± (15.49)	0.33 b ± (0.056)	2.8 b ± (0.025)	54 c ± (0.766)	6 c ± (0.356)
LSD Values		0.21	135.0	61.4	0.05	0.27	4.3	1.0

NOT/P: Number of tillers per plant; SH/P: Shoot height per plant
SFW/P: Shoot fresh weight per plant; SDW/P: Shoot dry weight per plant
RFW/P: Root fresh weight per plant ; RDW/P: Root dry weight per plant
NOL/P: Number of leaves per plant.
LOLB/P : Length of lateral branch per plant; NOLB/P: number of lateral branches per plant
LA/L: Leaf area per leaf; R/SDWR: Root/shoot dry weight ratio; ROG/W: Rate of growth per week
PC: Percent colonisation ; NOS/g soil : Number of spores per gram soil

¶ Means ± standard errors.
* Numbers followed by different letters in a given column are significantly different according to Fisher's LSD through ANOVA test.

1.3.4
Interactions between Glomus fasciculatum, peanuts and conditions of substrate soil (Treatments A2, B6, C8; Plate 1.3.1).

Considering the interactions of ***Glomus fasciculatum*** versus the conditions of substrate soil on peanut growth, it was found that with ***Glomus fasciculatum***, peanut growth showed a significantly greater increase ($P \leq 0.05$) in the autoclaved soil amended with original soil microbiota compared with peanuts grown in the autoclaved soil and non-autoclaved soil as measured by the indices: number of tillers per plant, shoot height per plant, number of lateral branches per plant, number of leaves per plant, root fresh weight per plant and rate of growth per week. Growth of peanuts on autoclaved soil with added back microbiota exceeded that on non-autoclaved soil on the additional index of root/shoot dry weight ratio. ***Glomus fasciculatum***-inoculated peanuts in the autoclaved soil displayed a significant growth increase compared with non-autoclaved natural soil as indicated by indices of number of leaves per plant and root/shoot dry weight ratio (Treatments A2, B6, C8; Plate 1.3.1 ; Table 1.3.6).

The levels of colonisation and sporulation of ***Glomus fasciculatum*** were significantly higher ($P \leq 0.05$) in autoclaved soil amended with soil microbiota compared with the other two substrate soils. However, the % colonisation and sporulation of this fungus was still significantly higher ($P \leq 0.05$) in autoclaved soil compared with non-autoclaved soil (Table 1.3.6).

In general, one can conclude from these results that both ***Glomus mosseae*** and ***Glomus fasciculatum*** were infective to ***Arachis hypogaea*** L. [var. *hypogaea* cv. Florunner], but ***Glomus mosseae*** was the major sporulator with highest % colonisation level and more effective in increasing peanut growth on several indices. The autoclaved soil amended with soil microbiota was the best substrate for both AM fungi and peanut growth.

Table 1.3.6
The interaction of *G. fasiculatum* vs. conditions of substrate soil on growth indices and % colonisation and sporulation.

Growth indices ¶		NOT/P (cm)	SH/P (cm)	SFW/P (g)	SDW/P (mg)	NOLB/P (No.)	LOLB/P (cm)	NOL/P (No.)
substrate	Autoclaved Soil + Micro-organisms	2.0 a* ± (0.222)	35.5 a ± (1.437)	7.0 a ± (0.492)	770.5 a ± (15.811)	5.0 a ± (0.772)	7.2 a ± (0.701)	31 a ± (3.134)
substrate	Autoclaved Soil	1.0 b ± (0.209)	30.1 b ± (1.609)	6.2 a ± (0.588)	595.5 b ± (15.52)	7.0 b ± (0.431)	6.7 a ± (0.443)	22 b ± (1.724)
substrate	Non-autoclaved Soil	1.0 b ± (0.179)	27.8 b ± (1.479)	6.1 a ± (0.613)	675 ab ± (12.461)	7.0 b ± (0.613)	6.8 a ± (0.592)	25 c ± (2.467)
LSD Values		0.3	4.0	1.6	141.0	1.0	1.0	3.0

Growth indices ¶		LA/L (cm2)	RFW/P (mg)	RDW/P (mg)	R/SDWR Ratio	ROG/W (cm)	PC (%)	NOS/gSoil (No.)
substrate	Autoclaved Soil + Micro-organisms	1.5 a* ± (0.18)	994.4 a ± (11.097)	251 a ± (14.63)	0.33 a ± (0.088)	4.2 a ± (0.020)	73 a ± (0.651)	9a ± (0.359)
substrate	Autoclaved Soil	1.5 a ± (3.983E-3)	668.7 b ± (9.632)	221 ab ± (15.638)	0.37 a ± (0.037)	2.8 b ± (0.037)	63 b ± (0.534)	7 b ± (0.0.266)
substrate	Non-autoclaved Soil	1.5 a ± (5.061E-3)	548.1 b ± (9.267)	173 b ± (10.649)	0.26 b ± (0.037)	2.6 b ± (0.038)	51 c ± (0.669)	4c ± (0.260)
LSD Values		0.21	135.0	61.4	0.05	0.27	4.3	1.0

NOT/P: Number of tillers per plant; SH/P: Shoot height per plant
SFW/P: Shoot fresh weight per plant; SDW/P: Shoot dry weight per plant
RFW/P: Root fresh weight per plant ; RDW/P: Root dry weight per plant
NOL/P: Number of leaves per plant.
LOLB/P: Length of lateral branch per plant; NOLB/P: number of lateral branches per plant
LA/L: Leaf area per leaf; R/SDWR: Root/shoot dry weight ratio; ROG/W: Rate of growth per week
PC: Percent colonisation ; NOS/g soil: Number of spores per gram soil

¶ Means ± standard errors.
* Numbers followed by different letters in a given column are significantly different according to Fisher's LSD through ANOVA test.

A = Autoclaved substrate soil amended with soil microbiota

1: Glomus mosseae-inoculated peanut plant

2: Glomus fasiculatum-inoculated peanut plant

3: Control

B = Autoclaved soil substrate

4: Glomus mosseae-inoculated peanut plant

6: Glomus fasiculatum-inoculated peanut plant

5: Control

C = Non-autoclaved soil substrate

7: Glomus mosseae-inoculated peanut plant

8: Glomus fasiculatum-inoculated peanut plant

9: Control

Plate 1.3.1
Comparison of vegetative growth among mycorrhizal peanut plants and controls in the three soil conditions.

1.4
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 both macronutrients and micronutrients especially phosphorus (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,1983) and fungus sporulation (Douds and 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* and AM fungi, and the tripartite relationship of host-*Rhizobium*-AM fungi is unlike either dipartite symbiosis. When legumes are symbiotic with both *Rhizobium* 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 (see Materials and Methods; 1.3). Such virgin soil, which has never been cultivated with peanuts before, was not anticipated to incorporate any efficient strains of *Rhizobium* that could develop symbiosis with peanuts. The soil properties were also discouraging to build a tripartite relationship of peanut-*Rhizobium*-AM fungi as nodulation of leguminous plants is adversely influenced by the poor content of macronutrients (NPK) or micronutrients (for example molybdenum, boron, zinc, manganese and cobalt). Also, the formation of the nodule may be influenced by pH of the soil, salinity and antagonistic microorganisms (Subb Rao, 1982), soil P content (Farnco,1977) and fungicide applications (Anderson, 1978). Whatever the reason no rhizobial nodulation was observed in any peanut plant through out the experiments. Therefore to screen the mycorrhizal development and effects on growth of peanut without

these complications, the trials in this study were limited to the dipartite rather than a tripartite relationship.

Visual estimates of the growing peanuts indicated that growth responses to AM fungal inoculation by (*Glomus mosseae* and *Glomus fasciculatum*) were the same for all treatments and controls in the first month. This may be because the AM fungi either grew initially as parasites or were slow to get established. Clearly initially they were not effective in increasing the transfer of P and perhaps other heavy immobile elements which can not move readily by diffusion to the rhizosphere 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 which the control peanut plants were extremely stunted and grew poorly. So only after an initial lag phase do AM fungi become beneficial symbiotic microorganisms that increased the growth and plant biomass of mycorrhizal host plant. There is evidence that this is caused by their increasing P uptake mainly (Mosse, 1973b).

The influence of AM fungi on growth indices revealed that *Glomus mosseae* was the most effective fungus in stimulating peanut growth when compared with *Glomus fasciculatum*. The *Glomus mosseae*-inoculated peanuts were generally taller and more vigorous than those of the *Glomus fasciculatum*-inoculated treatments. This could be attributed to the fact that *Glomus mosseae* is considered to be more effective in alkaline and calcareous soils such as the substrate soil used in these experiments (with a pH of 7.90, ≈1 % $CaCO_3$ and 3782 µg Ca g^{-1} soil) than *Glomus fasciculatum* which is more adapted to acid soils (Hayman,1983 ; Siqueira *et al.*, 1984 ; Khaliel, 1990 ; Safir *et al.* 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 mechanism and interaction of soil microorganisms with AM fungi are not well understood, the work reported here reinvestigated the

question of whether the improved growth obtained by mycorrhizal plants inoculated with crude inoculum (roots and soil of plant infected with an AM fungus) is due to AM fungus alone or to the cumulative effects of the mycorrhizal fungus and the associated original soil microorganisms. Generally nonmycorrhizal and mycorrhizal peanut growth was less in non autoclaved soils compared with autoclaved soils and amended autoclaved soils 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 were detected in the root tissue. So there is therefore 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 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 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 amended 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 peanuts because less microbial inter and/or intraspecific competition would be expected in this soil. At the same time it seemed also to improve mycorrhizal formation as indicated by the significantly higher % colonisation and spore population number ($NOSG^{-1}S$) 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 (Lopez-Aguillon and Mosse, 1987 ; Hepper *et al.*, 1988). Also, the AM fungi in the nonsterile 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 (Paulitz and Linderman, 1991). This argument could explain the low percent colonisation 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 ***Glomus mosseae*** over ***Glomus fasciculatum*** in the autoclaved substrate soil amended with soil microbiota. This finding is similar to the results obtained by Azcon *et al.* (1990). They observed that soil microorganisms increased the infection by ***Glomus mosseae*** and decreased the establishment of ***Glomus fasciculatum*** in the roots of ***Medicago sativa.*** The results also showed that the soil microbiota amendments to the autoclaved soil contributed to the success of mycorrhizal sporulation (production of spores per g soil) and high percent colonisation of peanut plants which would have had a positive impact on mycorrhization of ***Arachis hypogaea*** L. and supports the observations of earlier workers that some soil microorganisms were found to enhance sporulation of arbuscular mycorrhizal fungi (Ross, 1980), mycorrhizal root colonisation (Azcon-Aguilar and Barea 1985) and mycorrhizal plant growth (Meyer and Linderman, 1986). Although it is clear that soil microbiota are able to suppress mycorrhizal responses, the mechanism(s) responsible for this phenomenon remains a mystery (Johnson, 1993). Therefore, further studies are encouraged towards understanding these interactions to manage favourable conditions for development of AM fungi.

Peanut plants in non autoclaved soil showed only reduced growth

despite the fact that there was no disease in the plants grown in non autoclaved soil. This soil contains many more microbes than either of the other soils because of autoclaving and only adding back few microbes. It will also contain indigenous AM fungi which were removed by autoclaving or filtering in other two soils. So possible reasons for reduced growth in non autoclaved soil compared to both autoclaved soil treatments are:

- Less nutrients available because of microbial competition.
- Less nutrients available because autoclaving releases some.
- Indigenous AM fungi suppresses mycorrhization.
- Combination of these.

At the same time these factors are not compensated for by increased microbial mineralisation, because when absent in autoclaved soil, plants did better than in non-autoclaved soil.

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. 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 Chapter 1 have established that inoculation with the two AM fungi had different effects on the growth indices of peanuts in substrate soils with the same and different microbial biomass content. Also, it documented that the introduced AM fungus *Glomus 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 while they do better in autoclaved soil, but generally they do best in autoclaved soil with added back microbiota (lacking indigenous AM fungi). These findings establish the potential for mycorrhization with *Glomus*

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.

CHAPTER 2

Mycorrhizal Dependency and Mineral Uptake of the Peanut.

2
Mycorrhizal Dependency and Mineral Uptake of the Peanut

2.1 INTRODUCTION

Some plant species require either a mycorrhizal association or addition of P and perhaps some other nutrients to maintain growth. These are the obligately mycorrhizal species or mycorrhizal dependent species. Plant mycorrhizal dependency has been defined as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of soil fertility" (Gerdemann, 1975). Plenchette *et al.* (1983) proposed a new method for calculating mycorrhizal dependency known as relative mycorrhizal dependency (RMD) index by which values range from 0 % to 100 % as plants range from fully independent to fully dependent on mycorrhizas for growth. They suggested that RMD can be measured by expressing the difference between the dry mass of the mycorrhizal plant and the dry mass of the nonmycorrhizal plant as a percentage of the dry mass of the mycorrhizal plant.

Mycorrhizal dependency of plants is affected by several factors, including root morphology (Mosse and Hayman, 1980 ; Maronek *et al.*, 1980 and Hayman, 1983), plant species or varieties (Krishna *et al.*, 1985), and phosphorus levels in the soil and within the host tissues (Strobel *et al.*, 1982 ; Lopes *et al.*, 1985). Some legume plants with long roots receive less benefit from mycorrhizal formation than legumes with short roots (Crush, 1974). However, it is difficult to explain mycorrhizal dependency on the basis of root morphology, root number, or root surface area alone. Hall (1975) reported that

tomato is highly dependent on mycorrhizas yet this plant had the longest roots and the largest root surface area of the species he tested.

Phosphorus level in the soil and in the host tissue is one of the most important factors affecting the mycorrhizal dependency of different plant genera (Menge *et al.*, 1980, 1982 ; Strobel *et al.*, 1982, Lopes *et al.*, 1985). Several attempts have been made to determine mycorrhizal dependency either at native P concentration or at various added phosphate concentrations (Habte and Manjunath, 1991). A plant species characterised as having a particular degree of mycorrhizal dependency in one soil is bound to have an entirely different degree of mycorrhizal dependency in another soil depending on the concentration of available P in the soils (Aziz and Habte, 1987). Therefore, a clear evaluation of the mycorrhizal dependency of plant species is best accomplished when AM fungi and host species are allowed to interact across a gradient of established soil P concentrations (Habte and Manjunath, 1991).

The study of RMD of AM fungal hosts would be useful when considering practical mycorrhization of crop plants in low-input agricultural production systems where chemical fertilisers, if available, may not be economically affordable. Knowledge on the mycorrhizal dependency of host species is essential to a predictable management of the AM symbiosis for enhanced plant productivity (Habte *et al.*, 1993). Therefore this work was undertaken to study mycorrhizal dependency of ***Arachis hypogaea*** L. [var. *hypogaea* cv. Florunner] across a range of soil P fertility with emphasis on beneficial physical and chemical effects generated by ***Glomus mosseae*** which may enhance the nutrition and growth of the peanut within the tested range of soil P levels.

2.2
Methods

2.2.1
Methods and Experimental Design

The autoclaved substrate soil amended with the original soil microbiota was formulated as described previously (Chapter 1; Methods; 1.2.1.3) for use in these experiments. The original native P content was determined after autoclaving of the soil and mixing with peat moss (Materials and Methods; 1.3.1) by the method of Olsen *et al.* (1954). The base line was found to be 6 µg P g^{-1} soil.

Six P levels were prepared by adding superphosphate. The soil P levels used were (1st) 6 µg P g^{-1} [the P base line content in soil/peat moss mixture substrate without addition of superphosphate, see Materials and Methods; 1.3.1; (2nd) 30 µg P g^{-1} soil (24 µg P as superphosphate); (3rd) 60 µg P g^{-1} soil (54 µg P as superphosphate); (4th) 120 µg P g^{-1} soil (114 µg P as superphosphate); (5th) 240 µg P g^{-1} soil (234 µg P as superphosphate) and (6th) 480 µg P g^{-1} soil (474 µg P as superphosphate). The conversion of weight of added superphosphate to weight of phosphorus was calculated on the basis that superphosphate fertiliser contains 46% P_2O_5, of which 43.7% is phosphorus. The range of fertiliser treatments was based on the reference value for the rate of superphosphate application to peanut fields in Saudi Arabia which is 350 kg superphosphate ha^{-1} (equivalent to 30 µg P g^{-1} soil when converted to P) and the fact that P fixation as calcium phosphate in alkaline calcareous Saudi soils is 75% (Al-Mustafa 1989).

Adjusted P fertilised substrate soils were apportioned into 1.0 kg plastic pots. *Glomus mosseae*, maintained on Sudangrass as described before

[Materials and Methods; 1.1], served as inoculum. Ten g of the Sudangrass root -soil mixture were placed at 5-cm under the soil surface of each of the 120 pots that constituted the six mycorrhizal treatments. Each of the 120 nonmycorrhizal pots received 10 g of autoclaved mycorrhizal inoculum. The original soil microbiota other than the native mycorrhizal fungi were added back to all mycorrhizal and nonmycorrhizal pots as described previously [Chapter 1; Methods, 2.1.1.3]. The pots were placed randomly in the greenhouse. The experiment was conducted in a completely randomised design with 40 replications per treatment (20 mycorrhizal and 20 nonmycorrhizal) in each of the six P treatments for a total of 240 pots.

For each of the six P treatments, mycorrhizal and nonmycorrhizal plant height was recorded at weekly interval for the last 5 wk, before harvest. At harvest, after 14 wk, the plants were carefully freed from soil and fine rootlets of 5 randomly selected mycorrhizal plants from each P treatment were collected to determine the percent colonisation (PC). Collected composite samples of ≈ 1 g roots were washed with tap water and fixed in FAA solution in plastic vials for at least 24 h and stained in 0.05 % lactophenol according to the method of Phillips and Hayman (1970). Development of the AM fungus was assessed through PC and $NOSG^{-1}S$ indices.

The whole plants including shoot and root systems (n = 29; 12 mycorrhizal and 17 nonmycorrhizal) were weighed to determine both the FWP^{-1} and DWP^{-1} for each P treatment. The difference in fresh and dry weight per plant between mycorrhizal and nonmycorrhizal plants within each P treatment was expressed as percent increase over nonmycorrhizal plants as follows:-

$$\% \text{ Increase} = (M - NM)(NM)^{-1} \times 100 \quad (9)$$

2.2.2
Collection of Samples and Determination of Nutrient Content

Six plants were randomly selected as replicate samples for each P treatment (n = 6; 3 mycorrhizal, 3 nonmycorrhizal). Selected plant samples were cut at soil level to detach the shoots from the roots. The root systems were excluded from the analysis to avoid ion contamination that may have resulted from soil particles adhering to the roots. The analysis was carried out using all the above ground parts (stems, branches and leaves). Before analysis of the samples was carried out, the plants were washed, dried and ground as described previously (Materials and Methods; 1.4).

Calculation of P % increase:

The difference in P content between mycorrhizal and nonmycorrhizal within each P treatment were worked out as % increase from the respective nonmycorrhizal nutrient content as per the following equation:

P % increase =

$$(\text{P content of M} - \text{P content of NM})(\text{P content of NM})^{-1} \text{ X } 100 \qquad (10)$$

2.2.3
Relative Mycorrhizal Dependency

Relative mycorrhizal dependency (RMD) was worked out by expressing the difference between the mean dry weight (DW) of mycorrhizal and mean DW of nonmycorrhizal as a percent DW of mycorrhizal for each soil P level according to the following equation formulated by Plenchette *et al.* (1983).

$$\text{RMD \%} = ((\text{DW of M}) - (\text{DW of NM}))(\text{DW of M})^{-1} \text{ X } 100 \qquad (11)$$

A plant with a RMD of 100 % is considered to be 100 % mycorrhizal dependent.

Such plants are unable to grow in the absence of mycorrhizal fungi. A RMD value of 0 % will occur when nonmycorrhizal plants grow as well as mycorrhizal (i. e. there is no significant difference in their dry weights).

2.2.2 Data Analysis

Data were analysed by regression analysis, comparing regression equations and elevations (Zar,1984). Student's t-test was used to compare means pair-wise of mycorrhizal and nonmycorrhizal peanut plants of the same treatment (within treatment means)

2.3 RESULTS

2.3.1 Measurements

The results of this study were focused on the increasing shoot height measured during each wk commencing from the 10th wk in the 14 wk growth period, the fresh weight per plant (FWP^{-1}) and dry weight per plant (DWP^{-1}), nutrient contents of shoot system (in mg N, P, K, Na, Ca, Mg, and in µg Fe, Cu, Mn, and Zn), percent mycorrhizal colonisation (PC), and relative mycorrhizal dependency (RMD) determined at harvest after 14 wk.

2.3.1.1 After the 10th wk of growth

The shoot height of mycorrhizal plants consistently increased with added P (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 86% of the variation in response to added P. The shoot height of nonmycorrhizal peanut plants consistently increased with

added P (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 99% of variation in response to added P. The shoot height of mycorrhizal plants was significantly greater than nonmycorrhizal peanut plants at all P levels (with the exception of 480 µg P g^{-1} level, see Methods 2.2.1), according to Student's t-tests at $P \leq 0.05$ level (Table 2.3.1). Although the shoot height increased with both P fertilisation and AM fungal inoculation (Table 2.3.1 ; Fig. 2.3.1), the difference in percent increase in shoot height between mycorrhizal and nonmycorrhizal peanut plants decreased with increased amounts of added P (Table 2.3.1 ; Fig. 2.3.1). Mycorrhizal plants were 27 % taller than nonmycorrhizal plants exposed to unamended substrate soil at 6 µg P g^{-1} but were only 1 % taller in soil with the highest P application rate (480 µg P g^{-1}) in the 10th wk (Table 2.3.1 ; Fig. 2.3.1).

2.3.1.2
After the 11th wk of growth

The shoot height of mycorrhizal plants consistently increased with added P (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 75.5 % of the variation in response to added P. The shoot height of nonmycorrhizal peanut plants consistently increased with added P (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 99.1 % of the variation in response to added P.

The shoot height of mycorrhizal peanut plants was significantly greater than nonmycorrhizal according to Student's t-tests at $P \leq 0.05$ at all P treatments excluding the 480 µg P g^{-1} treatment (Table 2.3.1). An increase of 34 % and 2 % in shoot height was found in mycorrhizal plants grown at the 6 µg P g^{-1} and 480 µg P g^{-1} treatments, respectively (Table 2.3.1). Mycorrhizal plants grown at

the 6 µg P g^{-1} treatment had almost the same shoot height as nonmycorrhizal peanut plants grown at 480 µg P g^{-1} treatment.

2.3.1.3
After the 12th wk of growth

The shoot height of mycorrhizal plants consistently increased with added P (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 75.6 % of the variation in response to added P. The shoot height of nonmycorrhizal peanut plants consistently increased with added P (Table 2.3.1.). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 83.8% of the variation in response to added P. The shoot height of mycorrhizal peanut plants was significantly greater than nonmycorrhizal according to Student's t-tests at $P \leq 0.05$ level at all P treatments with exception of the 480 µg P g^{-1} (Table 2.3.1).

2.3.1.4
After the 13th wk of growth

The shoot height followed approximately the same pattern as above. The shoot heights of both mycorrhizal and nonmycorrhizal peanut plants consistently increased with added P. (Table 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 92.5 % of the variation in response to added P. The height of nonmycorrhizal peanut plants consistently increased with added P (Table 2.3.1 ; Fig. 2.3.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 98 % of the variation in response to added P. The mycorrhizal plant height was significantly greater than nonmycorrhizal peanut plants at all P levels according to Student's t-tests at $P \leq 0.05$ level with exception of the 480 µg P g^{-1} treatment

(Table 2.3.1). The difference in plant height between mycorrhizal and nonmycorrhizal peanut plants decreased from ≈ 38 % at the 6 µg P g^{-1} treatment to ≈ 0 % at the 480 µg P g^{-1} treatment (Table 2.3.1).

2.3.1.5
After the 14th wk growth period

In general, it could be concluded that increases in shoot height of both mycorrhizal and nonmycorrhizal peanut plants showed a strong linear relationship ($P \leq 0.05$) over the 14th wk growth period at each P level. Regression analysis accounted for 93 % and 98.2 % of the variation in response to added P for mycorrhizal and nonmycorrhizal peanut plants, respectively.

Comparison of the slopes of the mycorrhizal and nonmycorrhizal plants at each P treatment indicated significant difference ($P \leq 0.05$) at the 6, 30, 60, and 120 µg P g^{-1} levels. No significant differences in slopes were found at 240 and 480 µg P g^{-1} levels. However, comparison for elevations of mycorrhizal and nonmycorrhizal peanut plants indicated a highly significant difference ($P \leq 0.05$) at the 240 µg P g^{-1} level only.

Table 2.3.1
Shoot height experessed as absolute values and as a % difference of mycorrhizal peanut plants over nonmycorrhizal grown at six phosphorus levels (see Methods; 2.2.1) in autoclaved soil amended with original soil microbiota.

P levels / (SHP-1) ¶	6µg (cm)	30µg (cm)	60µg (cm)	120µg (cm)	240µg (cm)	480µg (cm)
10th wk						
Mycorrhizal	30.0* ±(5.2)	30.8* ±(3.3)	31.4* ±(2.3)	32.8* ±(2.3)	33.3* ±(4.7)	34.3 ±(4.9)
Nonmycorrhizal	22 ±(1.9)	22.5 ±(3.9)	23 ±(2.2)	24.8 ±(3.7)	27.7 ±(1.7)	33.7 ±(2.3)
% Increase	27	27	27	24	17	1
11th wk						
Mycorrhizal	34.4* ±(0.7)	34.8* ±(1.0)	35.1* ±(2.1)	36.0* ±(3.2)	36.3* ±(2.1)	36.6 ±(4.7)
Nonmycorrhizal	22.8 ±(1.7)	23.4 ±(1.2)	24.5 ±(1.2)	26.3 ±(3.4)	30.5 ±(2.7)	35.9 ±(2.8)
% Increase	34	33	30	27	16	2
12th wk						
Mycorrhizal	36.5* ±(1.5)	36.8* ±(2.2)	37.4* ±(5.8)	38.0* ±(4.4)	38.4* ±(2.1)	38.7 ±(3.3)
Nonmycorrhizal	23.9 ±(2.1)	24.5 ±(2.5)	30 ±(1.8)	31.4 ±(1.1)	33.4 ±(1.7)	37.8 ±(2.9)
% Increase	35	33	20	17	13	2
13th wk						
Mycorrhizal	39.1* ±(1.3)	39.2* ±(1.1)	39.5* ±(6.9)	39.5* ±(2.8)	40.9* ±(1.3)	41.40 ±(1.8)
Nonmycorrhizal	24.3 ±(2.1)	25 ±(3.1)	26.8 ±(3.2)	28.9 ±(3.33)	34.9 ±(1.9)	41 ±(2.6)
% Increase	38	36	32	27	15	1
14th wk						
Mycorrhizal	41.8* ±(2.9)	42.1* ±(2.6)	42.3* ±(5.6)	42.6* ±(6.6)	43.5* ±(1.7)	44 ±(2.0)
Nonmycorrhizal	25.9 ±(1.6)	26.8 ±(1.5)	28.4 ±(1.6)	30.6 ±(2.2)	37.31 ±(1.4)	44 ±(2.8)
% Increase	38	36	33	28	14	0

¶ Means shoot height of mycorrhizal and nonmycorrhizal peanuts ± S.D.
Mean pairs in a single wk column with an asterisk [*] are significantly different at $P \leq 0.05$ according to a Student's t-test.

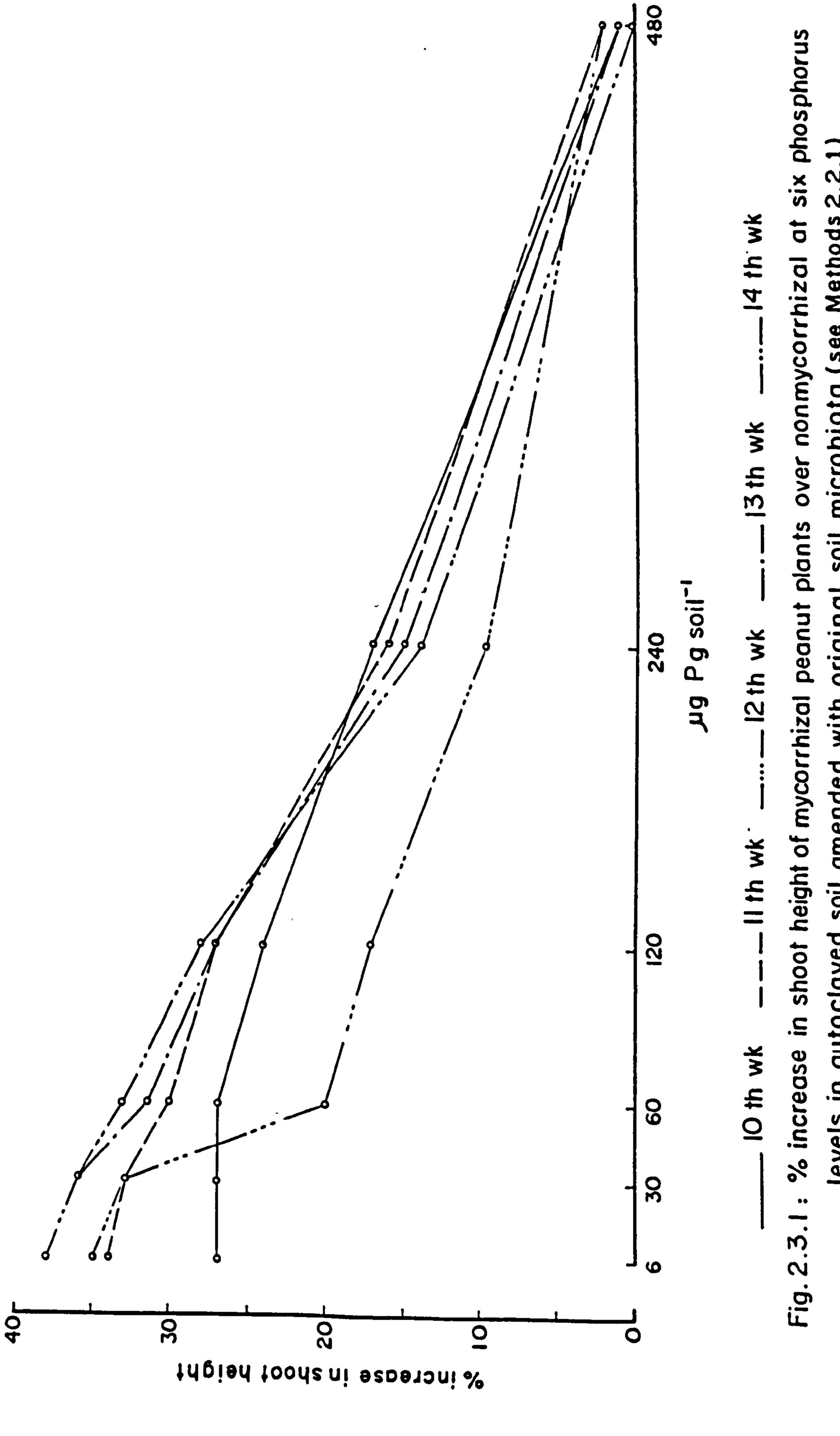


Fig. 2.3.1 : % increase in shoot height of mycorrhizal peanut plants over nonmycorrhizal at six phosphorus levels in autoclaved soil amended with original soil microbiota (see Methods 2.2.1).

2.3.1.6
Fresh and dry weights

After 14 wk, mycorrhizal peanut plants had significantly greater FWP^{-1} and DWP^{-1} compared with nonmycorrhizal grown at the same P level up to 240 µg P g^{-1} added P. The differences in FWP^{-1} and DWP^{-1} between mycorrhizal and nonmycorrhizal peanut plants decreased as the concentration of the P increased, from 96 % and 121 %, respectively, at the 6 µg P g^{-1} level to 1 % and 0 % respectively at the 480 µg P g^{-1} level (Appendix D ; Table 2.1). FWP^{-1} and DWP^{-1} of mycorrhizal peanut plants grown in the same substrate soil without added P fertilisation (6 µg P g^{-1}) were equal to that of nonmycorrhizal grown at 240 µg P g^{-1} level. For plants with mycorrhiza, linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 90 % of variation in FWP^{-1} in response to P fertilisation. For plants without mycorrhiza, linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 100 % of the variation in FWP^{-1} in response to P fertilisation. For plants with mycorrhiza, linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 91 % of the variation in DWP^{-1} in response to P fertilisation, while for nonmycorrhizal plants, linear regression analysis indicated a highly significant ($P \leq 0.05$) relationship that accounted for 100 % of variation in DWP^{-1} in response to added P.

Pair-wise comparisons of mean of FWP^{-1} and DWP^{-1} of mycorrhizal and nonmycorrhizal peanut plants within each P treatment indicated highly significant ($P \leq 0.05$) differences in four treatments when P was applied at level

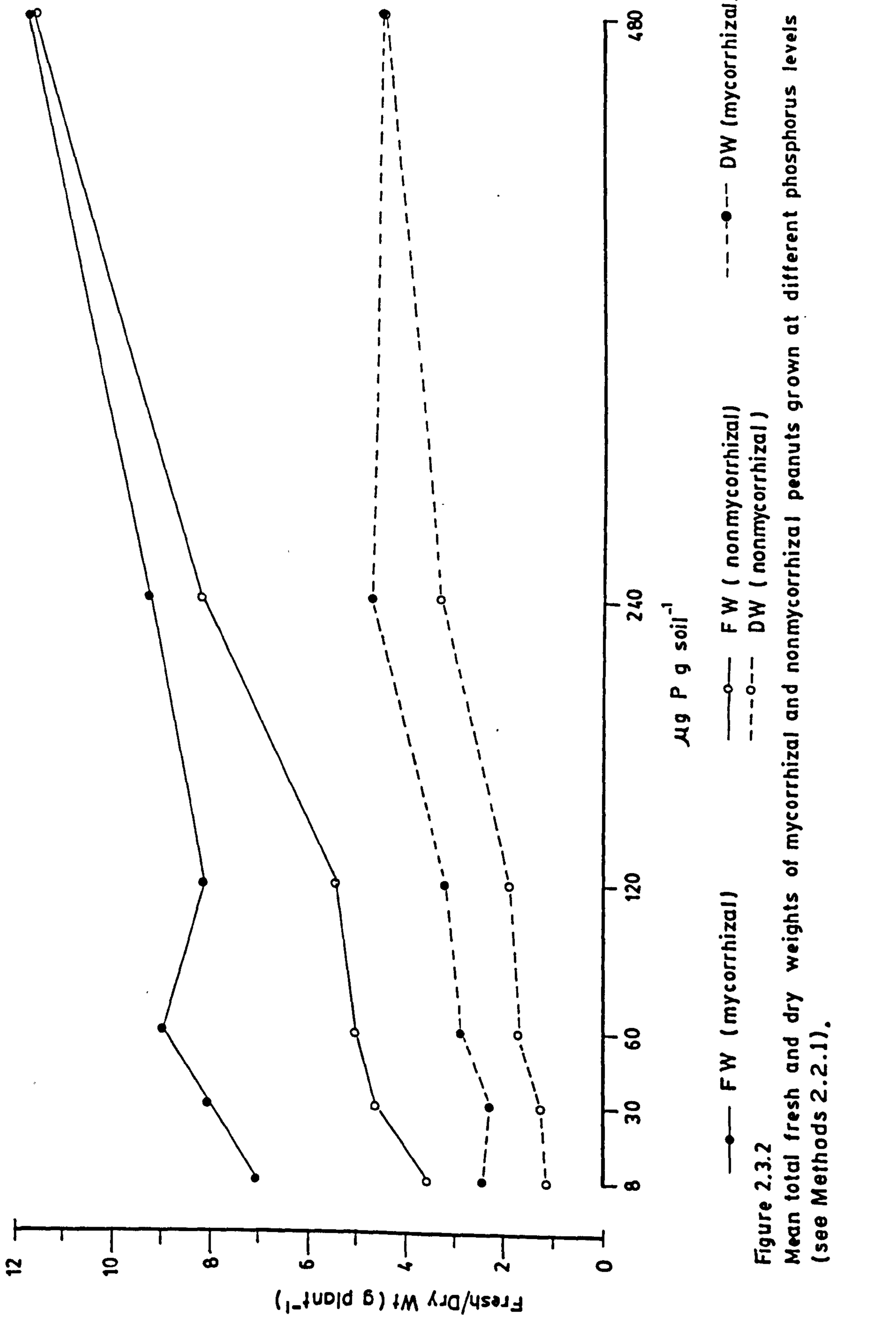


Figure 2.3.2
Mean total fresh and dry weights of mycorrhizal and nonmycorrhizal peanuts grown at different phosphorus levels (see Methods 2.2.1).

≤ 120 µg P g^{-1} according to a group of t-tests. However, the effect of phosphorous increased in excess of 120 µg P g^{-1} level was diminished and eventually eliminated so no difference at 480 µg P g^{-1} was detected (Fig. 2.3.2).

2.3.2 Nutrient uptake

The mineral content of mycorrhizal and nonmycorrhizal peanut plants grown at different level of P application are presented in Table 2.3.3. & Table 2.3.4.

2.3.2.1 Nitrogen [N]

Analysis of variance of regression of N in shoots of mycorrhizal peanut plants indicated no significant relationship ($P = 0.05$) with added P. The statistical analysis of the N content of nonmycorrhizal peanut plants indicated a highly significant ($P \leq 0.05$) linear relationship between increase in N with increasing P fertilisation. The linear regression accounted for 72.9 % of the increase in N with added P. Student's t test indicates that mycorrhizal plants had significantly greater ($P \leq 0.05$) N concentration in their shoots than nonmycorrhizal peanut plants grown at the same P level when P was added the added at 60 µg g^{-1} or less according to Table 2.3.2.

2.3.2.2 Phosphorus [P]

In mycorrhizal and nonmycorrhizal peanut plants, the amount of absorbed P tended to increase with increasing P fertilisation. The analysis of variance of the regression of mean P content indicated a highly significant ($P \leq 0.05$) linear relationship in the absorbed P content with added P. The linear regression accounted for 73.5 % and 94.2 % for mycorrhizal and nonmycorrhizal peanut

Table 2.3.2
Mean concentration of nitrogen, phosphorus, potassium, sodium, calcium and magnesium [in mg/ g] in mycorrhizal and nonmycorrhizal peanuts grown at six phosphorus levels (see Methods; 2.2.1) in autoclaved soil amended with original soil microbiota.

Phosphorus Levels(treatments)		6	30	60	120	240	480
Nutrients content	VAM(+/-)¶	(µg /g soil)	(µg /g soil)	(µg /g soil)	(µg /g soil)	(µg /g soil)	(µg /g soil)
Nitrogen [N]	+	15.9±(0.07)*§	18.6 ± (0.12)*	12.8±(0.07)*	13.0 ± (0.03)	10.1 ± (0.03)	13.8 ± (0.06)
mg/g dry wt	-	10.6 ± (0.15)	10.5 ± (0.08)	9.9 ± (0.12)	11.4 ± (0.06)	13.9 ± (0.03)*	14.2 ± (0.06)
Phosphorus [P]	+	2.1 ± (0.18)*	2.8 ± (0.06)*	3.6 ± (0.03)*	4.0 ± (0.06)*	4.3±(6.7E-3)*	5.2 ± (0.12)
mg/g dry wt	-	0.6 ± (0.08)	1.1 ± (0.08)	2.1 ± (0.06)	2.3 ± (0.02)	2.5 ± (0.05)	4.9 ± (0.04)
% Increase	%	250	155	71	74	72	6
Potassium [K]	+	10.70±(0.09)*	15.30±(0.06)*	19.90±(0.06)*	10.3 ± (0.03)	11.3 ± (0.02)	12.9 ± (0.09)
mg/g dry wt	-	9.0 ± (0.12)	9.6 ± (0.12)	7.1 ± (0.09)	11.7 ± (0.06)	12.5 ± (0.03)	13.2 ± (0.13)
Sodium [Na]	+	0.5±(5.84E-3)	0.8 ± (0.02)	0.7 ± (0.03)	0.9 ± (0.02)	0.6±(5.8E-3)	0.8 ± (0.03)
mg/g dry wt	-	0.8±(8.9E-3)	0.9±(5.8E-3)	0.9 ± (0.01)	0.7 ± (0.06)	1.0 ± (0.01)	1.2 ± (0.07)
Calcium [Ca]	+	24.9±(0.03)*	22.8 ± (0.12)*	19.1 ± (0.17)*	18.4 ± (0.06)*	17.4 ± (0.22)	17.8 ± (0.06)
mg g-1 dry wt	-	19.6±(0.09)	17.6 ± (0.1)	16.0 ± (0.04)	15.0 ± (0.06)	19.5 ± (0.09)	20.4 ± (0.06)
Magnesium [Mg]	+	5.0±(0.09)*	7.9 ± (0.07)*	5.6 ± (0.06)*	6.4 ± (0.06)*	4.5 ± (0.06)	5.1 ± (0.15)
mg/g dry wt	-	4.0 ± (0.12)	5.4 ± (0.02)	5.2 ± (0.07)	5.0 ± (0.07)	6.3 ± (0.07)	5.1 ± (0.12)

Means are readings of three replicates ± standard errors (S.E.).
§ Asterisks[*] denote a significant effect at $P \leq 0.05$ according to Student's t-test.
¶ (+/-) corresponds to mycorrhizal and nonmycorrhizal peanuts.

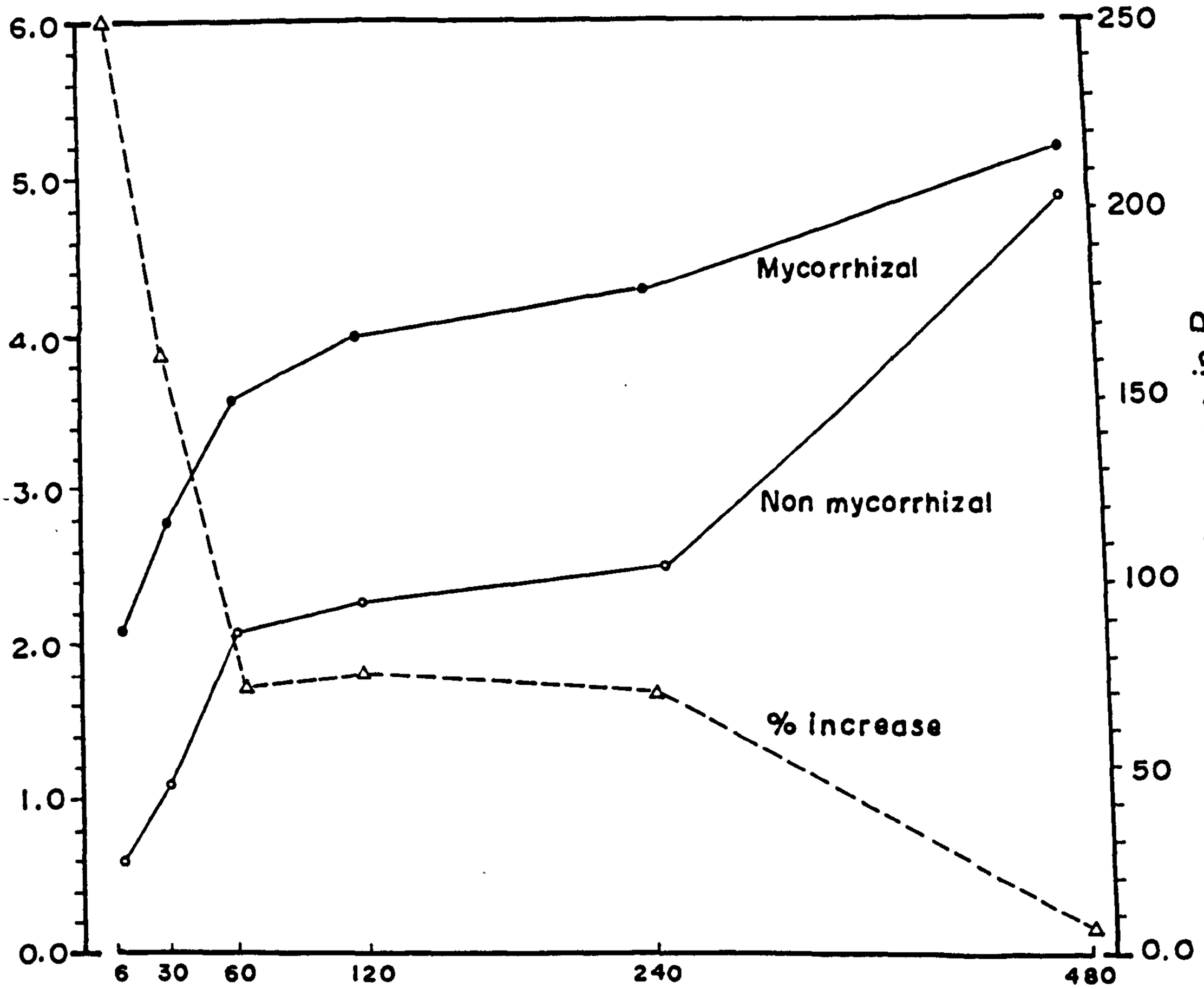


Figure 2.3.3
Phosphorus uptake and increase in P content of mycorrhizal compared with nonmycorrhizal plants (%) at a range of P concentrations (see Methods 2.2.1).

plants respectively, of the absorbed P with applied P. Mycorrhizal plants had significantly greater P concentration in their shoots than nonmycorrhizal peanut plants grown at the same P level according to Student's t-tests for all P treatments with an exception at the 480 µg P g^{-1} level. Phosphorus concentration in the shoots of mycorrhizal peanut plants grown in soil without added P (6 µg P g^{-1}) was approximately three fold that of nonmycorrhizal peanut plants exposed to the same soil. The P concentration absorbed by nonmycorrhizal peanuts grown at the highest P level (480 µg P g^{-1}) was almost equal to that of mycorrhizal peanut plants grown in the same soil (Table 2.3.2 ; Fig. 2.3.3).

2.3.2.3 *Potassium* [K]

There were highly significant increases in concentrations of K in mycorrhizal peanut plants compared to nonmycorrhizal when the added P rate ≤ 60 µg P g^{-1} according to Student's t-tests ($P \leq 0.05$). Nevertheless, the difference decreased as P fertilisation dose level increased to more than 120 µg P g^{-1} (Table 2.3.2). No linear relationship between K concentration and application of P was present.

2.3.2.4 *Sodium* [Na]

Although nonmycorrhizal plants had slightly higher Na concentrations in their shoots than mycorrhizal, the differences between mycorrhizal and nonmycorrhizal peanut plants were not statistically significant in any of the P treatments (Table 2.3.2). Linear regression analysis indicated no significant linear relationship between Na concentrations and added P in mycorrhizal, but it showed a highly significant ($P \leq 0.05$) linear relationship for nonmycorrhizal peanut plants with Na and added P.

Table 2.3.3
Mean concentration of iron, copper, manganese, and zinc [in µg/g] in mycorrhizal and nonmycorrhizal peanuts grown at six phosphorus levels (see Methods; 2.2.1) in autoclaved soil amended with original soil microbiota.

Phosphorus Levels(treatments)		6	30	60	120	240	480
Nutrients content	VAM(+/-)¶	(µg/g soil)	(µg/g soil)	(µg/g soil)	(µg/g soil)	(µg/g soil)	(µg/g soil)
Iron [Fe]	+	176.7±(0.15)*	656.9 ± (0.15)*	236.5 ± (0.67)*	425.9±(0.52)*	316.7±(0.19)*	321.3 ± (0.58)
(µg/g dry wt)	-	141.5 ± (0.23)	103.1 ± (0.18)	222.5 ± (0.35)	373.2 ± (0.21)	273.5 ± (0.87)	512.8 ± (0.67)*
Copper [Cu]	+	4.2 ± (0.12)*	4.2 ± (0.12)*	3.4 ± (0.07)*	2.3 ± (0.06)	2.3 ± (0.07)	1.9 ± (0.03)
(µg/g dry wt)	-	1.2 ± (0.58)	2.3 ± (0.09)	1.2 ± (0.09)	2.4 ± (0.09)	2.2 ± (0.07)	3.2 ± (0.09)*
Manganese [Mn]	+	43.7 ± (0.67)	79.0 ± (0.37)*	67.6 ± (0.35)*	68.8 ± (0.15)*	33.9 ± (0.17)	72.2 ± (0.88)*
(µg/g dry wt)	-	45.6 ± (0.88)	35.1 ± (0.29)	49.2 ± (0.35)	28.7 ± (0.13)	40.4 ± (0.25)	48.9 ± (0.29)
Zinc [Zn]	+	25.0 ± (0.58)*	27.0 ± (0.15)*	22.5 ± (0.06)	20.5 ± (0.06)	18.1 ± (0.09)	24.7 ± (0.67)*
(µg/g dry wt)	-	16.8 ± (0.17)	16.4 ± (0.06)	23.3 ± (0.47)	26.3 ± (0.03)	20.2 ± (0.09)	19.2 ± (0.13)

Means are readings of three replicates ± standard errors (S.E.).
§ Asterisks[*] denote a significant effect at $P \leq 0.05$ according to Student's t-test.
¶ (+/-) corresponds to mycorrhizal and nonmycorrhizal peanuts.

2.3.2.5 *Calcium* [Ca]

Generally, mycorrhizal peanut plants accumulated more Ca in their shoots than nonmycorrhizal at all P treatments except the 480 µg P g^{-1}. The differences were statistically significant as the P level exceeded 120 µg P g^{-1} (Table 2.3.2). No significant linear relationship between Ca concentrations and P fertilisation was present.

2.3.2.6 *Magnesium* [Mg]

Mycorrhizal peanut plants had greater Mg concentrations in their shoots than nonmycorrhizal plants at all P treatment with exception of the 240 and 480 µg P g^{-1} treatments. The Mg concentrations in the mycorrhizal plants had a tendency to increase as the amounts of applied P increased. By contrast, the Mg content of nonmycorrhizal peanut plants showed no tendency to increase or decrease with increased amounts of P added (Table 2.3.2). No significant linear relationship between Mg concentration and P fertilisation was present.

2.3.2.7 *Iron* [Fe]

Mycorrhizal peanut plants absorbed significantly more Fe compared with nonmycorrhizal plants in all P treatments except the highest P level (480 µg P g^{-1}) where the accumulation of Fe was high in nonmycorrhizal peanut plants (Table 2.3.3). Linear regression analysis indicated no significant relationship between Fe concentrations and P fertilisation but in mycorrhizal, it indicated a significantly ($P \leq 0.05$) linear relationship that accounted for 77.1 % of the variation between Fe and P increases in nonmycorrhizal peanut plants.

2.3.2.8 *Copper* [Cu]

Mycorrhizal plants absorbed significantly more Cu than nonmycorrhizal peanut plants when P level ranged between 6 to 60 µg P g^{-1} (Table 2.3.3). Regression analysis indicated a highly significant ($P \leq 0.05$) ***inverse*** linear relationship that accounted for 68.3 % of the variation between Cu concentration and P increase for mycorrhizal plants. No significant linear relationship between Cu concentration and P fertilisation was detected for nonmycorrhizal peanut plants.

2.3.2.9 *Manganese*[Mn]

There were significantly higher Mn concentrations in mycorrhizal compared to nonmycorrhizal peanut plants with the exception of the 6 µg P g^{-1} and 240 µg P g^{-1} treatments (Table 2.3.3). Regression analysis showed no significant relationship between Mn concentration and P fertilisation.

2.3.2.10 *Zinc* [Zn]

Mycorrhizal plants accumulated significantly greater quantities of Zn in their shoot systems than nonmycorrhizal peanut plants did at the 6 µg P g^{-1}, 30 µg P g^{-1} and 480 µg P g^{-1} treatments (Table 2.3.3). No significant linear relationship was demonstrated between Zn uptake and P fertilisation. The Zn absorbed by mycorrhizal plants exposed to the original soil (6 µg P g^{-1}) was higher than the Zn absorbed by nonmycorrhizal peanut plants grown at the 480 µg P g^{-1} treatment.

2.3.3 *Percent colonisation*

After the 14th wk all plants inoculated with *Glomus mosseae* were colonised. The

average % colonisation (PC) of plants grown at the 6, 30, 60, 120, 240 and 480 µg P g^{-1} levels was 86 %, 82 %, 80 %, 75 %, 55 %, and 39 %, respectively (Fig. 2.3.3). Fertilisation with P therefore consistently decreased percent colonisation (Fig. 2.3.3). Linear regression analysis indicated a highly significant ($P \leq 0.05$) ***inverse*** relationship between amount of added P and PC. The regression accounted for 97.4 % of the variation in PC in response to P fertilisation.

2.3.4
Mycorrhizal Dependency

Relative mycorrhizal dependency (RMD), based on the total dry weight, ranged from a maximum of 55 % in plants grown at the lowest P level of 6 µg P g^{-1} to a minimum of ≈ 0 % in plants grown at the 480 µg P g^{-1} level (Fig. 2.3.4).

Linear regression analysis indicated a highly significant ($P \leq 0.05$) ***inverse*** relationship between amount of added P and RMD. The regression accounted for 92.7 % of the variation in the mycorrhizal dependency in response to added P. The results indicate that ***Arachis hypogaea*** is significantly mycorrhizal dependent when it is exposed to P fertilisation ≤ 240 µg P g^{-1} (Fig. 2.3.4).

The results of this work suggest a low threshold P requirement level, within which mycorrhizal dependency of ***Arachis hypogaea*** is inclined to be significantly pronounced (Fig. 2.3.4).

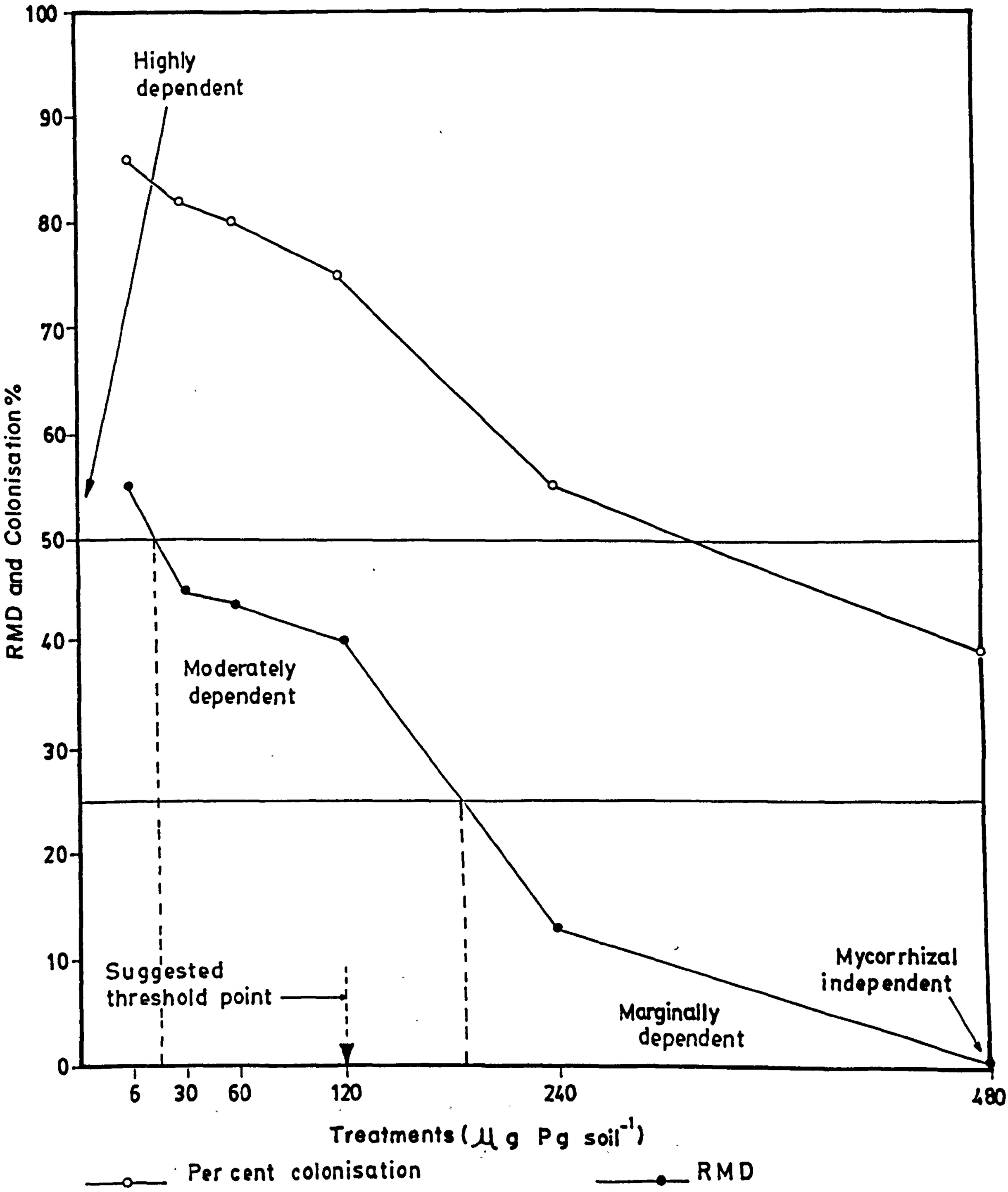


Fig. 2.3.4
Relative mycorrhizal dependency and percent colonisation of peanut at a range of phosphorus concentrations (see Methods; 2.2.1).

2.4 DISCUSSION

Phosphorus application and mycorrhization of *Arachis hypogaea* led to morphological, physiological and chemical differences arising between P-fertilised and *Glomus mosseae*-inoculated peanut plants as discussed below:-

After 14wk, up to 120 µg P g^{-1} level, increases in added phosphorus in the soil brought about a steady but small increase in growth parameters (SHP^{-1}, FWP^{-1} and DWP^{-1}). The rate of increase in mycorrhizal and nonmycorrhizal plants was similar but mycorrhizal plants had on average about 34%, 75%, 87% growth increase over nonmycorrhizal for SHP^{-1}, FWP^{-1} and DWP^{-1} at each P level. Above 120 µg P g^{-1} level increases in added phosphorus in soil caused even greater amounts of growth than below 120 µg P g^{-1} but the effect of mycorrhization was steadily less marked so that at the highest P level the growth of nonmycorrhizal and mycorrhizal plants was not significantly different. There is evidence, that at high P fertilisation some AM fungi tend to depress host growth. For example Peng *et al.* (1993) observed that *Glomus intraradices* depressed the growth of citrus in high P soils. Johnson (1993) hypothesised that heavy fertilisation has the potential to develop inferior mycorrhizal associations that may be involved in host biomass decline. Johnson (1993) believes that effective management of AM fungi in agriculture may require manipulation of AM fungal communities through inoculation or cultural practices that favour proliferation of the most beneficial AM fungi. The results of this investigation support Cooper's (1975) assumptions that mycorrhization shows significant growth enhancement when P levels are low, but when the soil P level is high nonmycorrhizal plants tend to grow as well as the mycorrhizal plants.

It appeared that P levels in the soil and in the host tissue were important factors affecting mycorrhizal dependency of *Arachis hypogaea* as found in other

plant genera (Menge *et al.*, 1980, 1982 ; Strobel, *et al.*, 1982 and Lopes *et al.*, 1985). Using the AM-dependency scale proposed by Habte and Manjunath (1991), peanut was highly dependent on the AM fungus (RMD > 50 %) at 6 µg P g^{-1}, moderately dependent (RMD 50 — 25 %) between 15 — 185 µg P g^{-1}, marginally dependent (RMD ≤ 25 %) between 185 to ≤ 480 µg P g^{-1} and mycorrhizal independent (with 0 % RMD) at 480 µg P g^{-1}.

It was evident also from the results of this study that AM inoculation and P fertilisation influenced P uptake by peanut plants. Below 480 µg P g^{-1}, nonmycorrhizal plants took up much less P and grew much less than mycorrhizal plants. The sigmodial response for nonmycorrhizal plants to P is typical of nonmycorrhizal plants of coarse-rooted species (Bolan *et al.*,1983). Mycorrhization with the AM fungus almost eliminated the sigmoidial effect and phosphorus uptake was significantly greater in mycorrhizal than in nonmycorrhizal plants at the same P level for all treatments except the 480 µg P g^{-1}, indicating that P can not be so effectively taken up by peanut without *Glomus mosseae* inoculation. This supports the previous evidence of Krishna and Bagyaraj (1984), Parvathi *et al.*(1985) and Middleton *et al.* (1989) that peanut is dependent upon a mycorrhizal association for good growth and uptake of nutrients — especially P (Abbott and Robson, 1984). When mycorrhizal and nonmycorrhizal plants were compared, the greatest % increase (250 %) in P uptake was found in mycorrhizal peanut plants grown at 6 µg P g^{-1}. Phosphorus fertilisation decreased % increase in the tissue P concentration down to 6.1 % in the 480 µg P g^{-1}. The P content of mycorrhizal plants grown in soil without added P fertilisation (6 µg P g^{-1}) was only slightly less than that absorbed by nonmycorrhizal plants grown in soil fertilised with the second highest P dosage (240 µg P g^{-1}). The nearly similar growth indices (SHP^{-1},

FWP^{-1} and DWP^{-1}) of mycorrhizal plants grown at 6 µg P g^{-1} and nonmycorrhizal plants grown at 240 µg P g^{-1} reflected these similarities of P uptake. It has been reported that AM fungi can improve plant P absorption by the external hyphal network which can explore the soil beyond the rhizosphere and absorb available phosphate that is normally inaccessible to the root hairs (Mosse, 1973b ; Alexander *et al.*,1984). Phosphorus-deficiency was probably due to the depletion of available phosphate in the rhizosphere of nonmycorrhizal peanut plants. High P content in plant tissue increases chlorophyll and photosynthesis (Allen *et al.*, 1981, Johnson *et al.*, 1982). Although chlorophyll and photosynthesis were not measured in the peanut plants in this investigation, the increased P content of mycorrhizal plants might have been responsible for increased growth by increasing photosynthesis or chlorophyll content. This hypothesis, could be supported by the finding that Mg (which is a constituent of chlorophyll), has significantly increased with *Glomus mosseae* inoculation.

The N content decreased with increasing P fertilisation in mycorrhizal peanut plants while fertilisation with P seemed to improve N uptake in nonmycorrhizal plants. Nitrogen content was significantly higher in mycorrhizal peanut plants compared with nonmycorrhizal plants when P application was below 120 µg P g^{-1}. Arbuscular mycorrhizal inoculation increased K content significantly when the applied P ≤ 60 µg P g^{-1}. This is in line with the observations of earlier workers who found that mycorrhizas increased K content in troyer citrange plants (Menge *et al.*, 1982) and in alfalfa plants (Nielsen and Jensen, 1983). However, above the 120 µg P g^{-1} level, both mycorrhizal and nonmycorrhizal plants had the same K concentrations. Sodium concentration in the nonmycorrhizal plants tended to be higher than those of mycorrhizal

peanut plants and its content increased with P fertilisation in nonmycorrhizal plants, but the differences between mycorrhizal and nonmycorrhizal plants were not significant. Further investigation is required to study the role of mycorrhizae on Na uptake although some reports have recorded lower Na concentrations in avocado with mycorrhizas (Menge *et al.*, 1980), mycorrhizal troyer citrange (Menge *et al.*, 1982) and mycorrhizal citrus seedlings (Timmer and Leyden, 1978). The mycorrhizal plants absorbed significantly more Ca below the 120 µg P g^{-1} level. Peanut pod development is known to be strongly depressed by inadequate Ca uptake (Cox *et al.*, 1982) especially in Ca deficient soils. Therefore when considering the future practical aspects of peanut mycorrhization in low-input sustainable agriculture, this enhanced Ca uptake phenomenon may contribute towards a solution to the poor filling of pods.

Mycorrhizal peanuts acquired a significant increase in Fe concentrations compared with nonmycorrhizal peanut plants, although Fe content had no linear relationship with added P increase. Mycorrhizal fungi increased Fe concentration in ***Ehrharta calycina*** (Killham and Firestone, 1983), alfalfa (Lambert *et al.*, 1980a) and peach seedlings (Menge *et al.*, 1980). There were significant differences between the Cu content of mycorrhizal and nonmycorrhizal peanut plants below 120 µg P g^{-1}. The Cu content of mycorrhizal plants decreased with P increase while the nonmycorrhizal peanut plants counterparts, generally, had the tendency to have decreased Cu contents with P increases. It seemed that ***Glomus mosseae*** inoculation tended to improve Cu uptake within the range where the peanuts were highly mycorrhizal dependent. It has been reported that Cu concentrations increase with mycorrhizal inoculation in several plants such as ***Ehrharta calycina*** (Killham and Firestone, 1983), alfalfa (Lambert *et al.*,1980a) and soybeans (Ross, 1971).

The results of this study showed that mycorrhizal plants contain more Mn

than nonmycorrhizal peanut plants beyond the 30 µg P g^{-1} level. The inconsistent Mn uptake by peanuts with P fertilisation appears to support the view of Heintze (1968) who reported that P reduces Mn toxicity by precipitation within the roots. Also Hashem (1995) found that mycorrhizal inoculation of *Vaccinium macrocarpon* has the ability to eliminate the toxicity of Mn by accumulation of Mn in roots or hyphae. The high concentrations of Mn in mycorrhizal plants seemed to be not toxic. Although the Zn content in the shoots of mycorrhizal peanut plants was very high when compared with nonmycorrhizal peanut plants, no signs of Zn toxicity were evident. Phosphorus fertilisation did not influence Zn content in nonmycorrhizal peanut plants. Additional work on Mn and Zn is needed to study further the role of mycorrhizal peanuts in uptake of these two nutrients.

Generally, the AM fungus *Glomus mosseae* enhanced macronutrients (N, P, K, Ca, & Mg) and micronutrients (Fe, Cu, Mn, & Zn) uptake especially at relatively higher mycorrhizal dependencies below 120 µg P g^{-1} dosage where the RMD ranged between 40 - 55 %. The acquisition of these nutrients could be attributed to functions of the external hyphae of the inoculant fungus *Glomus mosseae* (Kothari *et al.*, 1991). Probably, *Glomus mosseae* hyphae extending from mycorrhizal peanut plant roots had penetrated beyond the nutrients-depleted zone in the vicinity of the root and took up these nutrients from more distant parts of the soil especially when the root morphology of the peanut plants, which is characterised by poor development of root hairs is taken into account (Baylis,1970 ; Krishna and Bagyaraj, 1984). However, this rather speculative since the mechanism is not fully understood. Analysis of nutrient uptake reaffirms that growth stimulation of mycorrhizal peanut plants compared with their nonmycorrhizal counterparts may be attributed to improved accumulation of nutrients, particularly P.

Increased phosphorus fertilisation usually decreases *Glomus mosseae* colonisation (PC) and root length. McIlveen and Cole (1979) found that phosphate fertilisation results in a consistent decrease of PC on soybean roots inoculated with *Glomus mosseae*. In tomatoes, mycorrhizal colonisation decreased from 46.5 to 18.5 % with the addition of 0.5 to 2.4 meq. P plant^{-1} (Daft and Nicolson, 1972). In soybeans inoculated with *Glomus mosseae* a 100 % colonisation in soil without added P was reported (Asimi *et al.*,1979), but when P was added to the soil the % colonisation decreased with the amount of added phosphate and became minimal in soil receiving 1.0 g P kg^{-1}. In these experiments peanut was colonised at all P levels, and the results indicate that high mycorrhizal colonisation and positive difference in the growth of peanuts can occur at relatively high phosphate levels (Neck *et al.*, 1987). The % colonisation decreased from 86 % at 6 µg P g^{-1} to 39 % at 480 µg P g^{-1} as the amount of P added increased. However, regression analysis indicated a highly significant inverse relationship between % colonisation and added P. This is in agreement with the commonly observed phenomenon that P fertilisation decreases % colonisation. Nonetheless, some results have been presented else where demonstrating an interdependence of AM fungi and their host plants suggesting that different plants have different P requirements and that a given AM fungus has a maximum infectivity at different P levels depending on the host (Arias *et al.*, 1991). Surprisingly, at 480 µg P g^{-1} treatment the peanut showed high levels of mycorrhizal colonisation, but was mycorrhizal independent, indicating that a host may only depend on AM fungi in soils where P is inaccessible or unavailable except to the absorbing hyphae of the AM fungi. This result supports the views of Linderman & Hendrix (1982) and Habte & Manjunath (1991) who argued against the use of a single P concentration for

differentiating "AM fungi-dependent" from "AM fungi-responsive". This may be important when considering the practical aspects of the utilisation of mycorrhizal plants with phosphate fertilisation. Although it is well known that high P concentrations in the soil and/or in the host tissues often reduces % colonisation, the mechanism is not fully understood. Mosse (1973b) assumed that the reduction of mycorrhizal colonisation by adding soluble P may be due to P toxicity. She reasoned that high P concentrations in the host cells causes resistance to mycorrhizal colonisation. Also, reduction of % colonisation has been reported to be very variable according to the host plant or fungal isolate (Antunes and Cardoso, 1991).

Chapter 2 reveals that limited P fertilisation supported by *Glomus mosseae* mycorrhization could be a potent and inexpensive tool that can be used for managing sustainable peanut cultivation in soils where P availability is low especially in the tropical lands (Howeler *et al.*, 1987). Therefore matching mycorrhizal dependency of host plants with soil P input is, undoubtedly, a critical step of the efforts that must be directed towards maximising the beneficial effects of AM fungi in sustainable agriculture.

CHAPTER 3

Glomus mosseae Potential Biocontrol of ***Erwinia carotovora*** in the Peanut.

3

Glomus mosseae Potential Biocontrol of *Erwinia carotovora* in the Peanut

3.1 Introduction

Results in Chapters 1 and 2 provide further examples that plant development is improved by symbiosis with AM fungi. AM fungi associations are therefore not detrimental to their host in contrast to pathogenic infection. Furthermore reports of interactions between AM fungi and pathogenic organisms such as fungi and nematodes (Hussey and Roncadori, 1982 ; Dehne, 1982) have indicated that AM fungi may provide protection against disease progress. Zambolim and Schenck (1983) reported that *Glomus mosseae* appeared to increase tolerance to the effects of pathogenic, root-infecting fungi (*Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani*) on mycorrhizal soybean compared with nonmycorrhizal plants.

The interaction of mycorrhizal plants and pathogens is however considerably dependent on the fungal symbiont, host plant and pathogen involved. When the mycorrhiza offsets pathogen damage or reduces the pathogen population, the interaction is regarded as positive (Roncadori and Hussey, 1982). If the mycorrhiza enhance disease development, the interaction is considered negative. The interaction can be neutral if the mycorrhiza does not affect disease development or symbiosis or other activities of the two organisms.

Diverse bacteria cause many diseases in plants but there are limited reports dealing with AM fungi-bacterial interactions. *Erwinia carotovora* is known to cause diseases in vegetables, ornamentals and field crops. It produces

pecteolytic and other macerating enzymes that induce loss of structural integrity in host tissue and causes soft rot disease (Cother and Sivasithamparam, 1983). Garcia-Garrido and Ocampo (1988) studied the effect of the inoculation of tomatoes with ***Erwinia carotovora*** with or without AM fungal colonisation by ***Glomus mosseae*** in a greenhouse trial. They found that ***Erwinia carotovora*** decreased the growth of nonmycorrhizal tomato plants, but neither growth nor the percentage of total root length were reduced in mycorrhizal plants.

The mechanisms responsible for the interactions between pathogens and AM fungi are poorly understood. Unlike ectomycorrhizas, AM fungi have neither an external mechanical barrier, such as a fungus mantle nor do they apparently produce any antibiotics. Instead, most studies indicate that there are changes in the root tissues which may act to promote increased host resistance and are more likely to influence disease development than interactions in the rhizosphere (Hussey and Roncadori, 1982; Schenck, 1983). Nevertheless, a contradicting belief assumes that there is a direct interaction between the mycorrhizal fungus and the pathogen. For example Caron, *et al.* (1985) studied the interaction between ***Glomus intraradices*** and ***Fusarium oxysporum*** f. sp. *radicis-lycopersici* on tomatoes. They observed that the mycorrhizal fungus decreased root necrosis in tomato plants and reduced the population of ***Fusarium oxysporum.***

Development of induced resistance depends on a time lapse between exposure to the AM fungus and pathogen (Bartschi *et al*, 1981). In most studies, plants were inoculated with AM fungi before exposure to pathogens (Bagyaraj, 1984). However, under natural conditions both microorganisms frequently occur concurrently in the rhizosphere of the plants.

This Chapter endeavours to evaluate the interactions generated by simultaneous inoculation of a pathogenic microorganism (***Erwinia carotovora***)

and a mycorrhizal fungus (*Glomus mosseae)* on development, growth response and uptake macronutrients by *Arachis hypogaea* L. to see if there is likely to be any beneficial effects under natural conditions.

3.2 Methods

3.2.1 Methods and Experimental Design

Since the highest RMD value (55 %) was found at the P treatment with P base line of 6 µg P g^{-1}, the original substrate soil deprived of any P fertilisation was selected to conduct the trial of *Glomus mosseae* biocontrol of *Erwinia carotovora* in the peanut and other subsequent investigative experiments. To avoid possible interaction with naturally occurring soil-inhabiting pathogens, amendment with original soil microbiota was totally excluded in this trial. Forty, 15 cm diameter, plastic pots were filled with 3.0 kg autoclaved substrate soil as used previously. The native P content was 6 µg g^{-1}. Seeds of *Arachis hypogaea* were first surface-sterilised in 2 % sodium hypochlorite solution for two min., then rinsed in three changes of sterile distilled water. Thereafter, they were pregerminated on moistened filter papers inside Petri dishes. Each experimental pot was hand seeded with one pregerminated seed. The experiment involved the following four treatments, each with ten replicates in a completely randomised design.

- *Glomus mosseae*-inoculated treatment.
- *Erwinia carotovora*-inoculated treatment.
- Dual inoculation (both *Glomus mosseae* & *Erwinia carotovora*) treatment.
- Uninoculated (Control) treatment.

Inoculation with *Glomus mosseae* and *Erwinia carotovora* was done at the time of seeding. *Glomus mosseae* maintained on Sudangrass in sterile soil (Materials and Methods; 1.1) served as inoculum. Ten g of Sudangrass root-

soil mixture were completely spread and thoroughly mixed in the top 5 cm of the soil. Each of the other experimental pots were treated with 10 g of autoclaved inoculum.

The bacterium ***Erwinia carotovora*** subsp. *carotovora* (Jones) Bergey, Harrison, Breed, Hammer and Huntoon was donated by the Plant Pathology Unit, College of Agriculture, King Saud University, Saudi Arabia. The bacterium was suspended in sterile distilled water. The suspension containing 7.0×10^9 bacteria ml^{-1} was prepared from cultures grown on nutrient agar (see Appendix B - 1, for details) for 24 h at 37 °C. The pots received 10 ml pot^{-1} of this suspension, while other experimental pots were given 10 ml pot^{-1} distilled water.

All pots were placed in the greenhouse. During the experiment, populations of the inoculant bacterium were assessed at 1 wk, 3 wk and 5 wk intervals. Rhizosphere soil (1.0 g) was taken from three experimental pots from each of the ***Erwinia carotovora***-inoculated treatments. The soil samples were suspended in distilled sterile water and shaked mechanically for 20 min. Then a series of dilutions were prepared. The number of bacteria in suitable dilutions of the soils were counted by the plate-count technique and related to 1.0 g wet rhizosphere soil. The numbers of bacteria g^{-1} soil were processed statistically and graphically by log-transformation. Plants were carefully uprooted for harvesting after 8 wk. Growth indices including shoot height per plant, root dry weight per plant, shoot dry weight per plant and rate of growth per week were ascertained. Spore collection to determine mycorrhizal population density was carried out by wet-sieving and decanting (Gerdemann and Nicolson, 1963). Number of the AM fungal spores was expressed as per gram soil ($NOSG^{-1}S$). Roots from mycorrhizal plants were preserved in the FAA fixative solution, cut

into 1-cm root segments, cleared and stained (Phillips and Hayman, 1970) to determine mycorrhizal colonisation progress by calculating a percent colonisation (PC) index. The macronutrients viz. nitrogen, phosphorus and potassium (NPK) concentrations in the shoot were determined and expressed in percent dry weight as described in detail in Materials and Methods; [1.4]. The % increase in *Glomus mosseae* and *Erwinia carotovora* inoculation over *Erwinia carotovora* inoculation (infected) or control (healthy) plants for each measured index was calculated as follows:

$$\%\ \text{increase dry weight} = \big((\textit{Glomus mosseae}\ \&\ \textit{Erwinia carotovora}\ \text{inoculation}) - (\textit{Erwinia carotovora}\ \text{inoculation (or) Control})\big)\big((\textit{Erwinia carotovora}\ \text{inoculation (or) Control})\big)^{-1} X\ 100. \quad (12)$$

3.2.2 Data Analysis

Data were statistically analysed by one-way ANOVA, with Fisher's LSD test being used to determine significant differences. The NPK data were first transformed to natural Logarithms before statistical analysis.

3.3 RESULTS

Glomus mosseae only inoculation significantly stimulated the growth of mycorrhizal peanuts compared with the controls (no mycorrhizal and bacterial inoculations) as indicated by growth indices of shoot height per plant, root dry weight per plant, shoot dry weight per plant and rate of growth per week (Plate 3.3.1 ; Figs 3.3.1 & 3.3.2 ; Appendix D ; Table 3.1).

When comparing peanut plants infected with *Erwinia carotovora* with the control peanuts, the biomass as indicated by growth indices of shoot height per plant, root dry weight per plant, shoot dry weight per plant and rate of growth per week showed marked reductions in presence of the pathogen (Figs 3.3.1 &

3.3.2 ; Appendix D ; Table 3.1). The % increases of controls over the diseased plants were 18.8, 60.4, 31.1 and 85.6% for shoot height per plant, root dry weight per plant, shoot dry weight per plant and rate of growth per week respectively (Appendix D ; Table 3.1). Morphologically the stems of the infected peanut plants were light brown with fewer branches, leaves reduced in size and number and a general stunted growth (Plate 3.3.1).

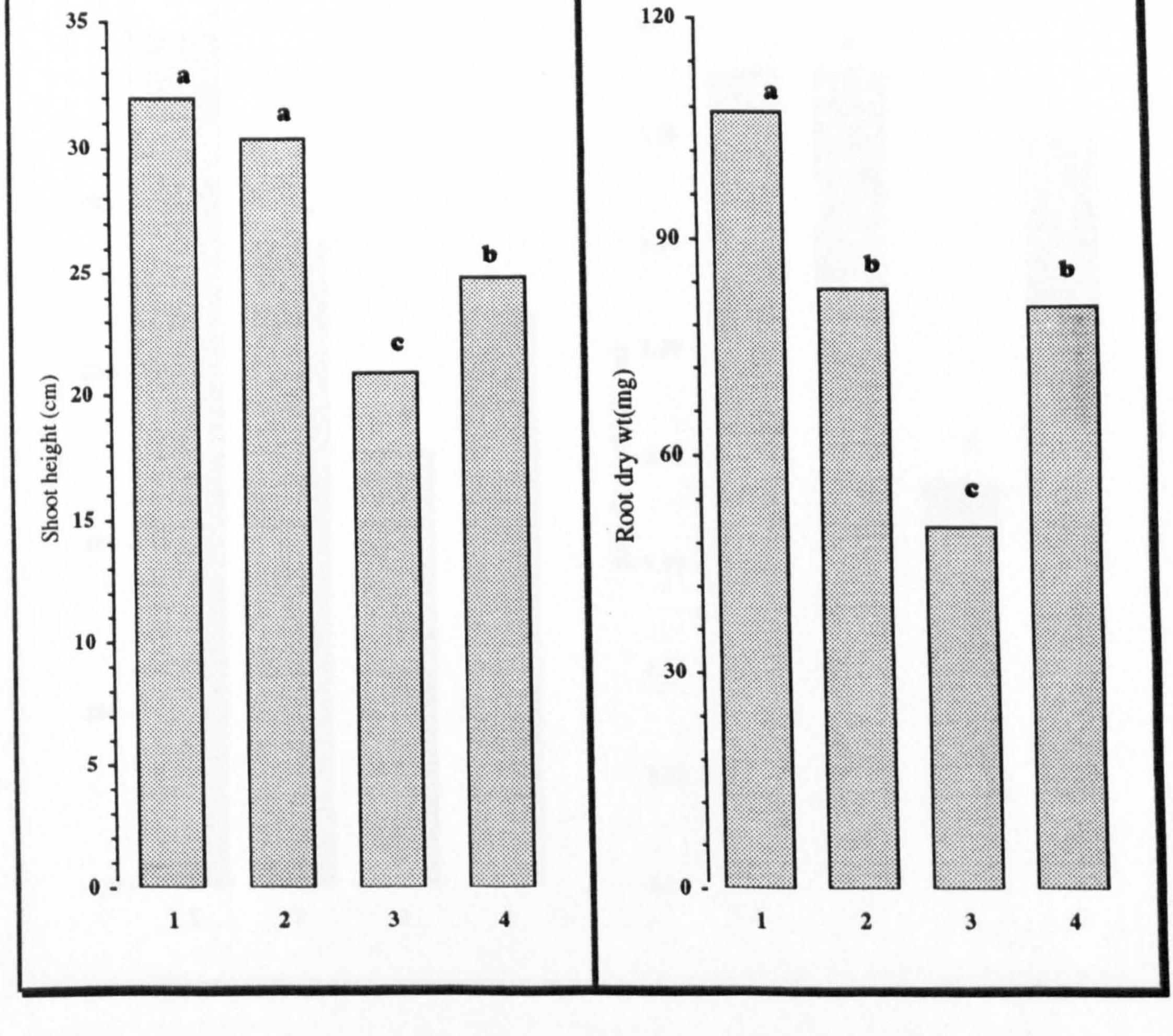
Comparing ***Glomus mosseae***-inoculated peanuts with ***Glomus mosseae+Erwinia carotovora*** dual inoculated plants, the AM fungus seems to be competent in partly excluding some of the effects of ***Erwinia carotovora*** because growth indices of root dry weight and shoot dry weight per plant that show significant differences with % increases over dual inoculation of 29.2 and 31.5% for root dry weight per plant and shoot dry weight per plant respectively [Figs 3.3.1 (a) & 3.3.2 (b) ; Appendix D ; Table 3.1]. Sporulation and colonisation levels were also not significantly lower in dual inoculated peanut plants compared with ***Glomus mosseae*** alone [Fig. 3.3.3 (a ; b) ; Appendix D ; Table 3.1]. So ***Erwinia carotovora*** neither affected spore population density ($NOSG^{-1}S$) nor mycorrhizal development (PC) in the host plant roots.

Comparing the dual inoculation with the ***Erwinia carotovora***-infected plants, the AM fungal symbiont seemed to compensate for any harm caused by the bacterium. The measured growth indices displayed significant ($P \leq 0.05$) higher biomass and provided % increases of 38.3, 40.6, 36.6 and 55.5%, respectively, when compared with the ***Erwinia carotovora***-infected plants (Appendix D ; Table 3.1), while the % increases for these indices were 22.4, 3.0, 12.5 and 9.2%, respectively, compared with the control plants. The % increases of the dual inoculation over the ***Erwinia carotovora***-infected plants and the controls may indicate that the fungus was still capable of enhancing the host growth in spite of the presence of the bacterium.

The phosphorus and potassium contents were significantly higher in *Glomus mosseae*-inoculated peanuts, compared with *Glomus mosseae* and *Erwinia carotovora*-dual inoculated peanut plants [Fig 3.3.4 (b, c) ; Appendix D ; Table 3.1]. The macronutrient (NPK) content was significantly ($P < 0.05$) higher in peanuts dual inoculated with *Glomus mosseae* and *Erwinia carotovora* when compared with *Erwinia carotovora*-infected peanuts giving % increases of 17.9, 103.9 and 26.6 % for NPK respectively. The dual inoculated peanut plants also surpassed the NPK uptake of the controls giving % increases of 13.6, 16.8 and 12.6 %, respectively [Fig. 3.3.4 (a-b-c) ; Fig. 3.3.5 ; Appendix D ; Table 3.1]. The NPK % differences of controls over *Erwinia carotovora* -infected plants were 4.5, 670 and 11.1% respectively (Fig. 3.3.5 ; Appendix D ; Table 3.1).

The numbers of the bacteria in the rhizospheric zone tended to decrease with time, although the bacterial populations were consistently lower ($P < 0.05$) in the rhizosphere of plants with mycorrhiza than the rhizosphere of those without mycorrhiza (Fig. 3.3.5 ; Appendix D ; Table 3.2).

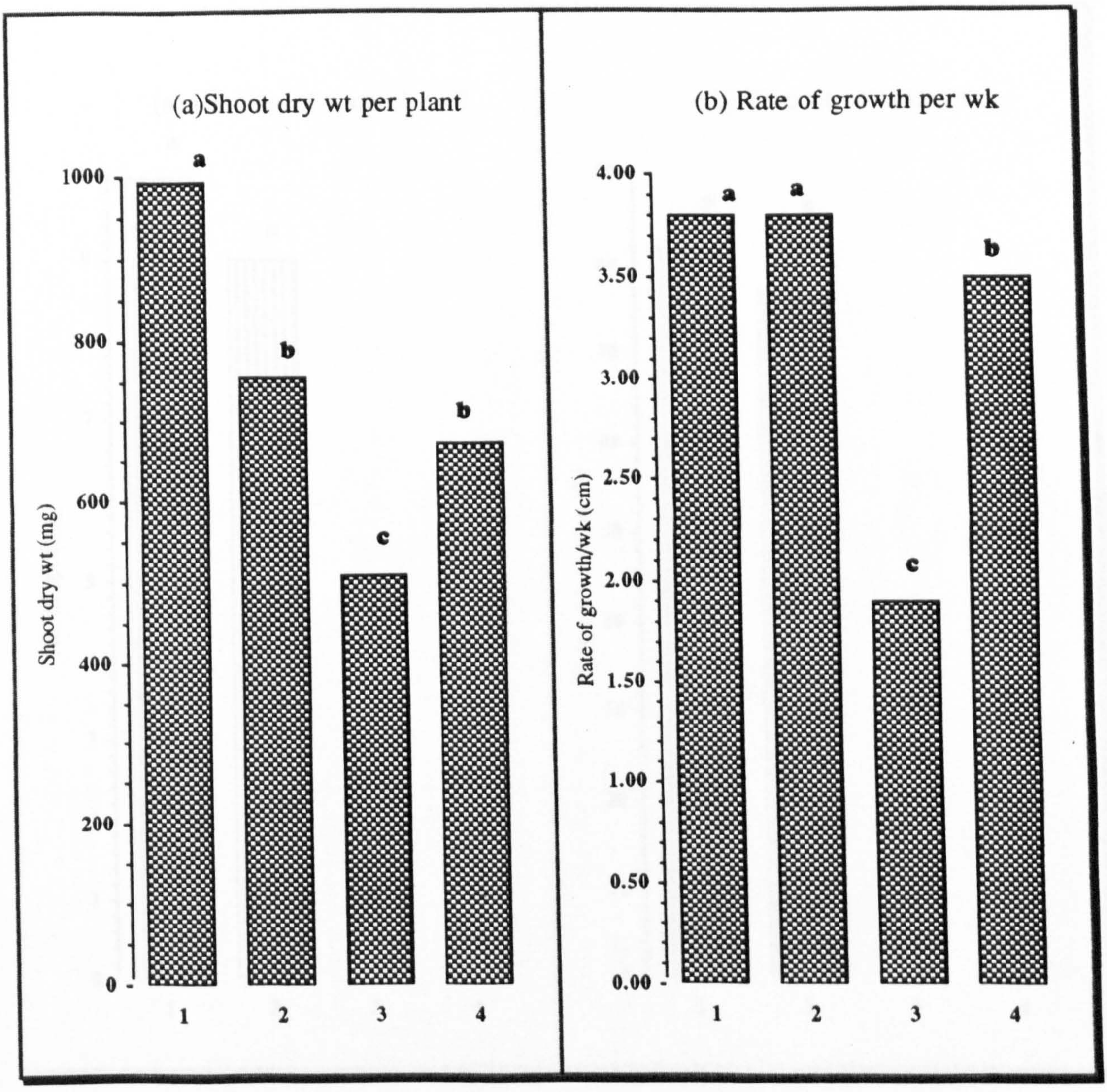
These results also suggest that *Glomus mosseae* can protect peanut seedlings against the effects of the pathogen *Erwinia carotovora* when both the AM fungus and bacterium were inoculated simultaneously and may indicate that *Glomus mosseae* associations can offer protection against the effects of invasion by this bacterium under natural conditions.



1 = *Glomus mosseae* ; 2 = *G.mosseae* + *Erwinia carotovora* ; 3 = *Erwinia carotovora* ; 4 = Control.

Bars marked with different letters are significantly different according to Fisher's LSD test ($P \leq 0.05$).

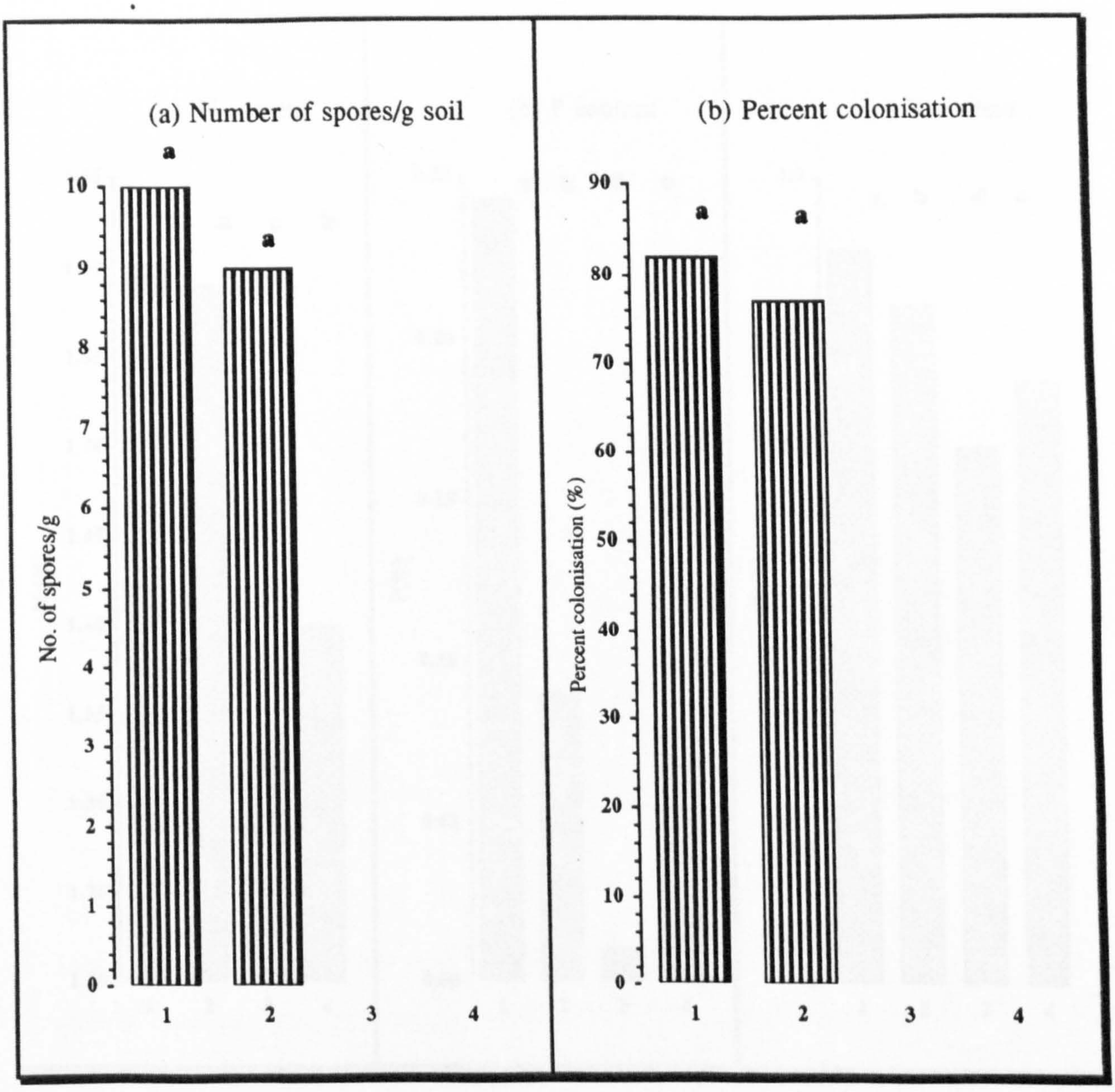
Figure 3.3.1
Growth indices of shoot height per plant and root dry weight per plant for *G. mosseae*-mycorrhizal peanut plants in presence or absence of *E.carotovora*.



1 = *Glomus mosseae* ; 2 = *G.mosseae* + *Erwinia.carotovora* ; 3 = *Erwinia carotovora* ; 4 = Control.

Bars marked with different letters are significantly different according to Fisher's LSD test ($P \leq 0.05$).

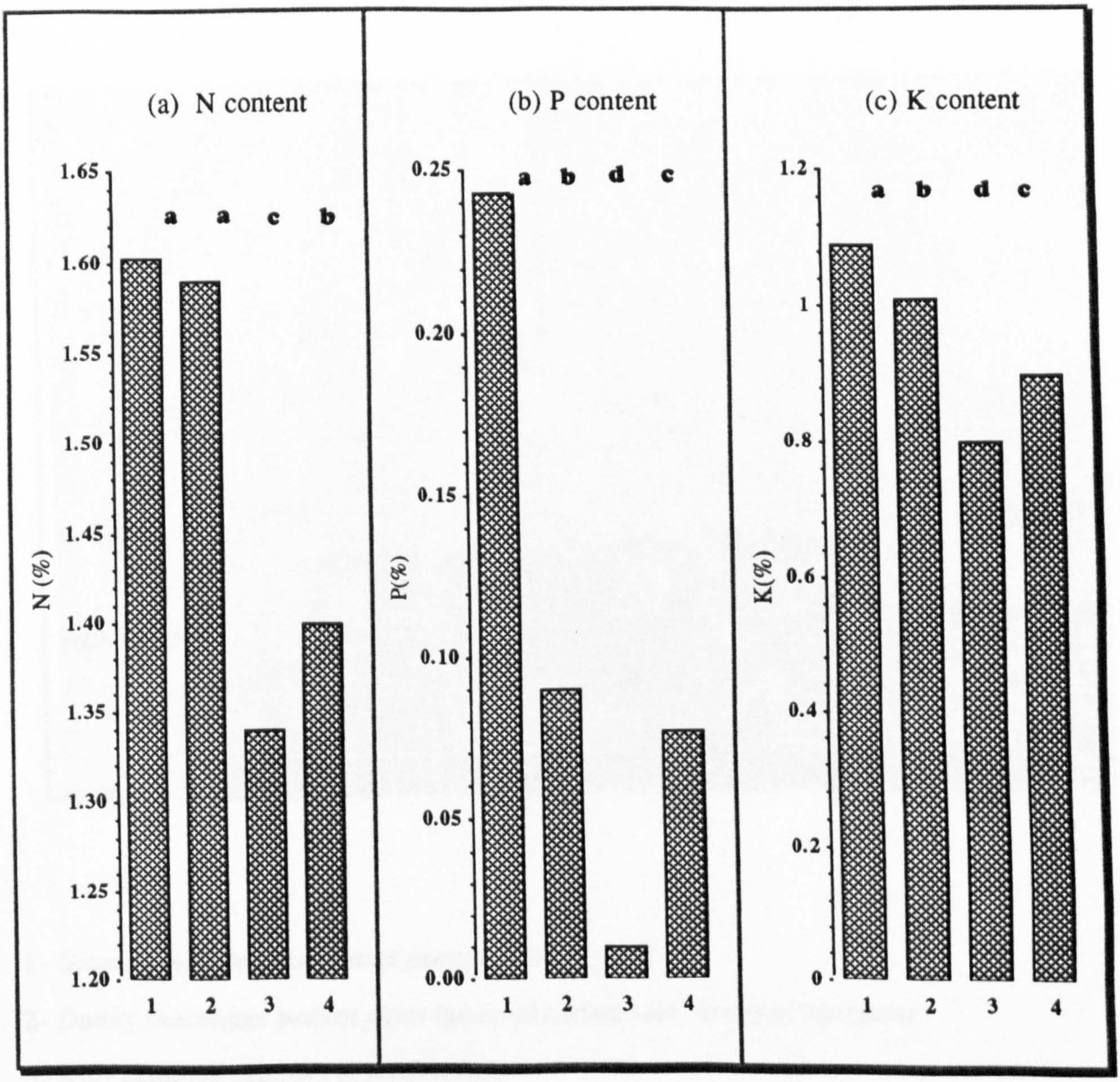
Figure 3.3.2
Growth indices of shoot dry weight per plant and rate of growth per week for *G. mosseae*-mycorrhizal peanuts in presence or absence of *E.carotovora*.



1 = *Glomus mosseae* ; 2 = *G.mosseae* + *Erwinia carotovora* ; 3 = *Erwinia carotovora* ; 4 = Control.

Bars marked with different letters are significantly different according to Fisher's LSD test ($P \leq 0.05$).

Figure 3.3.3
Spore density and percent colonisation of *G. mosseae* in presence or absence of *E.carotovora*.



1 = *Glomus mosseae* ; 2 = *G.mosseae* + *Erwinia carotovora* ; 3 = *E. carotovora* ;
4 = Control.

Bars marked with different letters are significantly different according to Fisher's LSD test ($P \leq 0.05$).
NPK data transformed to natural logs for stastistical analysis, but original means presented here.

Figure 3.3.4
NPK uptake by *G. mosseae*-mycorrhizal peanuts in presence or absence of *E.carotovora*.

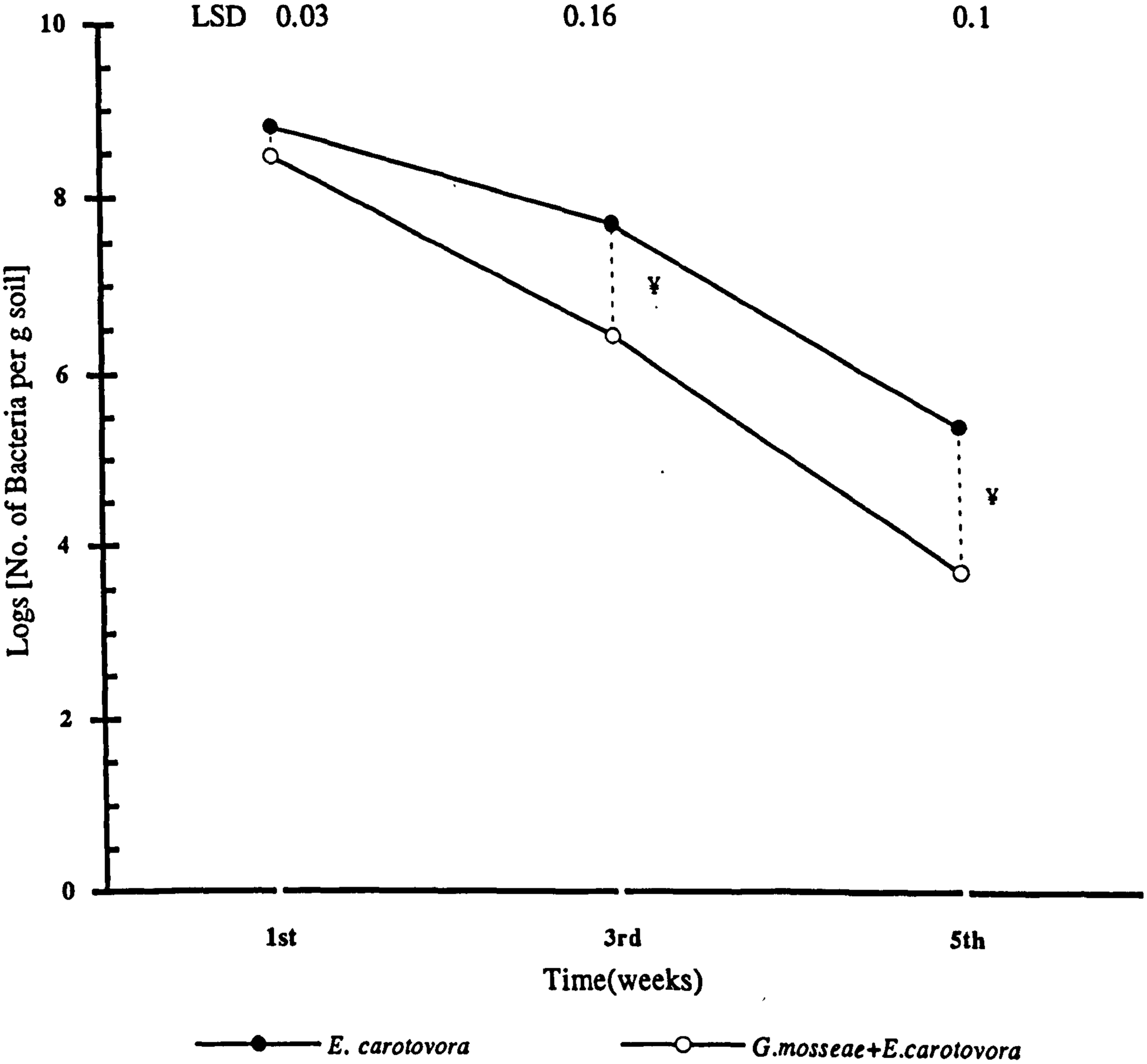
1- ***Glomus mosseae*-inoculated peanut plant.**

2- ***Dually inoculated peanut plant (mycorrhization and bacterial infection).***

3- ***Uninoculated peanut plant (Control).***

4- ***Erwinia carotovora*-infected peanut plant.**

Plate 3.3.1
Growth comparison among peanut treatments.



¥ Hi-Lo dotted lines indicate significant difference ($P<0.05$) between bacterial population when mycorrhizal or nonmycorrhizal

Figure 3.3.5
Populations of *E.carotovora* from rhizosphere of mycorrhizal and nonmycorrhizal peanuts vs. time.

3.4 DISCUSSION

Data presented in Chapter 3 indicate that variations in morphological, chemical and biological parameters showed significant differences between healthy and *Erwinia carotovora*-infected peanut plants. *Erwinia carotovora* depressed the dry weight biomass and other growth indices of nonmycorrhizal peanuts. At the same time the mycorrhizal peanuts infected with the bacterium showed significant increases in dry weight and other measured parameters compared with uninfected nonmycorrhizal controls. The lowest content of N, P and K was detected in *Erwinia carotovora*-infected peanut plants compared with *Glomus mosseae* and dual inoculation treatments. The dual inoculation with the pathogenic bacterium seemed not to affect the AM fungus sporulation and colonisation levels as clearly shown by NOSG^{-1}S and PC indices. The biocontrol of *Erwinia carotovora* by *Glomus mosseae* is likely to be accomplished through three ways as discussed below:

First, these results indicate that *Glomus mosseae* inoculation may provide biocontrol indirectly by enhancing NPK uptake and probably other nutrients (Chapter 2, this thesis) resulting in enhanced nutritional status of the peanuts that was able to ward off or tolerate the bacterial infection. This interpretation may supports Dehne's (1982) view that the protective effect afforded by the AM fungi against pathogens and their effects can be attributed to promoted growth achieved through better plant nutrition.

Second, the results also indicate that the AM fungus affected quantitatively the bacterial populations in the rhizosphere of the dual inoculation treatment and caused them to decrease significantly compared with the single bacterial inoculation. This reduction in bacterial numbers in the rhizosphere indicates a direct biocontrol effect. Also, in the dual inoculation treatment, the pathogenic

bacterium failed to suppress significantly the AM fungus sporulation and colonisation levels. These findings may also indicate that there is, probably, a direct interaction between the mycorrhizal fungus and the pathogen. Perhaps the fungus produced suppressive metabolites. Similar assumptions have been made by other workers (Caron *et al.*, 1985 ; Garcia-Garrido and Ocampo, 1988).

Finally, AM fungi have been reported to increase plant root concentrations of antimicrobial compounds like phytoalexins (Morandi, *et al.*,1984). This phenomenon may involve an improvement of disease resistance for plants with AM fungi to soil borne pathogens. Results obtained with nematodes (Morandi, 1987) strengthen this hypothesis. Therefore, physiologically, the increased resistance of mycorrhizal peanuts may be a result of specific alterations in the physiology of peanut roots due to the microbial metabolism of the AM fungus that may promote defence mechanisms.

The results reported in this Chapter provide clear evidence that the AM fungus confers biocontrol against the bacterial pathogen ***Erwinia carotovora*** when they are inoculated simultaneously into soil which peanut seedlings are growing. This could possibly indicate potential biocontrol effects under field conditions as both microorganisms occur concurrently in natural rhizosphere zones. Since the mechanisms of biocontrol of plant pathogens are complex and difficult to unravel, then to explain fully the possible mechanisms of the biocontrol of the nominee endomycorrhiza further research and investigative studies are called for.

CHAPTER 4

Effect of Simulated Salinity Stress on mycorrhizal Peanut.

4

Effect of Simulated Salinity Stress on Mycorrhizal Peanut

4.1 Introduction

Arid and semi-arid soils make up 40 % of the total global area (Fisher and Turner, 1978). A problem of high salinity often occurs in these soils and can be a significant factor limiting agricultural productivity (Flowers *et al.*, 1977). Also, a further complication challenging agriculture today is the decreasing availability of good quality irrigation water due to the relatively high content of dissolved salts (Mantell *et al.*,1985).

The accumulation of salts inhibits or checks the growth of crops in an area of 50 million hectares in the agricultural areas of arid and semi-arid regions (Carter,1975). There are several ways to alleviate the harmful impact. Salts can be leached by flooding, salt-resistant plant varieties can be bred (Heyster and Nabors,1981a) or the ability of plants to tolerate the salinity can be increased (Dehan and Tal, 1978). Due to shortage of waters in such lands it seems the more feasible strategy to produce salinity-resistant plant varieties and/or to enhance the ability of the plant to tolerate the salinity (Yeo and Flowers, 1980).

The accumulation of soluble salts in the rhizosphere will reduce the water potential and consequently reduce the availability of water to plants. In addition the uptake of these salts will, doubtless, affect the physiological processes of plants growing in these environments (Heyster and Nabors, 1981b). Therefore, the plant when exposed to high salinity, tends to change

metabolic activity to produce certain organic compounds such as sucrose, synthesise amino acids especially proline and accumulate salts inside the cells to reduce the internal water potential to counterbalance the soil water potential to maintain cell turgor. This phenomenon is known as osmotic adjustment or osmoregulation.

Among the biological approaches to enhance plant growth in saline conditions, the role of AM fungi has been investigated before. Most native plants and crops of arid and semi-arid areas are mycorrhizal (Pond *et al.*, 1984), and It has been suggested that AM fungal colonisation might enhance salt tolerance of some crops (Estaun,1990). Recent studies have indicated that some plants such as lavender (***Lavandula spica*** L.), tomato (***Lycopersicon esculentum*** Mill.), onion (***Allium cepa*** L.) and bell pepper (***Capsicum annum*** L.) show increased growth under saline conditions when their roots were colonised by AM fungi (Azcon *et al.*, 1976 ; Hirrell and Gerdemann, 1980 ; Pond *et al.*, 1984). Rosendahl and Rosendahl (1991) studied the role of AM fungi in the protection of cucumber plants (***Cucumis sativus*** L.) against stress induced by sodium chloride. Plants were subjected to salt stress by exposing the root systems to increasing NaCl concentrations and then allowed to recover in distilled water. The degree of wilting was estimated during the experiment according to a wilting index based on the following characters: 0, no wilting; 1, leaves soft; 2, leaves and stalk soft; 3, stalk bent; 4, the whole plant is soft and hangs. The recovery was calculated as the regression coefficient of the regression line between the square root of time (from when plants were replaced in distilled water) and wilting index. They found that both AM fungi (***Glomus spp***) that were tested were able to protect the plants from salt stress compared with nonmycorrhizal plants. They suggested that the presence of AM fungi in the roots might alter the osmotic balance of cells as AM fungi are likely

to influence the composition and concentration of amino acids and carbohydrates in the host plant.

Since peanut is known to benefit from mycorrhizal association with significant increase in dry matter yield (Daft and EL-Giahmi, 1976 ; Chapters 1, 2 & 3, this thesis), and increased uptake of P and other elements (Krishna and Bagyaraj, 1982 ; Rao *et al.*, 1990 ; Chapter 2, this thesis), it would be interesting to discover if AM fungi have any additional beneficial effects in reducing salinity stress imposed on ***Arachis hypogaea*** L. [var. *hypogaea* cv. Florunner] in saline conditions. Therefore, this trial was structured to scrutinise some physiological and growth salt stress-indicating parameters viz. free proline content, leaf relative water content, nutrient uptake and biomass yield in mycorrhizal plants in saline conditions. To the best of my knowledge, such study of ***Glomus mosseae***/peanut symbiosis under saline sodic conditions has not previously been investigated.

2.2
Methods

2.2.1
Seedlings Preparation, Mycorrhization and Experimental Design

The experiment was conducted using the autoclaved substrate soil amended with the original soil microbiota formulated as described in Chapter 1. As it was discovered that the highest relative mycorrhizal dependency of peanut was at the original substrate soil with a P base line of 6 µg P g^{-1} accordingly, the substrate soil without further P fertilisation was selected for this trial. The concentration of the original soluble salts of this soil was 280 µg ml^{-1} with electrical conductivity (EC_{soil}) of 0.094 dSm^{-1} (see Table 2.2.1). The substrate

soil was apportioned into 2.0 kg plastic pots whose drainage holes were lined with perforated polyethylene bags.

Five salinity levels for the irrigation of pots were prepared by adding sodium chloride (NaCl) to distilled water. The NaCl concentrations were 0, 0.1, 0.2, 0.3 and 0.5 M. The electrical conductivities of the salinised irrigation waters and the leachates were determined by a conductivity meter model LF 56 (Wis - Techn. Wekslåtten. D812 Weilheim).

Seeds of *Arachis hypogaea* were surface-sterilised and pregerminated as described above (Chapter 3; Methods, 3.2.1). Pregerminated seeds were transplanted into each of 50 pots that constituted the five levels of salinity stressed mycorrhizal peanut treatments on top of a bed of 5 g of inoculum. The crude inoculum consisted of chopped roots and soil from a 4-months old pot culture of *Glomus mosseae* propagated on Sudangrass as per the procedure devised by INVAM (see Materials and Methods; 1.1). Pregerminated seeds were also transplanted to make 50 salinity stressed nonmycorrhizal peanuts pots. Each of the salinity stressed nonmycorrhizal peanuts experimental pots received 5 ml of the crude inoculum filtrate which was sieved through a 37 µm sieve openings to remove mycorrhizal fungal spores to assure similar microbial population in all pots. The plants were established for 2 wk prior to irrigation with the salt solutions in the greenhouse. The experiment was conducted in a completely randomised design, with 10 salinity stressed mycorrhizal peanuts and 10 salinity stressed nonmycorrhizal peanuts at each of the five salinity treatments for a total of 100 pots. Twenty pots with peanuts watered with distilled water were used as controls. All pots were fertilised with half strength Hoagland's solution (Hoagland and Arnon 1950) on a biweekly basis.

2.2.2
Salinity Acclimatisation and Salinisation Procedures

To avoid salinity shock and to acclimatise peanut seedlings and the AM fungus to high NaCl concentrations, salinity stress was imposed on the seedlings by applying the saline irrigation waters progressively. Each treatment was watered with the lowest NaCl concentration then with the next higher concentration until each treatment reached its designed irrigation concentration. The acclimatisation took 2.5 wk to get to 0.5M treatment. The treatments were irrigated twice wk^{-1} with its appropriate saline irrigation concentration in such a way to avoid leaching. To maintain NaCl salinisation at the correct level, before applying each subsequent saline irrigation, all the pots were leached with distilled water (≈ 700 ml pot^{-1}) to prevent salt accumulating beyond the experimental concentration. The experiment was terminated after 9 wk of salt treatment.

2.2.3
Physiological and Growth Salt Stress-indicating Parameters

2.2.3.1
Determination of free proline content

The determination was conducted as per the method of Bates (1973).

- Approximately 0.5 g of peanut leaves were homogenised in 10 ml of 3 % sulfosalicylic acid solution.
- The homogenate was filtered through Whatman no. 2 filter paper.
- Then the reaction mixture was prepared in a test tube as follows:-
  - (a) 2 ml leaf filtrate.
  - (b) 2 ml acid-ninhydrin (see Appendix A - 1, for details).
  - (c) 2 ml glacial acetic acid.

- This mixture was heated for 1 h at 100 °C.
- The reaction was terminated in an ice bath.
- The reaction mixture was extracted with 4 ml toluene by mixing vigorously with a micro stirrer for 20 s.
- Toluene (containing chromatophores) was separated from water using separating funnel, warmed to room temperature and the absorbancy read at 520 nm using toluene as blank in spectronic 20 (BAUSCH & LOMB) spectrophotometer.
- The proline concentration was determined from a standard curve and calculated as follows:

$$\left((\mu g \text{ proline } ml^{-1} \text{ X ml toluene}) \div (115.5 \mu g \ \mu mole^{-1})\right)\left((g \text{ sample} \div 5)\right)^{-1}$$

$$= \mu moles \ g^{-1} \text{ of fresh weight material.} \quad (13)$$

2.2.3.2
Determination of leaf relative water content

A leaf from a third branch of the main stem from 3 salt stressed mycorrhizal plants and 3 salt stressed nonmycorrhizal plants at each salinity level was selected and removed at 10:00 h in the morning. The fresh weight was recorded. The leaves were submerged in distilled water in Petri dishes and kept for 5 h in the dark. The saturated weight (SW) was determined. The leaves were oven-dried at 60 °C for 48 h and then weighed. The leaf relative water content (LRWC) was calculated according to the formula devised by Turner and Kramer (1980).

$$LRWC = (FW - DW)(SW - DW)^{-1} X\ 100 \quad (14)$$

2.2.3.3
Determination of fresh and dry weights

The whole plant including shoot and root system was weighed to determine the fresh weight per plant (FWP^{-1}), then oven-dried at 60 °C for 48 h to determine the dry weight per plant (DWP^{-1}) for each NaCl salinisation treatment.

2.2.4
Determination of Plant Nutrient Content

At harvest, the plant samples were collected, washed and ground as outlined before [Materials and Methods; 1.4]. The nutrient contents in shoot systems (stems, leaves and petioles) for the salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts within each treatment were appraised. The determinations of nitrogen, phosphorus, potassium, calcium and sodium were conducted by the methods described in Jackson (1958). The nutrient content was calculated per gram dry weight.

2.2.5
Mycorrhizal Assessment

For determination of percent colonisation of roots and determination of number of spores per gram soil (see Materials and Methods; 1.6.3 and 1.6.4.).

2.2.6
Calculation of Relative Response

The difference in FWP^{-1}, DWP^{-1}, proline content, leaf relative water content, nitrogen, phosphorus, potassium, calcium and sodium between the salinity stressed mycorrhizal peanuts (SMP) and salinity stressed nonmycorrhizal peanuts (SNMP) within each treatment was expressed as relative response (= % increase) using the following equation:

$$\text{Relative Response (\%)} = \left((SMP) - (SNMP)\right)\left((SNMP)\right)^{-1} X\ 100 \qquad (15)$$

3.4
Data Analysis

Data were analysed using simple regression analysis comparing regression equations and elevations. Student's t-test was used to compare mean pairwise of salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts of the same treatment (within each salinisation level).

4.3
RESULTS

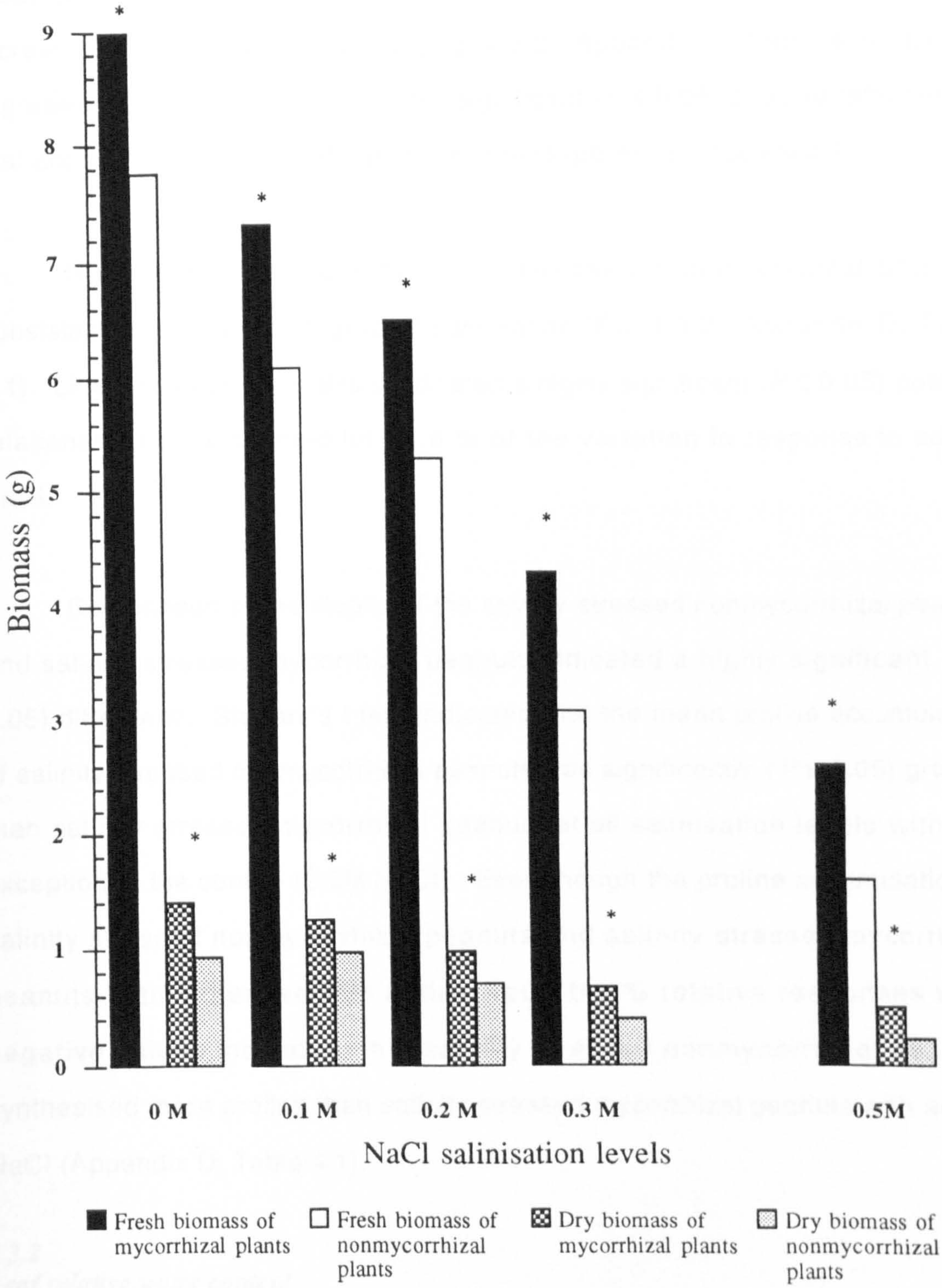
4.3.1
Peanut biomass

For, salinity stressed mycorrhizal peanuts, linear regression analysis indicated a highly significant ($P \leq 0.05$) negative linear relationship that accounted for 93.5 % of the variation in FWP^{-1} in response to increasing NaCl salinity. For salinity stressed nonmycorrhizal peanuts, linear regression analysis indicated a highly significant ($P \leq 0.05$) negative linear relationship that accounted for 86.1 % of the variation in FWP^{-1} in response to added NaCl.

For salinity stressed mycorrhizal peanuts, linear regression analysis indicated a highly significant ($P \leq 0.05$) negative linear relationship that accounted for 86.7 % of the variation in DWP^{-1} in response to NaCl salinity. For salinity stressed nonmycorrhizal peanuts, linear regression analysis indicated a highly significant ($P \leq 0.05$) negative linear relationship that accounted for 63.8 % of the variation in DWP^{-1} in response to added NaCl.

Comparison of regression of slopes of FWP^{-1} and DWP^{-1} of salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts indicated no significant differences ($P = 0.05$). However, comparison of elevations indicated that salinity stressed mycorrhizal peanuts had significantly greater ($P \leq 0.05$) FWP^{-1} and DWP^{-1} than salinity stressed nonmycorrhizal peanuts. The relative responses in FWP^{-1} and DWP^{-1} between salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts tended to form a near-linear increase with increasing salinisation levels.

Salinity stressed mycorrhizal peanuts had significantly greater fresh and dry biomass compared with salinity stressed nonmycorrhizal peanuts grown at the same salinisation level at all NaCl treatments. The mean dry weight per plant (DWP^{-1}) of salinity stressed mycorrhizal peanuts grown at the highest salinisation level (0.5 M) was more than two fold that of salinity stressed nonmycorrhizal peanuts grown in the same salinisation level (Fig. 4.3.1 ; Appendix D; Table 4.1).



Asterisks denote significance according to Student's t tests

Figure 4.3.1
Effect of salinity stress on root and shoot biomass of mycorrhizal and nonmycorrhizal peanut plants.

4.3.1
Free proline content

Mean proline content of salinity stressed mycorrhizal peanuts consistently increased with gradual salinisation (Fig. 4.3.2 ; Appendix D; Table 4.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) positive relationship that accounted for 86.4 % of the variation in response to added NaCl.

Mean proline accumulation of stressed nonmycorrhizal peanuts consistently increased with gradual salinisation (Fig. 4.3.2 ; Appendix D; Table 4.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) positive relationship that accounted for 95.6 % of the variation in response to added NaCl.

Comparison of the slopes of the salinity stressed nonmycorrhizal peanuts and salinity stressed mycorrhizal peanuts indicated a highly significant ($P \leq 0.05$) difference. Student's t test indicated that the mean proline accumulation of salinity stressed nonmycorrhizal peanuts was significantly ($P \leq 0.05$) greater than salinity stressed mycorrhizal peanuts at all salinisation levels with the exception of the control (0.0M NaCl). Even though the proline accumulation of salinity stressed nonmycorrhizal peanuts and salinity stressed mycorrhizal peanuts both increased with added NaCl, the % relative responses were negative values indicating that salinity stressed nonmycorrhizal peanuts synthesised more proline than salinity stressed mycorrhizal peanuts with added NaCl (Appendix D; Table 4.1).

4.3.2
Leaf relative water content

The leaf relative water content (LRWC) of salinity stressed mycorrhizal peanuts

consistently decreased with gradual salinisation (Fig. 4.3.3 ; Appendix D; Table 4.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) negative relationship that accounted for 85.7 % of the variation in response to added NaCl. The leaf relative water content of salinity stressed nonmycorrhizal peanuts, also consistently decreased with gradual salinisation (Fig. 4.2.3 ; Appendix D; Table 4.1). Linear regression analysis indicated a highly significant ($P \leq 0.05$) negative relationship that accounted for 96.3 % of the variation in response to added NaCl.

The leaf relative water content of salinity stressed mycorrhizal peanuts was significantly greater than salinity stressed nonmycorrhizal peanuts at all salinisation levels. Comparison of the slopes of the salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts indicated a highly significant difference ($P \leq 0.05$). The % relative responses in leaf relative water content between salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts tend to increase with increased amount of NaCl (Appendix D; Table 4.1).

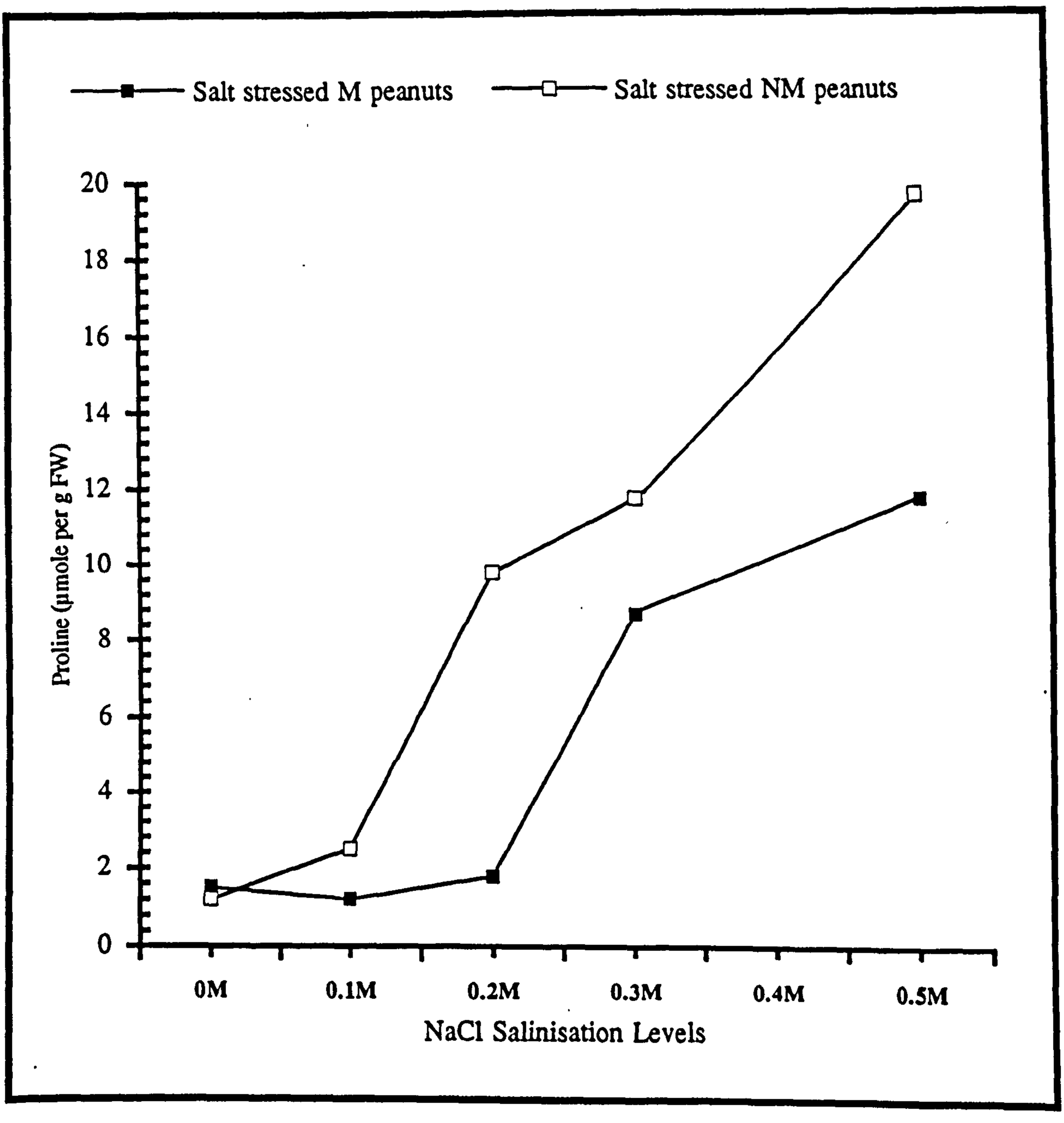


Figure 4.3.2
Proline concentration of salt stressed mycorrhizal and salt stressed nonmycorrhizal peanut plants.

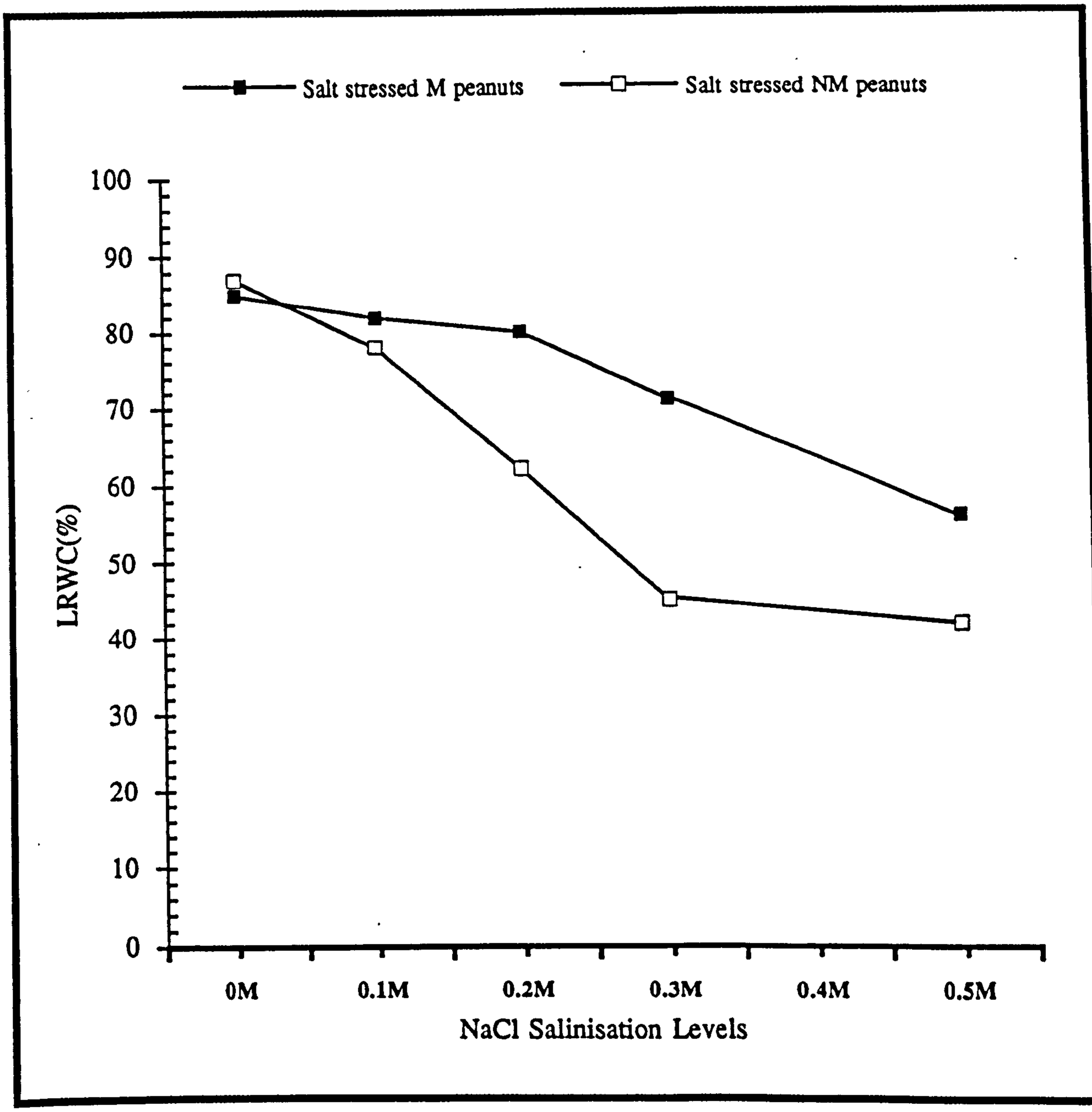


Figure 4.3.3
Leaf relative water content of salt stressed mycorrhizal and salt stressed nonmycorrhizal peanut plants.

4.3.4
Mycorrhizal assessment

Linear regression analysis indicated a highly significant ($P \leq 0.05$) negative relationship between amount of added NaCl and mycorrhization as measured by spore production ($NOSG^{-1}S$) and mycorrhizal colonisation (PC). The regression accounted for 87.6 % and 79.3 % of the variation in spore density and colonisation index, respectively, in response to added NaCl (Fig. 4.3.4). Colonisation and spore density decreased from an average of 93 % and 12 spores g^{-1} at control treatment (0.0 M) to 45 % and 2 spores g^{-1} at highest salinisation level (0.5 M) (Appendix D; Table 4.1). The highest salinisation level almost completely eliminated sporulation but only reduced colonisation by the AM fungus to half that measured at 0 NaCl.

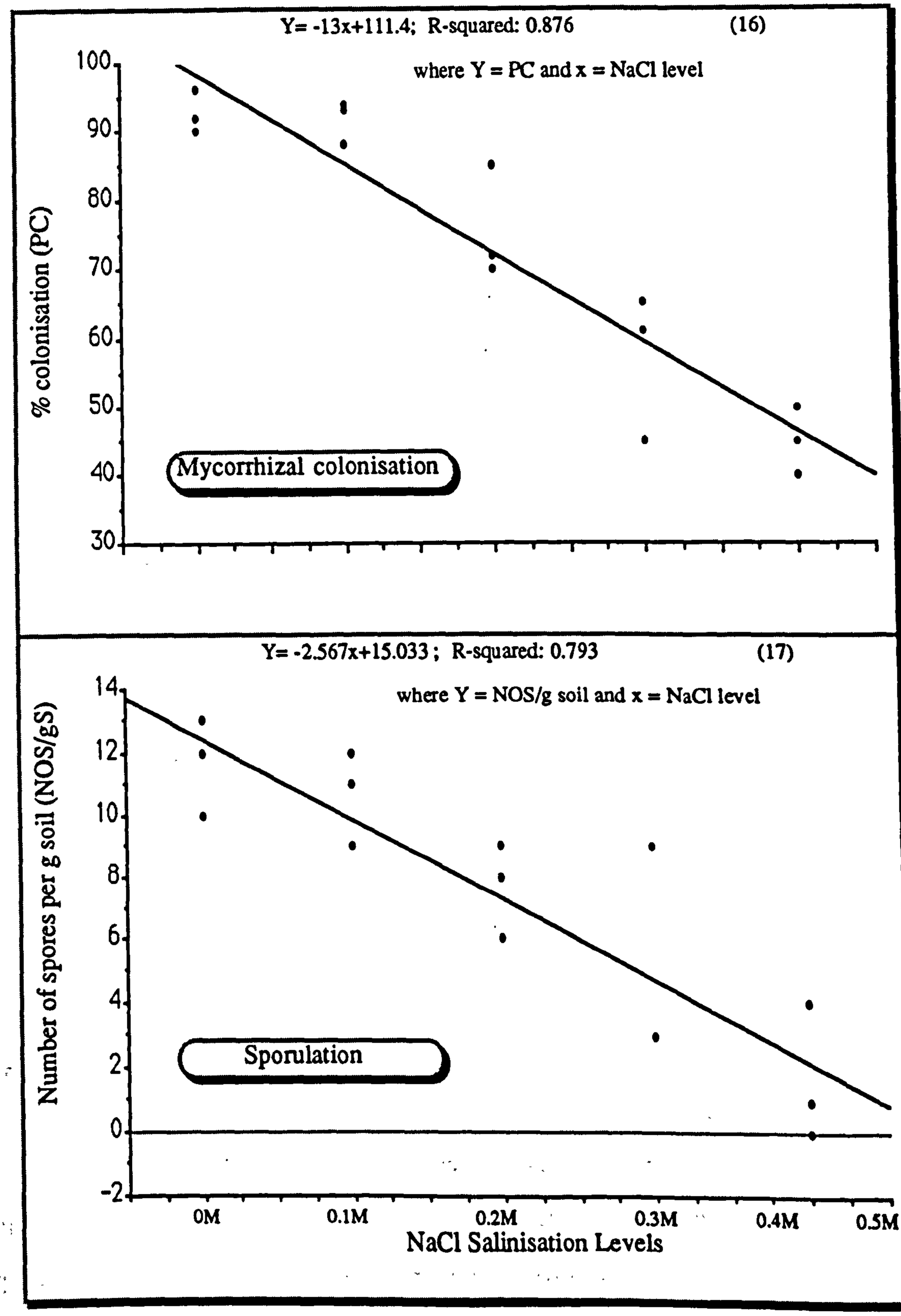


Figure 4.3.4
Decrease of sporulation and mycorrhizal colonisation as a function of increasing salinity stress.

4.3.5 Nutrient uptake

4.3.5.1 Nitrogen

Analysis of variance of the regression of mean nitrogen content in the shoot system of salinity stressed mycorrhizal peanuts indicated insignificant linear relationship ($P = 0.05$) between N content and gradual NaCl salinity. The regression accounted for 26.8 % of the variation in N content in response to the gradual salinity. Also, there is no significant linear relationship between N content in salinity stressed nonmycorrhizal peanuts and added NaCl at $P = 0.05$.

The salinity stressed mycorrhizal peanuts had higher N content than salinity stressed nonmycorrhizal peanuts at the salinisation levels ≤ 0.1M and 0.3 M NaCl while salinity stressed nonmycorrhizal peanuts had significantly higher N content than salinity stressed mycorrhizal peanuts at salinisation 0.5M NaCl according to Student's t-test ($P = 0.05$). Generally the relative responses were positive, a trend that tends to decrease with added NaCl (Table 4.3.1). This indicated that salinity stressed mycorrhizal peanuts accumulated more N than salinity stressed nonmycorrhizal peanuts, but the relative response tends to decline as salinisation increased.

4.3.5.2 Phosphorus

Both salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts showed reduced P content with NaCl addition. Regression analysis indicated a highly significant ($P \leq 0.05$) negative linear relationship between gradual salinisation of both salinity stressed mycorrhizal peanuts and salinity

Table 4.3.1
Nitrogen content of mycorrhizal and nonmycorrhizal peanuts as influenced by sodium chloride salinity stress.

NaCl levels	VAM (+/-)¶	Nitrogen (mg per g DW)
0.0M	+ - (%) ¥	1.58* ± (.08) 1.1 ± (.06) 44
0.1 M	+ - (%)	1.5* ± (.05) 1.38 ± (.21) 9
0.2 M	+ - (%)	1.60 ± (.06) 1.50 ± (.06) 7
0.3 M	+ - (%)	1.60* ± (.06) 1.39 ± (5.8E-3) 15
0.5 M	+ - (%)	1.22 ± (.04) 1.37* ± (3.3E-3) -11
Significant linear correlation [P ≤ 0.05] §	+ -	(o) (o)

Mean pairs [± S.E.M.] within a column with an * are significantly different at (P=0.05) according to a Student's t test.
¶ (+/-) corresponds to mycorrhizal and nonmycorrhizal with the AM fungus *G. mosseae*.
¥ Relative response = {[SMP (+)] - [SNMP (-)]÷[SNMP (-)]} X100 (15).
§ Significant linear correlation positive (+ve), negative (-ve) or no correlation (o).

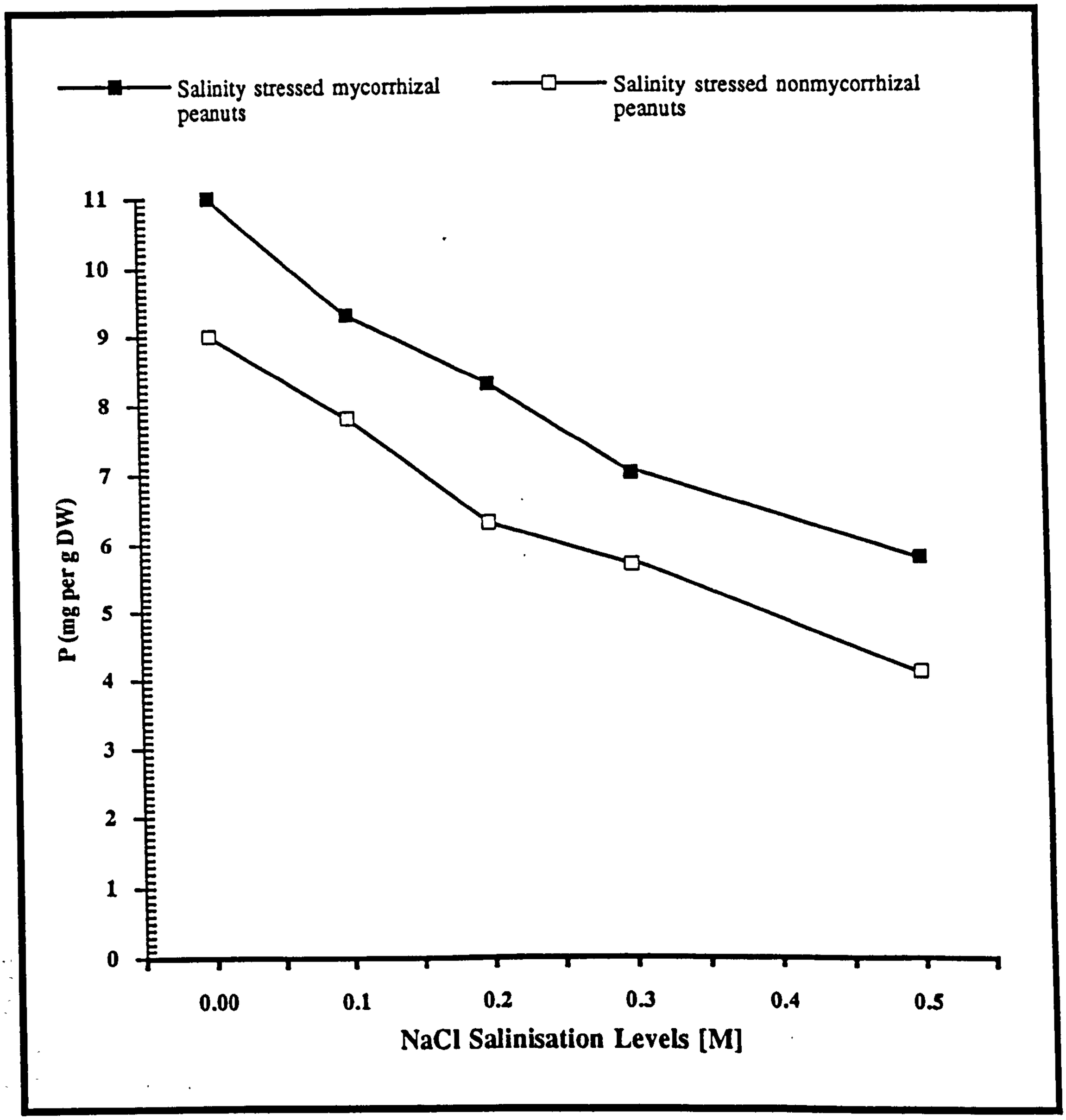


Figure 4.3.5
Effect of salinity stress on phosphorus in mycorrhizal and nonmycorrhizal peanuts.

stressed nonmycorrhizal peanuts that accounted for 94.5 % and 79.1 % respectively between P content and NaCl increase.

Student's t-tests indicate significantly higher P in salinity stressed mycorrhizal peanuts compared with salinity stressed nonmycorrhizal peanuts at all salinisation levels. The relative response were positive and ranged from 19 % at 0.1 M NaCl to 41 % at 0.5 M NaCl level, displaying a tendency to increase with gradual salinisation stress (Fig. 4.3.5 ; Appendix D; Table 4.2).

4.2.5.3 Potassium

There was no significant linear correlation between K content and gradual stress induced by NaCl for salinity stressed mycorrhizal peanuts ($P = 0.05$). However, a significant negative linear relationship was detected for salinity stressed nonmycorrhizal peanuts ($P = 0.5$) that accounted for 26.6 % of the variation in K content in response to gradual salinity.

The data suggest that gradual NaCl salinity stress increased Na uptake (Fig. 4.3.8) and decreased K. Salinity stressed mycorrhizal peanuts had significantly higher K content than salinity stressed nonmycorrhizal peanuts at salinisation levels of 0.1M and 0.3M with the relative response following a more or less a consistent positive pattern as the salinity increased (Fig. 4.3.6 ; Appendix D; Table 4.2). This indicated that the absorption of K by salinity stressed mycorrhizal peanuts was relatively better than salinity stressed nonmycorrhizal peanuts, but only at salinisation levels less than 0.3M.

4.2.5.4
Calcium

Simple regression analysis indicate significant positive linear correlation ($P < 0.05$) that accounted for 68.9 % and 81.4 % for salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts respectively between Ca content and NaCl addition.

At low salinisation level (NaCl = 0.1 M), the salinity stressed mycorrhizal peanuts took up significantly greater Ca than salinity stressed nonmycorrhizal peanuts. However, when the salinisation level reached ≥0.2 M, salinity stressed nonmycorrhizal peanuts accumulated significantly more Ca than salinity stressed mycorrhizal peanuts. The relative responses followed an inconsistent pattern. The Ca absorption by salinity stressed mycorrhizal peanuts was high at first, then the response trend reverses indicating greater Ca uptake by salinity stressed nonmycorrhizal peanuts (Fig. 4.3.7 ; Appendix D; Table 4.2). This inconsistency may indicate that Ca absorption needs further investigation in saline conditions.

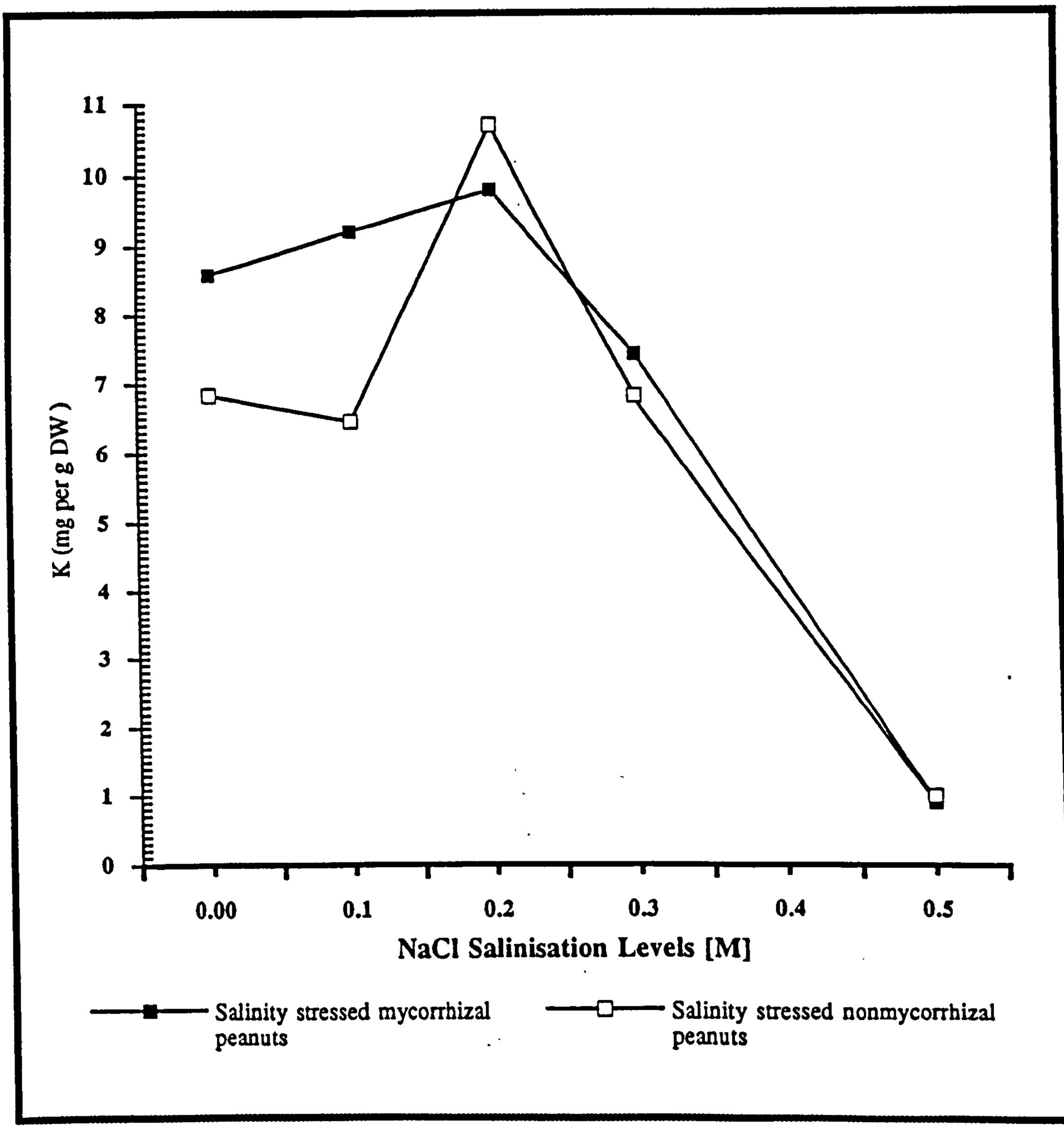


Figure 4.3.6
Effect of salinity stress on potassium in mycorrhizal and nonmycorrhizal peanuts.

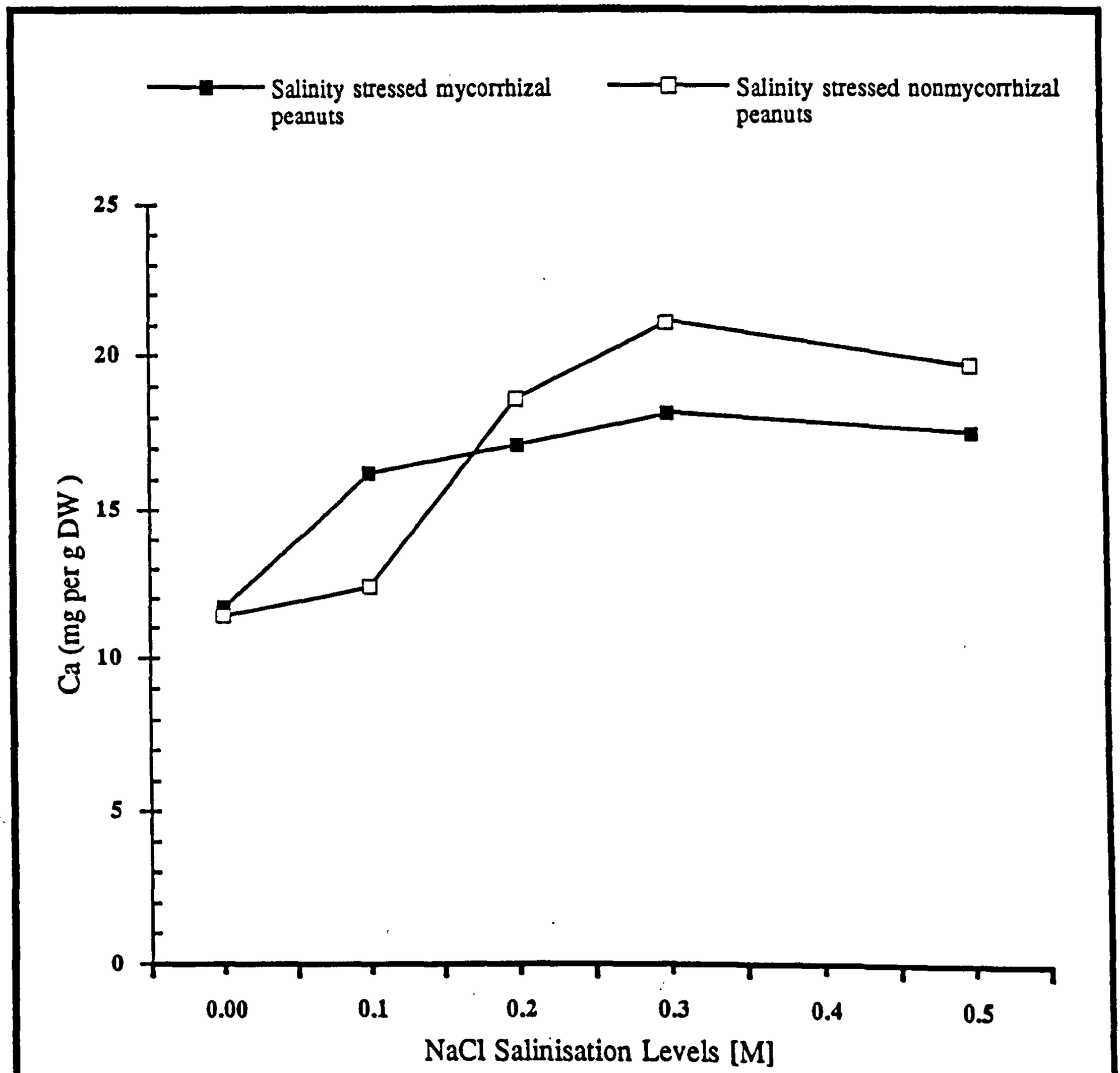


Figure 4.3.7
Effect of salinity stress on calcium in mycorrhizal and nonmycorrhizal peanuts.

4.3.5.5 Sodium

Analysis of the regression of mean Na contents in shoots of salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts indicated a highly significant ($P \leq 0.05$) positive linear relationship with increase of salinity stress. The linear regression accounted for 95.5 % in salinity stressed mycorrhizal peanuts and 93.9 % in salinity stressed nonmycorrhizal peanuts increases of added NaCl.

Salinity stressed mycorrhizal peanuts had significantly greater Na content in their shoots than salinity stressed nonmycorrhizal peanuts grown at all salinisation levels. The relative response follows a consistent positive increased trend as salinity increases (Appendix D; Table 4.2).

In summary from Table 4.3.1 and Appendix D (Table 4.3.2) the absorption of N by both salinity stressed mycorrhizal and nonmycorrhizal peanuts showed no correlation with salinisation at all. The absorption of P for both salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts was negatively correlated with gradual increase in salinisation. The accumulation of K in salinity stressed mycorrhizal peanuts did not show any correlation, but it displayed a negative correlation in salinity stressed nonmycorrhizal peanuts as NaCl increased. The uptake of Na and Ca by both salinity stressed mycorrhizal peanuts and salinity stressed nonmycorrhizal peanuts was positively correlated with the increased salinisation level.

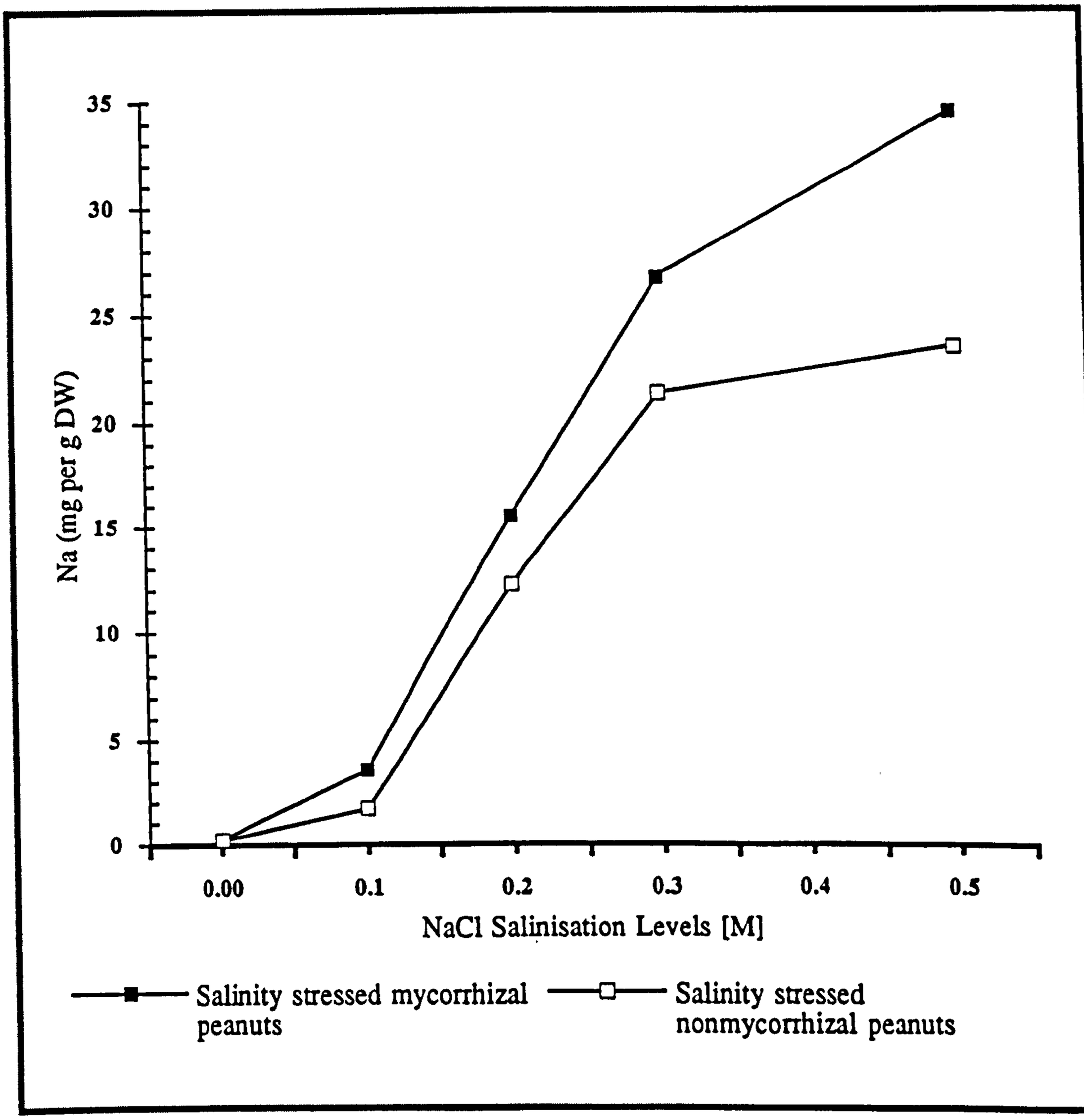


Figure 4.3.8
Effect of salinity stress on sodium in mycorrhizal and nonmycorrhizal peanuts.

For salinity stressed mycorrhizal peanuts the trends of relative responses for P, K and Na are generally positive indicating that salinity stressed mycorrhizal peanuts absorbed much of these nutrients compared with salinity stressed nonmycorrhizal peanuts. The trend of relative responses of Ca did not follow a definite trend throughout the salinisation treatments.

The results of measurements of free proline content, leaf relative water content, nutrient uptake and biomass yield indicate that inoculation of salt stressed peanuts with the AM fungus ***Glomus mosseae*** ameliorate the detrimental effects of induced by NaCl salinisation.

4.4 DISCUSSION

Chapter 4 of this study evaluated the response of *Glomus mosseae*- inoculated peanuts to simulated salinity stress. Salt stress reduced final biomass in both salt stressed mycorrhizal and nonmycorrhizal peanuts, but salt stressed mycorrhizal peanuts suffered reductions in biomass less than similarly salt stressed-nonmycorrhizal peanuts. The indices of fresh and dry biomass production indicate that *Glomus mosseae* markedly improved peanut yield under the same saline conditions. When exposed to the higher concentrations of sodium chloride, the final biomass of the plants was substantially reduced irrespective of the presence or absence of the mycorrhiza. It can not be determined from the results whether the plants grew consistently, albeit very slowly, during their period of exposure to salt or the salt inhibited growth completely and their yield was largely determined during the period of growth prior to salt exposure. Very few species of higher plants continue growth when exposed to concentrations which approach those of sea water which is approximately 0.5M sodium chloride (Flowers *et al.*, 1977), the higher concentration used with peanut.

The primary cause of reduced yield of plants at high salinity could arise from an adverse specific effect of Na and Cl on metabolism. However, it could also occur because of a reduced ability to maintain a normal water balance when a plant can not accumulate sufficient solutes to sustain the concentration gradient across the cell membrane as occurs in the absence of external salinity (osmoregulation). Sodium chloride stress reduced the leaf relative water content (LRWC) in both salt stressed mycorrhizal and nonmycorrhizal peanuts, but the LRWC was significantly higher in salt stressed mycorrhizal peanuts than

salt stressed-nonmycorrhizal at salinisation level ≥ 0.2 M. The index of LRWC indicated that *Glomus mosseae* fungus improved water content of stressed peanuts. This supports the view that symbiotic relationships formed between AM fungi and host plants improve water uptake (Mosse, 1973a ; Harley and Smith, 1983). Host mycorrhization can influence plant-water relations by reducing resistance to water transport (Allen, 1982) and may thus enhance plant drought tolerance (Allen *et al.*, 1981).

Typically under saline stress, total free amino acid and proline concentration increase in leaves (Barnett and Naylor, 1966). Proline accumulation under salt stress has been reported extensively. However, it is a matter of controversy whether it reflects regulatory adaptive process or if it indicates damage due to salt stress (Vartanian *et al.*, 1992). The results showed that both salt stressed mycorrhizal and nonmycorrhizal peanuts accumulated free proline that was positively linearly correlated to gradual increased NaCl stress. However foliar increases in free proline in salt stressed-nonmycorrhizal peanuts were significantly higher at salinisation level ≥ 0.2 M compared with their similarly stressed mycorrhizal counterparts. Stressed mycorrhizal peanuts osmotically adjust better compared with nonmycorrhizal plants. To achieve this osmoregulation, stressed mycorrhizal peanuts have to contain a greater concentration of solutes such as Na and/or synthesise more solutes such as proline. Stressed mycorrhizal peanuts contained higher Na, but less proline compared with nonmycorrhizal peanuts. This clearly shows that high Na accumulation was for the osmoregulation purposes, while reduced foliar proline in mycorrhizal peanuts suggests that the mycorrhizal fungus was able to alleviate the damage due to salts stress.

The mineral composition of plants exposed to salinity stress varies with the concentration of the salt solution, plant species, and the part of the plant

harvested. Apart from Na, salt stressed mycorrhizal peanut did not contain much more cations than nonmycorrhizal peanuts and generally, K content declined with the increasing NaCl. Significant negative correlations between salinity and K, Ca, Cu and Fe were discovered for barley (Hassan *et al.*, 1970a) and K, Ca, Cu, Fe and Mn for corn (Hassan *et al.*, 1970b). However at lower NaCl concentration, K content was maintained or somewhat increased in both mycorrhizal and nonmycorrhizal peanut plants and only above 0.2M NaCl was K then drastically reduced. The reduction was similar in both mycorrhizal and nonmycorrhizal plants. The maintenance of K up to a critical external NaCl concentration (typically 100-200 mM NaCl) is characteristic of nonhalophyte response and the results reported here suggest that mycorrhizal associations do not change this response or the critical concentration at which K uptake breaks down. Because of their greater Na uptake, the Na/K ratio was generally greater in mycorrhizal peanut plants

Although the results indicate that P content was reduced by salinity stress yet salt stressed mycorrhizal peanuts contained significantly more P than salt stressed-nonmycorrhizal peanuts at all salinisation levels. The mechanisms responsible for decreasing P uptake by plants exposed to saline conditions are not well understood (Mass and Nieman, 1978). The application of phosphorus has been clearly shown to improve yield and growth responses under saline conditions (Champagnol, 1979).

The results presented agree with previous reports (Azcon *et al.*, 1976 ; Hirrell and Gerdemann, 1980 ; Ojala *et al.*, 1983) in that AM fungi can greatly increase the yield of plants growing in phosphorus-deficient, saline soil especially in *Glomus mosseae* associations which has been shown to affect plant tolerance to environmental stress (Safir *et al.*, 1972). The mechanism of this benefit is likely to be mainly through improved P nutrition, as others have

reported (Gerdemann, 1968 ; Mosse, 1973b and Ojala *et al.*, 1983). The growth of plants exposed to osmotic stress may be reduced even more by nutritional imbalances. Since the results in Chapter 4 revealed that salt tolerance of peanut was enhanced by ***Glomus mosseae*** inoculation, then an alternative to fertilising with nutrients to correct mineral deficiencies in plants growing in saline soils would be to inoculate them with AM fungi.

The sporulation ($NOSG^{-1}S$) and colonisation (PC) levels of ***Glomus mosseae*** were inversely correlated with the NaCl salinisation. The decreased colonisation by the AM fungi under salt stress may be because of reduced germination of fungal spores (Hirrell, 1981). The reduction in biomass production with gradual NaCl stress is likely to correlate with mycorrhizal colonisation decrease. This supports previous evidence that the peanut plant is dependent upon mycorrhizal association for good growth (Krishna and Bagyaraj, 1982; 1984; Parvathi *et al.*, 1985; Middleton *et al.*, 1989; Chapter 2, this thesis).

Generally, the results of the measured indices in these experiments, indicated that mycorrhization of salt stressed peanuts with the AM fungus ***Glomus mosseae*** is likely to have a role in relieving stress induced by NaCl salinisation. Although the physiological mechanism(s) for increased salt and accompanied water stress remains unclear (Rosendahl and Rosendahl, 1991), the following speculative points may be considered to explain further the role of AM fungus in relieving the stress on peanuts:-

- The presence of AM fungi in the roots might alter the osmotic balance as AM fungi have been found to influence the composition of amino acids and carbohydrates (Rosendahl and Rosendahl, 1991).

• The AM fungus *Glomus mosseae* could also enhance peanut plants tolerance to salt stress through improved P uptake and probably improved K nutrition (Chapter 4, this thesis).

• The hyphae of AM fungi can penetrate the immediate area around the roots and may be absorbing water and nutrients from the soil solution at a lower water potential than that at the root surface (Rhodes and Gerdemann, 1975).

These findings suggest that AM inoculation is a challenging method for enhancing peanut yield, especially in soils where phosphorus deficiency, high pH (calcareous soil), are complemented by adverse saline conditions, which may arise either by low water availability and/or poor quality irrigation water because of relatively high salinity.

CHAPTER 5

Effect of Fungicides on Symbiotic Interaction between *Glomus mosseae* and the Peanut.

5

Effect of Fungicides on the Symbiotic Interaction between *Glomus mosseae* and the Peanut

5.1 Introduction

Natural soils contain a diverse microbiota, including fungi, many of which are beneficial organisms important in processes essential to soil fertility. These processes include cycling of soil nutrients and fertilisers and the direct transfer of nutrients to plants (Moorman, 1989). Other organisms of the soil microbiota may be detrimental to plant growth because they are pathogens (viz. Chapter 3). The common application of agrochemicals to plants to control pathogens and pests may adversely affect plants by indiscriminately attacking both detrimental and beneficial soil microorganisms.

Mycorrhizal fungi are certainly one of the beneficial soil microorganisms that are likely to be affected at least by fungicides (Aziz *et al.*, 1991 ; Nemec, 1985). For example, metalaxyl has been shown both to inhibit (Jabaji-Hare and Kendrick, 1987) and stimulate (Groth and Martinson, 1983) root colonisation by different AM fungi. From these studies it is evident that the effects of fungicides vary depending on the methods of application, efficiency, mode of action and chemistry of the fungicides, inherent nature of host plant involved, response of AM fungi and the prevailing environmental conditions (Sugavanam *et al.*, 1994)

According to Dodd and Jeffries (1989), our knowledge of the effects of fungicides on AM symbiosis is uncertain because of the lack of standardisation in the experimental methodology and by the fact that too few fungal species

have been tested under similar conditions. Variations could be because of differences in soils, host or AM fungal species. Even so, because of the enormous variety of crops, soils, and climates under which fungicides are used, it is too difficult to be able to recommend a universal standard experimental design for investigating the effects of fungicides on mycorrhizal fungi and mycorrhizal formation (Trappe *et al.*, 1984). A further complication is that the launching of new fungicides on the market is faster than mycorrhiza researchers can deal with them.

Plants with high mycorrhizal dependency and/or low soil phosphorus availablilty are two conditions that would maximise the potential for adverse agrochemicals effects on mycorrhizas and plant growth. Moorman, (1989) assumes that agrochemicals can affect AM fungi sufficiently to affect crop growth. Since AM fungi are vital symbionts of most field and natural vegetation, it seems to be obvious that the effects of fungicide on AM fungal formation and colonisation should be assessed before a fungicide can be used. Mycorrhizologists must collaborate with organic chemists, fungicide specialists and plant physiologists to investigate the underlying principles that control fungicide action.

Sugavanam *et al.* (1994) studied the effects of field application rates of six fungicides (Bavistin, Dithane, Emisan, Fytolan, Thiram and Ziram) on the formation and function of AM fungi using ***Arachis hypogaea*** L. as host plant. They found that Fytolan fungicide produced significant positive responses in mycorrhizal colonisation and sporulation levels with enhanced growth and yield of the host plant, while the other fungicides restricted mycorrhizal colonisation to different levels. The effects of these fungicides are sporulation varied depending on AM species.

Plantvax-75 is a systemic fungicide while Aspor is a contact one.

Although, in a preliminary report Giovannetti and Riess (1980) mentioned that Plantvax did not eliminate arbuscular mycorrhizal colonisation in onion, though it was significantly decreased.

In Saudi Arabia, Plantvax is used to control *Rhizoctonia sp* and rusts in wheat and vegetables and also used as seed dressing. While Aspor is used as a broad spectrum fungicide to control fungal diseases in vegetables and field crops. Apparently, the influence of these two fungicides on *Glomus mosseae*/peanut symbiosis have not been investigated. The purpose of this study was to investigate the residual toxicity of these two fungicides on the formation and function of the AM fungus *Glomus mosseae* and the symbiotic interaction with *Arachis hypogaea* L. [var. *hypogaea* cv. Florunner].

5.2 Methods

5.2.1 Methods and Experimental Design

Autoclaved substrate soil amended with original soil microbiota without added phosphorus fertilisation and where mycorrhizal dependency was therefore to be high (see Chapter 2), was used in this trial. Autoclaved soil plus soil microbiota, formulated as mentioned before [see Materials and Methods; 2.1.1.3], was distributed into plastic pots with drainage holes (15 cm diameter pot) lined with perforated polyethylene bags.

Two fungicides were used in this trial:-

- PLANTVAX- 75W is a systemic fungicide with 75 % active ingredient [oxycarboxin (5, 6-Dihydro 2-methyl-1, 4-oxathiin-3-carboxanilide-4, 4-dioxide)] manufactured by UNIROYAL Chemical; Division of Uniroyal Company, USA.
- ASPOR UTRA is a contact fungicide with protectant properties, containing 80

% active ingredient of zinc ethylene bisdithio-carbamate (zienb) packed by ADONIS Company, Lebanon.

Both fungicides were applied to the pots in form of powder and mixed thoroughly to obtain concentrations of 0; 50; 100; 150 and 200 µg active ingredient g^{-1} soil.

The inoculation of the experimental pots with the AM fungus *Glomus mosseae* was conducted by uniformly mixing into the pots 10 g crude inoculum produced as described before [see Materials and Methods; 1.1]. Surface disinfection, and pregermination of peanut seeds and planting of pregerminated seeds into pots were carried out according to the procedures outlined previously [Chapter 3; Methods, 3.2.1].

The 5 x 2 x 1 factorial experiment resulted from combinations of 5 levels of fungicide concentration, two fungicides and 1 AM fungus. The treatments were replicated 10 times and pots were distributed on greenhouse benches in a completely randomised design. Peanuts were grown for 8 wk.

At harvest, % colonisation (Phillips and Hayman, 1970), $NOSG^{-1}S$ collected by wet sieving and decanting (Gerdemann and Nicolson, 1963) and growth indices of shoot height per plant, FWP^{-1} and DWP^{-1} were assessed. Shoot P content was quantified spectrophotometrically as yellow phospho-vando-molybdate complex [see Materials and Methods;1.4.4.3].

5.2.2 Data Analysis

Data were subjected to a two-way ANOVA. Fisher's LSD was used to separate treatment means when F values were significant.

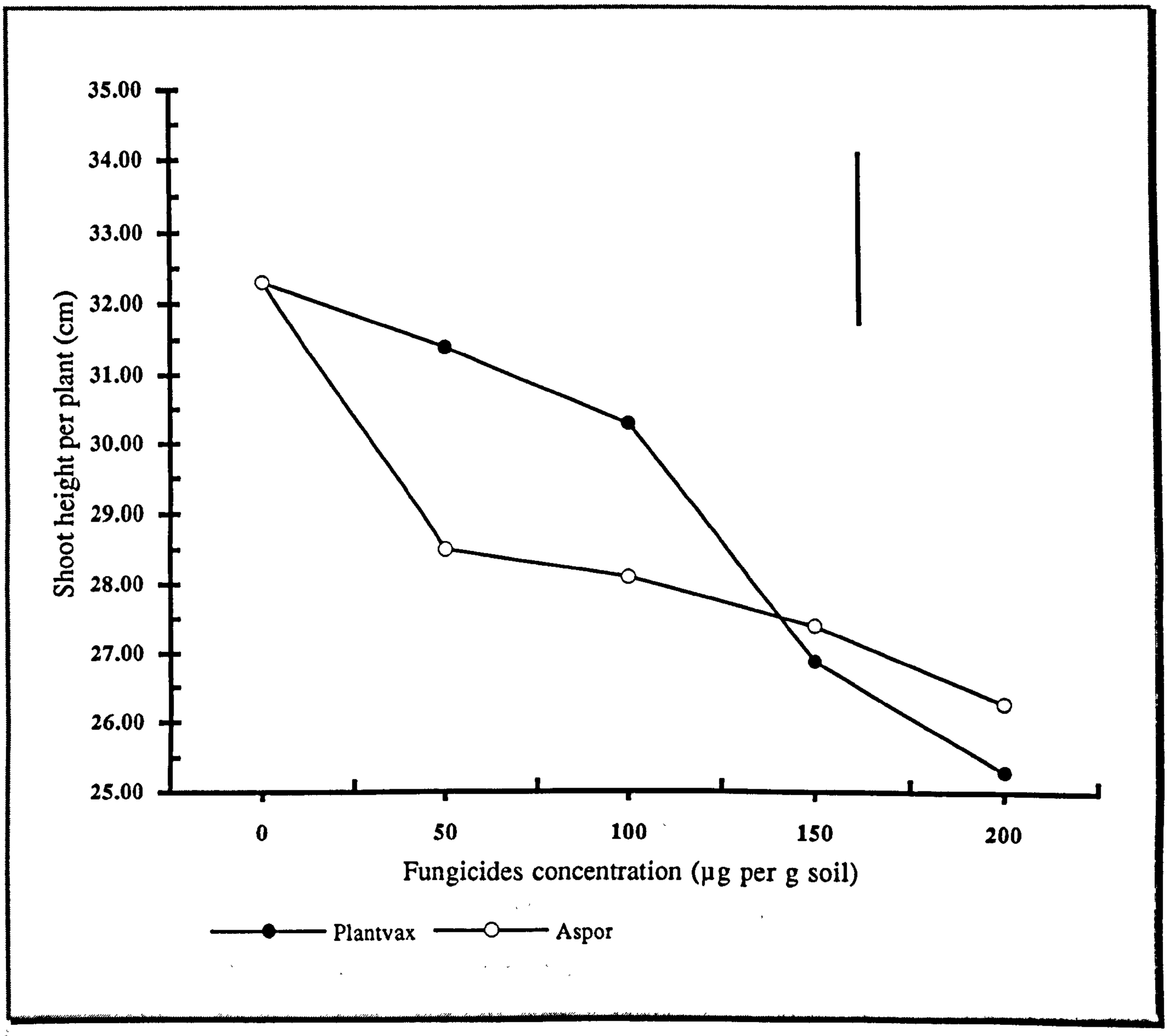
All graphics, statistical computations and word processing were run on an

Apple Macintosh LC personal computer using Microsoft Excel, StatView and MacWrite software, respectively, (see Appendix C - 1, 2 & 3, for details).

5.3 RESULTS

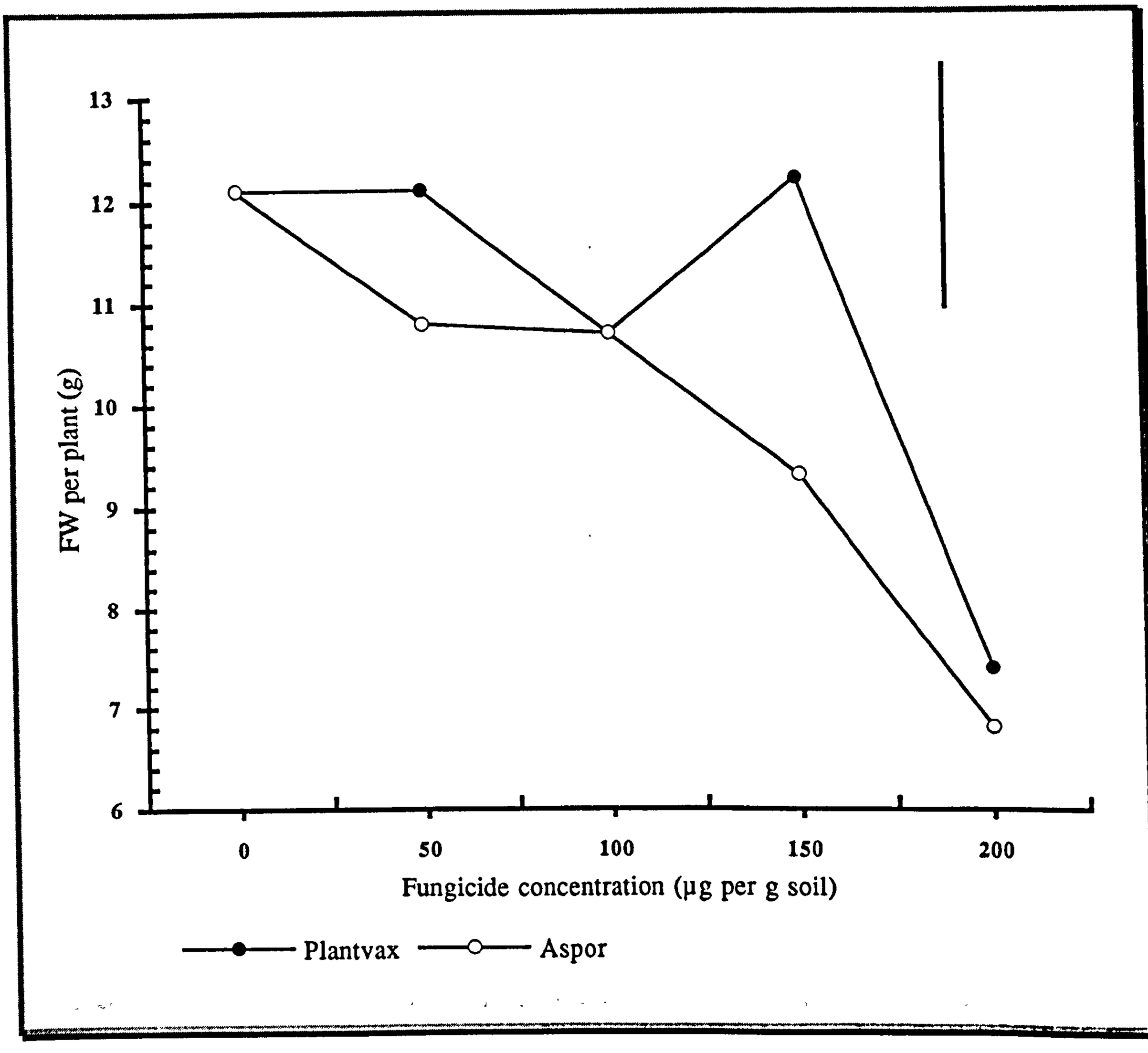
At the same concentration, there was no significant difference between Plantvax and Aspor on the growth parameters of shoot height per plant, FWP^{-1} and DWP^{-1}, but their concentration significantly affected the growth parameters. Plantvax significantly reduced the shoot height per plant when applied at the rate ≥150 μg g^{-1} while the Aspor significantly reduced the shoot height per plant at > 50 μg g^{-1} compared with the control. The concentration of Plantvax did not significantly reduce FWP^{-1} and DWP^{-1} except in the 200 μg g^{-1} treatment. By contrast Aspor significantly reduced both FWP^{-1} and DWP^{-1} indices compared with the control at both 150 μg g^{-1} and 200 μg g^{-1} treatments [Fig. 5.2.2 ; Appendix D; Table 5.2 a-b and Fig. 5.2.3 ; Appendix D; Table 5.3 a-b].

Plantvax and Aspor decreased shoot phosphorus concentration at all application levels and statistical analysis indicated that Aspor reduced the P concentration at an application level ≥ 100 μg g^{-1} more when compared with the effects generated by Plantvax [Fig. 5.2.4 ; Appendix D; Table 5.4 (a-b)].



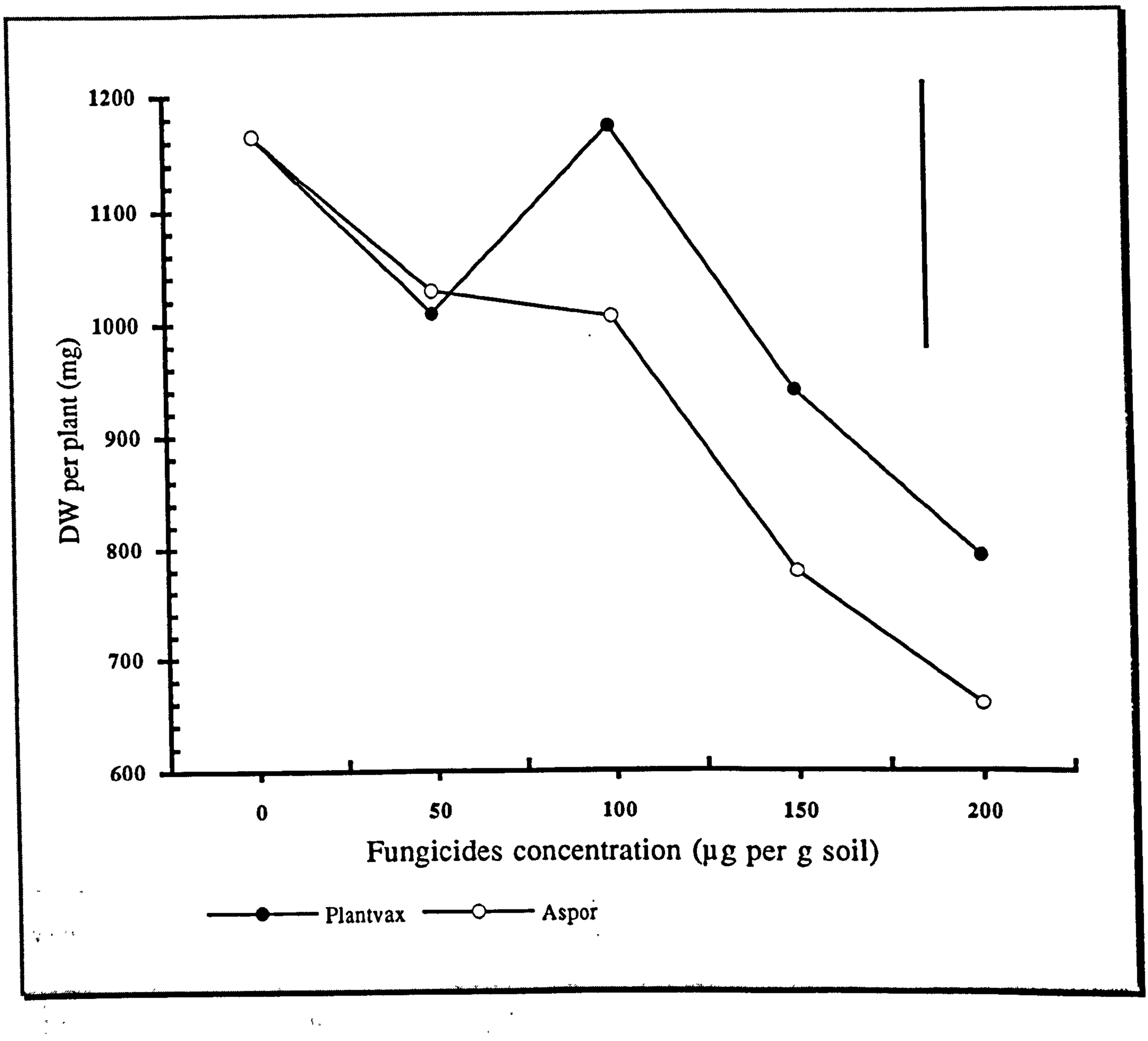
Vertical bar represents Fisher's LSD value.

Figure 5.3.1
Effect of fungicide application on shoot height per plant of mycorrhizal peanuts.



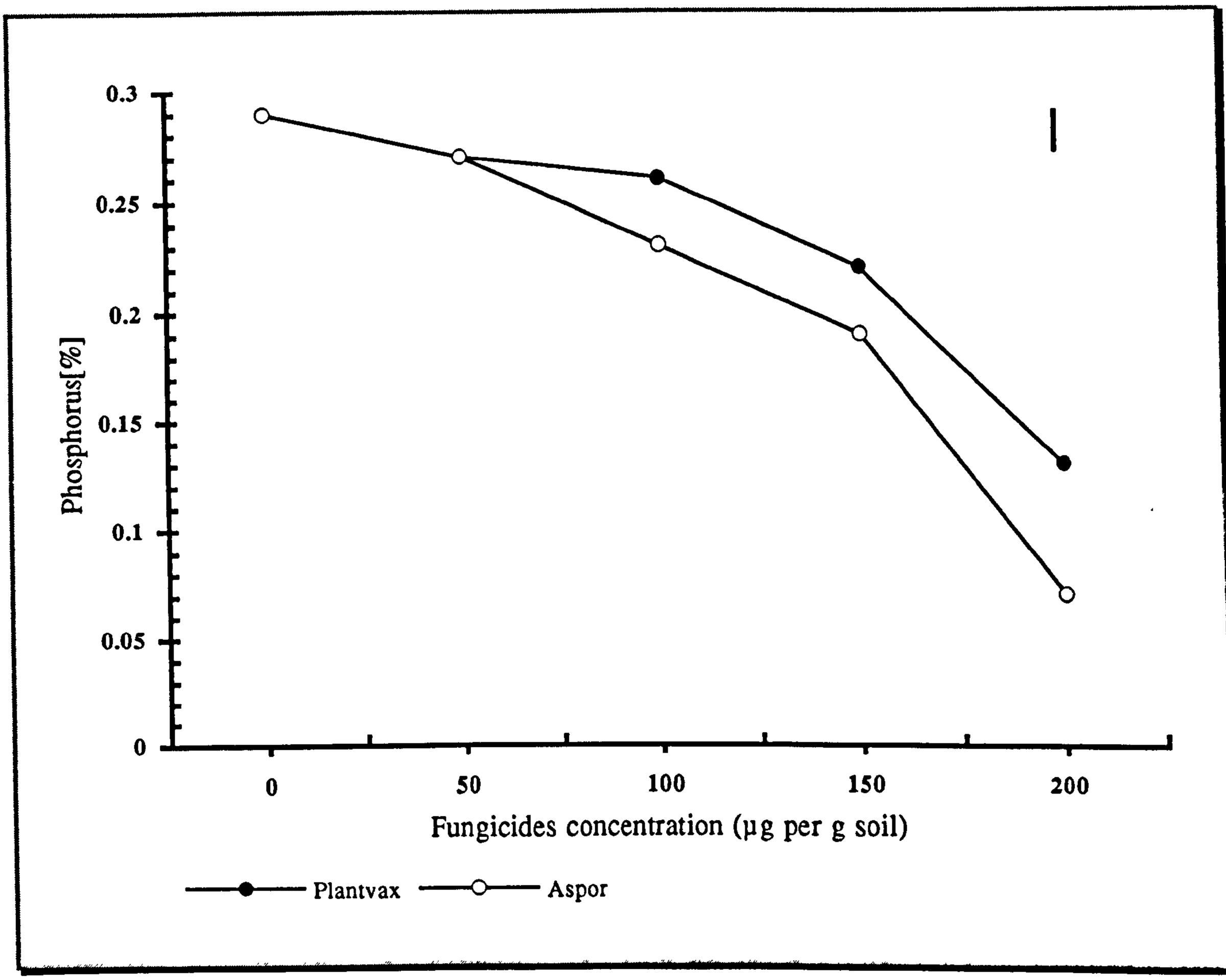
Vertical bar represents Fisher's LSD value.

Figure 5.3.2
Effect of fungicide application on fresh weight per plant of mycorrhizal peanuts.



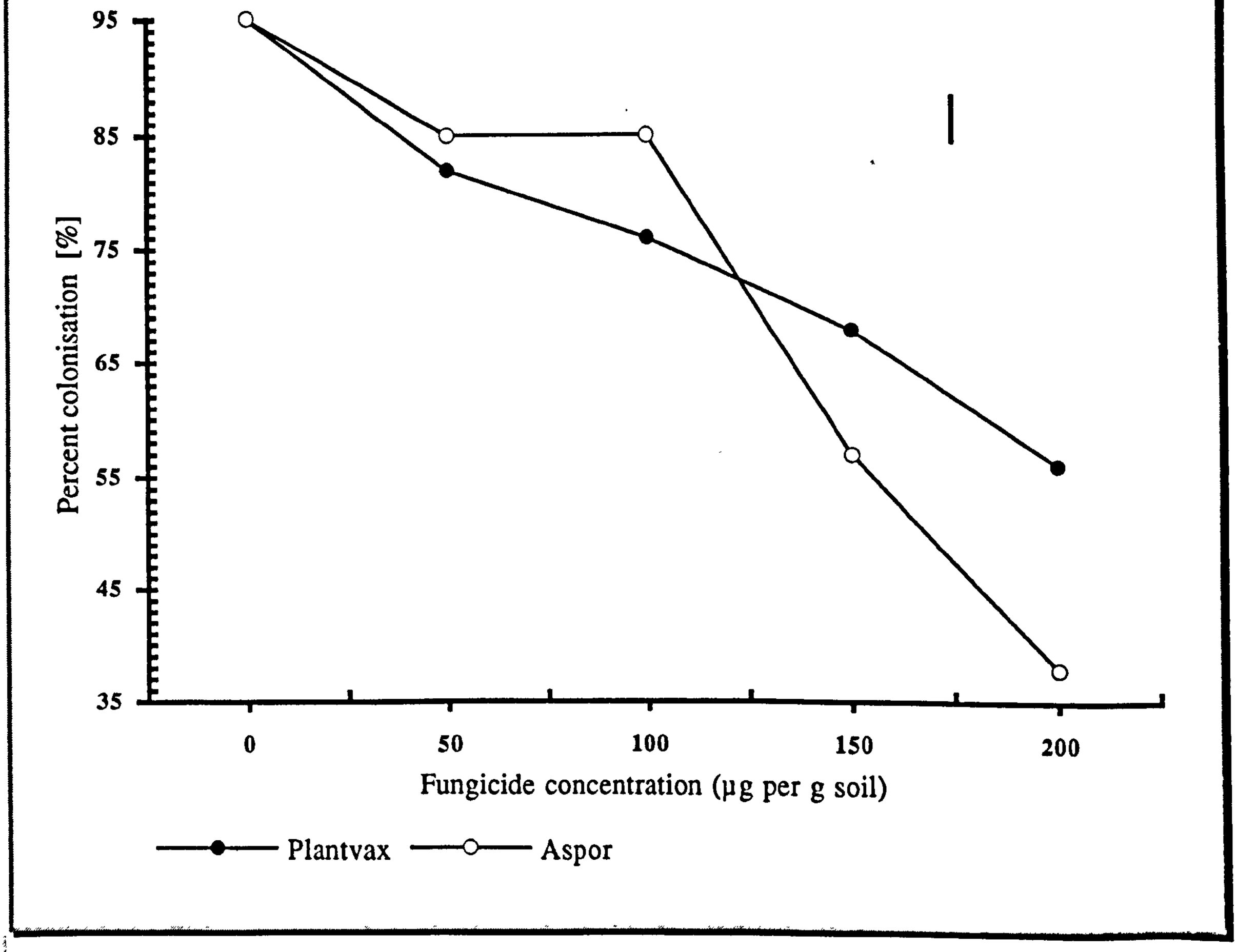
Vertical bar represents Fisher's LSD value.

Figure 5.3.3
Effect of fungicide application on dry weight per plant of mycorrhizal peanuts.



Vertical bar represents Fisher's LSD value.

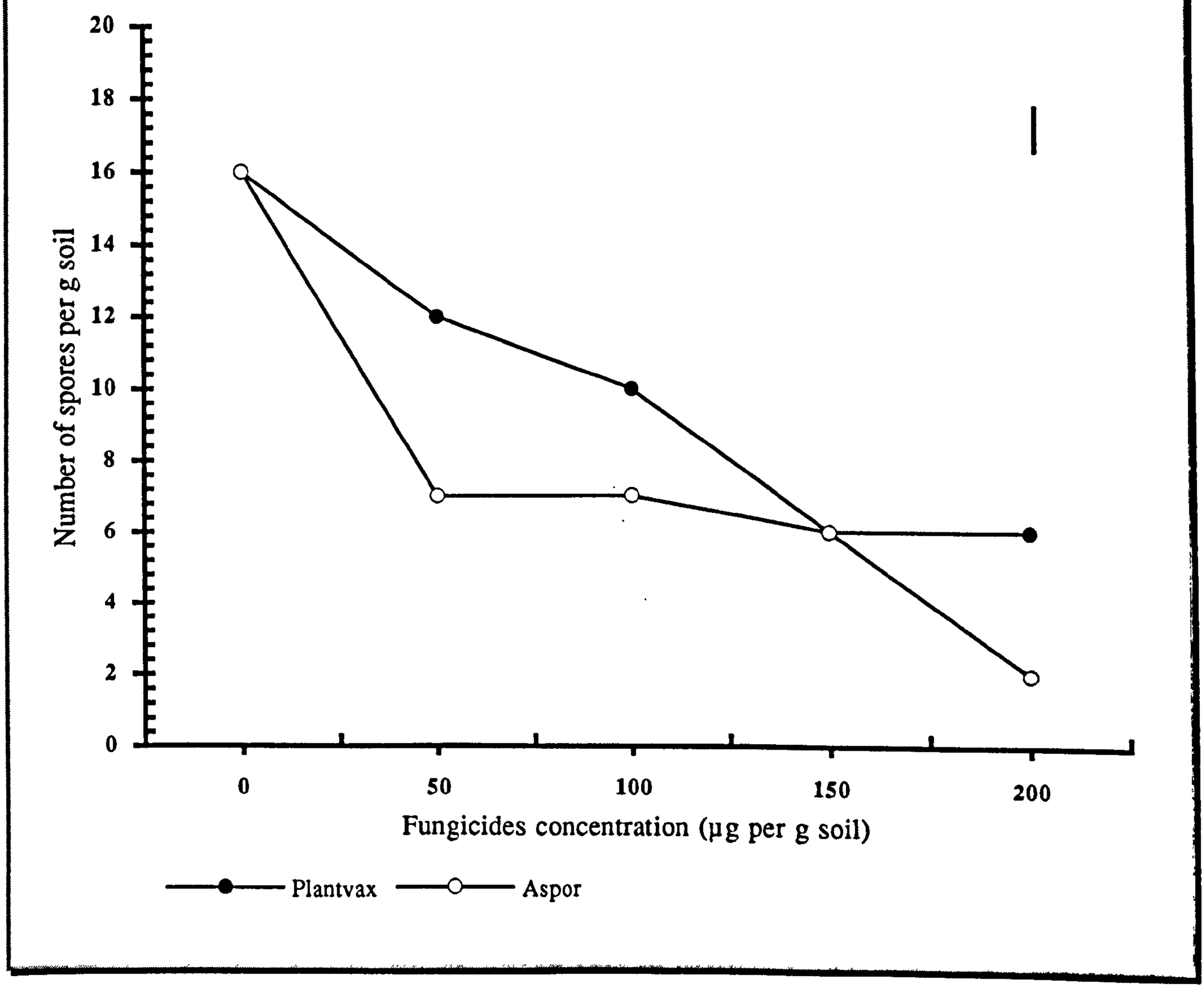
Figure 5.3.4
Effect of fungicide application on phosphorus content of mycorrhizal peanuts.



Vertical bar represents Fisher's LSD value.

Figure 5.3.5

Effect of fungicide application on percent colonisation of mycorrhizal peanuts.



Vertical bar represents Fisher's LSD value.

Figure 5.3.6
Effect of fungicide application on sporulation (number of spores per g soil) of the AM fungus.

Both contact and systemic fungicide followed the same pattern in decreasing mycorrhizal % colonisation levels (the PC index) at all fungicide application levels although, at an application level ≥ 100 µg g^{-1}, the effects produced by the contact fungicide (Aspor) were significantly greater than those produced by the systemic fungicide (Plantvax) [Fig. 5.2.5 ; Appendix D; Table 5.5 (a-b)]. Again both fungicides significantly reduced sporulation (the $NOSG^{-1}S$ index) at all tested fungicides levels. The effects of Aspor on $NOSG^{-1}S$ were significantly higher when compared with the effects of Plantvax at all application levels except in 150 µg g^{-1} treatment [Fig. 5.2.6 ; Appendix D; Table 5.6 (a-b)].

Generally, the results indicate that both fungicides tend to reduce AM fungal formation, P uptake in peanut especially at high application levels and reduce % colonisation levels and sporulation of the fungus. Nonetheless, the residual toxicity of the contact fungicide (Aspor) seems to be potentially more inhibitory to sporulation and % colonisation compared with that of the systemic fungicide (Plantvax).

5.4 Discussion

Results of Chapter 5, that deals with the effects of fungicides on symbiotic interaction of the AM fungus and peanut, show that the concentrations of the two fungicides, Aspor and Plantvax can inhibit the development of *Glomus mosseae* in the peanut by affecting both spore production and subsequent mycorrhizal colonisation of peanut roots. These effects lead to a reduction of phosphorus uptake and consequently a reduction in the final yield of plant biomass. It is obvious from these results, however, that the two types of fungicides used failed to completely eliminate the mycorrhizal formation in the peanut. This finding supports the view reported in literature that generally no fungicide is so harmful that it stops AM fungi formation completely (Gonçalves *et al.*, 1991 ; Sieverding, 1991). Unlike pathogenic fungi, AM fungi may not directly be affected by fungicide applications, but rather indirectly through chemical, physiological or biological changes of the host plant or the rhizosphere. Theoretically, since Plantvax is a systemic fungicide it might have been expected to influence the fungus formation inside the peanut root tissue (measured as.% colonisation) more adversely than the contact fungicide Aspor, but the results seem to indicate that the property of systemic translocatability of the fungicide does not appear to be related to its effect on mycorrhiza formation.

Dodd and Jeffries (1989) suggested that the results of greenhouse studies should be viewed with care when used as a screening process to investigate fungicide-AM fungi interactions. Sieverding and Leihner (1984) found significant positive correlations between the results of pot trials and field trials, but it is always difficult to relate pot experiments to field conditions and the effects of fungicides on AM fungi in pots may be over estimated on two counts:- First fungicides may be cycled within soil in pots and not washed out as in field

soil (Bateman and Nicholls 1982). In pots, fungicide cycling is caused by alternate upward movement of water during periods of soil drying and downwards movement after irrigation (Dodd and Jeffries, 1989). The texture of the potted soil used in these experiments was classified as sandy clay loam according to the particle size distribution. These properties would attenuate this cycling because it is not a compacted soil with high clay content, that usually, retains fungicides due to the high adsorption coefficients of such soils (Dodd and Jeffries, 1989). By contrast, it is rather free-draining soil with permeability of $1.8X10^{-3}$ cm s^{-1} that would allow deeper penetration and even distribution of the fungicides through out the potted soil. Since the experimental pots were with drainage holes, there is a possibility that fungicides were also washed out with excess irrigation water more or less resembling conditions in field soil. Accordingly, it is not thought that an artificially high concentration of fungicides developed in the root zone. The second point often raised against greenhouse studies is that they may overestimate the effect of fungicides due to the use of sterilised potting soil, which would undoubtedly, eliminate the biodegrading microorganisms. The absence of these microorganisms would greatly increase the persistence of fungicides compared with their persistence in field soil (Moorman, 1989). Again this problem is not anticipated in this study because the soil was amended with original soil microbiota (see Chapter 1).

The results of these experiments are likely to be relevant to field application conditions. These experiments indicate that the mycorrhizal association with the peanut in soils treated with relatively high dosages of Aspor and Plantvax fungicides may result in the loss of mycorrhizal benefits and is not likely to improve peanut growth substantially. Also, high dosages of these two fungicides may possess somewhat harmful effects on beneficial microorganisms of the added back original soil microbiota and eliminate the

synergistic microbiota-AM fungi reactions as revealed by this study (Chapter 1). Therefore these two fungicides must be avoided in any management strategy that aims to preserve arbuscular mycorrhizal fungi and associated beneficial soil microbiota. As mentioned in Chapter 3, further research and investigative studies are called for to explain fully the possible mechanism of the biological control of AM fungi to replace yield losses incurred by pathogens. Until then, from an agronomic and economic point of view, the chemical control of pathogenic fungi will be given preference over consideration regarding fungicidal effects on AM fungal population. Nevertheless, for socioeconomic reasons, and considerations regarding environmental contamination by fungicide applications these should be avoided as much as possible and chemical disease control should be replaced by biocontrol mechanisms.

Crews (1993) defined sustainable agriculture as one that operates on renewable energy sources while maintaining soil fertility, water resources, human health and species diversity. According to this definition I believe mycorrhization of the peanut as described in the results in this thesis contributes to a growing body of research aimed at understanding the mechanism and constraints of sustainable food producing system.

SUMMARY AND CONCLUSIONS

The work carried out in this thesis was designed to study the mycorrhizal status of the peanut in different soil conditions, mycorrhizal dependency and nutrient uptake, mycorrhizal potential in biocontrol, mycorrhizal response to salinity stress, and effects of fungicides on peanut/mycorrhiza association.

This comparative study indicates that *Glomus mosseae* and *Glomus fasciculatum* were infective to the peanut (*Arachis hypogaea* L.), but each AM fungus displayed a differential effectiveness. The AM fungus *Glomus mosseae* stimulated peanut growth more than *Glomus fasciculatum.* The number of tillers per plant, shoot height per plant, root fresh weight per plant, root dry weight per plant, root/shoot weight ratio, rate of growth per week, percent colonisation, and number of spores per g soil were all significantly higher in *Glomus mosseae*-inoculated peanut plants. *Glomus mosseae* was the better AM fungus for peanut mycorrhization compared with *Glomus fasciculatum* in autoclaved potted alkaline calcium rich soil amended with original soil microbiota. Therefore, *Glomus mosseae* and autoclaved soil with microbial filtrate amendments were used in the subsequent experiments of this study.

Phosphorus application promoted the growth of both mycorrhizal and nonmycorrhizal peanut plants, but increased fertilisation rate of P considerably decreased both percent colonisation and spore population density. A threshold response level to P application was found [P = 120 µg P g^{-1}], below which peanut was inclined to be moderately or highly mycorrhizal dependent denoting morphological, physiological and chemical differences arising between P-fertilisation and *Glomus mosseae*-mycorrhizal plants. Peanut RMD index tended to diminish when the P level exceeded 120 µg P g^{-1} indicating

that peanut inclines to be independent of the AM fungus as P application increased. Mycorrhizal dependent peanuts had increases of 17 – 38 % in plant height, 50 – 96 % fresh weight and 77 – 120 % in dry weight. Also, there were significant increase in nutrient uptake of N, P, K, Ca, Mg and micronutrients (Fe, Cu, Mn, and Zn). However, no difference in Na uptake was observed over nonmycorrhizal peanut plants.

Glomus mosseae proved to be efficacious in excluding the effects of *Erwinia carotovora*. The pathogenic bacterium restricted neither mycorrhizal spore population density nor mycorrhizal development in peanut roots. Generally, the AM fungus improved the nutritional status and decreased the susceptibility of peanut seedlings to the bacterial soft rot disease and significantly alleviated the disease symptoms.

When grown in increasing concentrations of NaCl, the salt stressed-nonmycorrhizal peanuts contained significantly higher free proline than salt stressed mycorrhizal plants. As NaCl salinity increased, the salt stressed mycorrhizal peanuts decreased, but their biomass was always greater compared with salt stressed-nonmycorrhizal peanuts. Their greater Na content suggested they could better osmotically adjust. Gradual increase in NaCl salinity tended to decrease both the AM fungus colonisation and sporulation levels. Although the fungus infectiveness was still relatively high (45 %), spore population was adversely affected (2 spores g^{-1}). The improved nutritional status of the peanut in salt stress conditions was attributed to the biological role of *Glomus mosseae* fungus. *Glomus mosseae* was also able to relieve salt stress enabling the plant to tolerate the effects of NaCl salinity.

At high application rates, both Plantvax and Aspor fungicides tended to adversely affect the growth parameters (viz shoot height $plant^{-1}$, fresh weight $plant^{-1}$ and dry weight $plant^{-1}$) and phosphorus uptake in the peanut. As a consequence peanut growth was reduced. However, the residual toxicity of the contact fungicide (Aspor) seemed to be potentially harmful to sporulation and %

colonisation of the AM fungus comparable to the systemic fungicide (Plantvax). This indicates that the mycorrhizal development in the peanut in soils treated with relatively high dosage of these two fungicides may result in the loss of these mycorrhization benefits and is unlikely to improve peanut growth substantially.

Sustainable agriculture depends on the maintenance of optimum physical, chemical and biological balances in the agrosystems. Disturbance to such systems through overgrazing, fertilisation, agrochemical application, contamination with salt and/or irrigation with poor quality salty waters would alter physical, chemical and biological soil processes that may influence balanced competition between organisms that is needed to sustain productivity (Campbell and Greaves, 1990). The present study illustrates that the AM fungus *Glomus mosseae* associated with increased growth of the peanut could be involved in tolerance of high pH (Chapter 1), synergistic interaction with indigenous beneficial soil microbiota (Chapter 1), increased nutrient uptake (Chapter 2), protection against a bacterial pathogen (Chapter 3) and relieving salt stress (Chapter 4). Nonetheless, these mycorrhization benefits are likely to be threatened by high fungicide application rates (Chapter 5).

From these experiments it is possible to conclude that the role of AM fungi may be valuable if peanut production is to return to the state where luxury inputs of phosphate fertiliser, fungicides and cost of desalinisation and reclamation of saline soils are decreased to levels that make growing of the crop economically viable, yet do not pollute the environment. I believe that the AM fungus *Glomus mosseae* is potentially capable of contributing to low-input sustainable agriculture as an alternative to conventional agricultural practices of peanut cropping especially in semi-arid zones of tropical and subtropical regions when peanut mycorrhization should be seriously considered when phosphorus is unavailable due to deficiency or soil fixation. Also this study encourages the potential feasibility of using limited fresh water supplies with

saline waters (e.g brackish water, seawater etc.) for mycorrhizal peanut and probably other crops in these regions.

If the results of this study can be extended to the field, inoculation of peanut plants with rhizosphere organisms such as AM fungi and *Rhizobium* may improve peanut production. Field trials with these rhizosphere symbionts in tropical soils are needed to test this hypothesis. This biotechnology may attract applied research interest for improving the fertilisation and field production of this essential oil-producing crop plant in P-deficient soils and is becoming of high interest as recently, mycorrhizologists and agronomists focus on the potential of AM fungi to improve yields and to reduce the use of fertilisers.

Although the main emphasis of this study was on the mycorrhizal development and effects on growth of peanut grown without the rhizobial symbiont, still there is increasing evidence that insufficient supply of mineral nutrients, particularly phosphorus and nitrogen is the main yield limiting factor in tropical area. Therefore mycorrhizal researchers are encourage to structure research programmes to admit rhizosphere symbionts such as *Rhizobia* and AM fungi into the design of sustainable agricultural systems. Manipulation of AM fungi in such systems could be achieved if research efforts were to be directed to identify those cultural and environmental stresses which could be alleviated by the use of AM fungi, through selection of efficient AM fungal species and/or strains in addition to provision of guidelines for practical application.

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APPENDICES

APPENDIX A [CHEMICALS]

1
Acid-ninhydrin

•-Acid-ninhydrin was prepared by warming 1.25g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid, with agitation, until dissolved. Storage was at 4°C [the reagent remains stable for 24 h only].

2
Diethylentriaminepentraacetic acid (DTPA)

This solution was prepared as follows:-

To prepare 10L of this solution, the following reagents were dissolved in approximately 200 ml of distilled water:-

Reagents:

- 149.2g of reagent grade $(HOCH_2CH_2)_3N$ triethanolamine (TEA).
- 19.67g of diethylentriamiepentraacetic acid (DTPA).
- 14.79g of $CaCl_2$. $2H_2O$

Sufficient time was given for the DTPA to dissolve. The pH was adjusted to 7.30 with 1:1 *N* HCl, while stirring and diluting to 10 L. (This solution is stable for several months).

3
Formalin-aceto-alcohol (FAA) fixative solution

Ethanol (95%)	500 ml
Glacial acetic acid	50 ml

Formaldehyde (40%)	100 ml
Water	350 ml

4

Hoagland's mineral salt solution

Stock solutions: Molar stock solutions of the following chemicals were made. The quantity of each stock solution placed in one litre of the final solution is given at the right.

M KH_2PO_4	1 ml
M KNO_3	5 ml
M $Ca(NO_3)_2$	5 ml
M $MgSO_4$	2 ml

Trace element solution: This solution was prepared by dissolving these quantities chemicals in 1 litre of water. One ml of this solution was added to each litre of the final solution.

H_3BO_3	2.86 g
$MnCl_2.4H_20$	1.81 g
$ZnSO_4.7H_20$	0.22 g
$CuSO_4.5H_20$	0.08 g
$H_2MoO_4.H_20$ (85%)	0.02 g

In addition, iron in the form of 0.5% iron tartrate was added at the rate of 1 ml to each litre of the final solution just before it was applied to the plants.

5

Lactophenol solution for staining

Glycerin	250 ml

Phenol	300 g
Lactic acid	250 ml
Water	300 ml

6
Mixed indicator

Reagents:

- Bromocrysol
- Methyl red
- o.1*N* NaOH
- Absolute alcohol

Each 0.1 g of bromocrysol green and methyl red were dissolved separately in 100 ml absolute alcohol. Then 2 ml of 0.1*N* NaOH were added to each indicator. Bromocrysol green and methyl red indicators were mixed in a ratio of 1:3 (v/v), respectively to form the so called mixed indicator.

7
Vanadate-molybdate reagent

- 22.5g of ammonium molybdate were dissolved in 400 ml distilled water
- 1.25g of ammonium vanadate were dissolved in 300 ml of boiling water.
- The ammonium vanadate solution was added to ammonium molybdate and left to cool to room temperature.
- Then 250 ml of conc. nitric acid was added. The solution was made up to 1000 ml with distilled water.

8
Weak acid solution

10 drops of 12N HCl in 200 ml H_2O

APPENDIX B [MEDIA]

1
Nutrient agar (Lab m)

Peptone	5.0 g
Beef extract	3.0 g
NaCl	12 g
Agar No.2	12 g
Distilled water	1000 ml
pH	7.3 ± 0.2

2
Peptone yeast extract agar

Proteose peptone	20 g
Yeast extract	2 g
$MgSO_4.7H_2O$	0.98 g
$CaCl_2$	0.059 g
Sodium citrate.$2H_2O$	1.0 g
Fe $(NH4)_2$ $(SO4)_2.6H_2O$	0.02 g
KH_2PO_4	0.34 g
$Na_2HPO_4.7H_2O$	0.355 g
Glucose	18 g
Agar	16 g
Distilled water	1000 ml

3
Potato dextrose agar

Potato extract	4 g
Dextrose	20 g
Agar No.1	15 g
pH 5.6	

APPENDIX C [SOFT WARES]

1
Graphics

Microsoft Excel®

©1985-1991

Version 3

From:

Microsoft Corporation

16011 NE 36th Way

Box 97017

Redmond, WA 98073-9717

2
Statistics

StatView™ 512+

©1986 Abacus Concepts, Inc.

From:

BrainPower Inc.

24009 Ventura Blud.

Suite 250

Calabasas, CA 91302

3
Word Processing

MacWrite® II Release 1.1

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From:

Claris Corporation.

5201 Patrick Henry Drive

Box 58168

Santa Clara, California 95052-8168

Table 2.1
Mean total fresh and dry weight per plant of fourteen-wk-old mycorrhizal and nonmycorrhizal peanut plants grown at six P levels, percent colonisation (PC), and relative mycorrhizal dependency percent (RMD%).

Phosphorus Levels / Parameters	6 (µg/g soil)	30 (µg/g soil)	60 (µg/g soil)	120 (µg/g soil)	240 (µg/g soil)	480 (µg/g soil)
Fresh Weight	(g/plant)	(g/plant)	(g/plant)	(g/plant)	(g/plant)	(g/plant)
I. Mycorrhizal	7.03*§	8.00*	8.95*	8.10*	10.20*	11.85
II. Nonmycorrhizal	3.58	4.63	5.00	5.40	8.20	11.76
%Increase	96	73	79	50	24	1
Dry Weight	(g/plant)	(g/plant)	(g/plant)	(g/plant)	(g/plant)	(g/plant)
I. Mycorrhizal	2.35*	2.25*	2.85*	3.12*	3.82	4.50
II. Nonmycorrhizal	1.07	1.24	1.60	1.86	3.32	4.48
% Increase	121	81	78	68	15	0
Percent colonisation (%)	86a∞	82a	80a	75a	55b	39c
RMD%	55a∞	45b	44b	40b	13c	0

§ Mean pairs in a column with an asterisk [*] are significantly different at $P \leq 0.05$ level according to a Student's t-test
∞ Mean numbers followed with same[letters] in a row are not significantly different different at $P \leq 0.05$

Table 3.1
Growth-indices, % colonisation and NPK content of mycorrhizal and nonmycorrhizal peanuts grown in autoclaved soil in presence or absence of *Erwinia carotovora*.

Indices ¶ / Treatments	SH/P (cm)	RDW/P (mg)	SDW/P (mg)	ROG/W (cm)	NOS/gSoil (No.)	PC (%)	N√ (%)	P√ (%)	K√ (%)
Glomus mosseae	**32.0 a** ±(0.47)	**107.4a** ±(8.42)	**993.9 a** ±(64.23)	**3.8 a** ±(0.122)	**10.0 a** ±(0.33)	**82a** ±(1.67)	**1.603 a** ±(8.8E-3)	**0.243 a** ±(0.015)	**1.088 a** ±(7.56E-3)
G. mosseae + E. carotovora	**30.4 a** ±(1.15)	**83.1 b** ±(7.77)	**756.0 b** ±(47.91)	**3.8 a** ±(0.11)	**9.0 a** ±(0.33)	**77 a** ±(3.21)	**1.59 a** ±(0.038)	**0.090 b** ±(0.021)	**1.013 b** ±(8.82E-3)
Erwinia carotovora	**20.9 c** ±(0.85)	**50.3 c** ±(4.13)	**510.2 c** ±(43.57)	**1.9 c** ±(0.10)	N/A ...	N/A ...	**1.34 c** ±(0.012)	**0.010 c** ±(5.487E-3)	**0.8 c** ±(0.029)
Control	**24.8 b** ±(0.85)	**80.7 b** ±(7.01)	**671.9 b** ±(28.82)	**3.5 b** ±(0.07)	N/A ...	N/A ...	**1.4 b** ±(0.044)	**0.077 d** ±(8.819E-3)	**0.90 d** ±(0.017)
%increase of Gm over Gm+Ec	5.3	29.2	31.5	0.5	6.8	6.1	0.8	170	7.4
%increase of Gm + Ec over Ec-infected	38.3	40.6	36.6	55.5	N/A	N/A	17.9	103.9	26.6
%increase of Gm + Ec over controls	22.4	3	12.5	9.2	N/A	N/A	13.6	16.9	12.6
%increase of controls over Ec-infected	18.8	60.4	31.7	85.6	N/A	N/A	4.5	670	11.1

SH/P: Shoot height per plant ; RDW/P: Root dry wt per plant.
SDW/P: Shoot dry wt per plant ; ROG/W: Rate of growth per week ; NOS/ g soil : Number of spores per g soil ; PC: % colonisation.
Gm+Ec : *Glomus mosseae* plus *Erwinia carotovora*; N/A : Not applicable.
¶ means ± S.E.M. with different letters in a given column are significant according to Fisher's LSD test ($P \leq 0.05$).
√ NPK data transformed to natural logs for statistical analysis, but means were presented here.

Table 3.2
Population number of *Erwinia carotovora* in the rhizosphere of mycorrhizal and nonmycorrhizal peanuts.

Time / Treatments	*1st wk*		*3rd wk*		*5th wk*	
	No.($x10^{-3}$)/g wet soil	LTD¶	No.($x10^{-3}$)/g wet soil	LTD	No.($x10^{-3}$)/g wet soil	LTD
Erwinia carotovora	650000	8.81 a	50000	7.7 a	250	5.39 a
G.mosseae + E. carotovora	300000	8.47 a	2700	6.43 b	5	3.69 b
Fisher's LSD values		0.03		0.161		0.099

Each figure is the mean of three replicates.
¶ LTD, Log-transformed data.
Means with different letters in a given coloumn are significant according to Fisher's LSD ($P \leq 0.05$) using log-transformation of data.

Table 4.1
Measured variables of mycorrhizal and nonmycorrhizal peanuts as influenced by sodium chloride salinity stress.

NaCl levels	VAM (+/-)¶	Measured indices					
		FW per plant (g)	DW per plant (mg)	Proline µmole/g	LRWC (%)	PC (%)	Spores /g soil (No)
0.0 M	+	9.00* ±(2.10)	1400* ±(30)	1.50±(.10)	85.00±(0.58)	93.00a±(1.76)	12.00a±(.88)
	-	7.77 ±(1.32)	933±(270)	1.20±(.11)	87.00±(0.88)	nd#	nd#
Relative response¥	(%)	**16**	**50**	**25**	**-2**	...	...
0.1 M	+	7.33* ±(.67)	1250*±(180)	1.20±(.09)	82.00*±(0.88)	92.00a±(1.86)	11.00a±(.88)
	-	6.07 ±(1.4)	967 ±(190)	2.50* ±(.12)	78.00±(0.58)	nd	nd
Relative response	(%)	**21**	**29**	**-52**	**5**	...	...
0.2 M	+	6.5* ±(0.72)	973* ±(130)	1.77 ±(.12)	80.00*±(0.58)	76.00b±(4.70)	8.00a±(.88)
	-	5.3 ±(1.60)	700 ±(170)	9.77* ±(.39)	62.00±(0.88)	nd	nd
Relative response	(%)	**23**	**33**	**-82**	**29**	...	...
0.3 M	+	4.33* ±(.55)	667* ±(220)	8.67 ±(.88)	71.00*±(0.58)	57.00c±(6.11)	5.00b±(2.0)
	-	3.07 ±(.47)	400 ±(100)	11.77* ±(.39)	45.00±(0.88)	nd	nd
Relative response	(%)	**41**	**67**	**-26**	**58**	...	...
0.5 M	+	2.6* ±(.06)	500* ±(320)	11.77 ±(.39)	56.00*±(0.88)	45.00c±(2.88)	2.00c±(1.2)
	-	1.57 ±(.81)	233 ±(90)	19.67* ±(.88)	42.00*±(0.88)	nd	nd
Relative response	(%)	**66**	**115**	**-40**	**33**	...	...
Significant Linear	+	(-ve)	(-ve)	(+ve)	(-ve)	(-ve)	(-ve)
Correlation [P< 0.05] §	-	(-ve)	(-ve)	(+ve)	(-ve)	...	...

Mean pairs [± S.E.M.] within a column with an * are significantly different at ($P \leq 0.05$) according to a Student's t test.
@ dS/m = mmoh/cm ¶ (+/-) corresponds to mycorrhizal and nonmycorrhizal with the AM fungus *G. mosseae*. # nd; not detected.
¥ Relative response = {[SMP (+)] - [SNMP (-)]÷[SSNMP (-)]} X100 (15). § Significant linear correlation positive (+ve), negative (-ve) or no correlation (o).

Table 4.2
Nutrients uptake of mycorrhizal and nonmycorrhizal peanuts as influenced by sodium chloride salinity stress.

NaCl levels	VAM (+/-)¶	Ion Uptake			
		Phosphorus (mg per g DW)	Potassium (mg per g DW)	Calcium (mg per g DW)	Sodium (mg per g DW)
0.0M	+	11*±(.06)	8.6*±(.06)	11.7±(.04)	0.24 ±(.002)
	-	9 ±(.06)	6.8 ±(.07)	11.4±(5.8E-3)	0.2 ±(3.12E+3)
Relative response ¥	(%)	**22**	**26**	**3**	**20**
0.1 M	+	9.3* ±(.03)	9.2*±(.11)	16.2*±(.01)	3.6* ±(.01)
	-	7.8 ±(.03)	6.5 ±(.02)	12.4±(3.3E-3)	1.7 ±(.04)
Relative response	(%)	**19**	**43**	**31**	**112**
0.2 M	+	8.*±(.04)	9.8 ±(.02)	17.1±(.01)	15.5* ±(.04)
	-	6.3±(.03)	10.7±(6.7E-3)	18.6*±(5.8E-3)	12.2±(5.8E-3)
Relative response	(%)	**32**	**-8**	**-8**	**27**
0.3 M	+	7.0*±(.06)	7.4*±(.03)	18.1±(5.8E-3)	26.7* ±(.05)
	-	5.7±(8.8E-3)	6.8±(.02)	21.1*±(5.8E-3)	21.3 ±(.04)
Relative response	(%)	**23**	**9**	**-14**	**25**
0.5 M	+	5.8*(±.11)	0.9±(.07)	17.5±(.03)	34.4* ±(.06)
	-	4.1±(.03)	1.0 ±(.02)	19.7*±(5.8E-3)	23.5 ±(.03)
Relative response	(%)	**41**	**-10**	**-11**	**46**
Significant linear	+	(-ve)	(o)	(+ve)	(+ve)
correlation [P≤0.05] §	-	(-ve)	(-ve)	(+ve)	(+ve)

Mean pairs [± S.E.M.] within a column with an * are significantly different at ($P \leq 0.05$) according to a Student's t test.
@ dS/m = mmoh/cm. ¶ (+/-) corresponds to mycorrhizal and nonmycorrhizal with the AM fungus *G. mosseae*.
¥ Relative response = {[SMP (+)] - [SNMP (-)]÷[SNMP (-)]} X100 (15). § Significant linear correlation positive (+ve), negative (-ve) or no correlation (o).

Table 5.1
Effect of fungicide application on shoot height per plant of mycorrhizal peanuts.

A. ANOVA table

Source	df	SS	MS	F-test
Fungicides (A)	1	12.96	12.96	1.73
Concentration (B)	4	508.46	127.11	16.95*
AXB	4	59.54	14.89	1.98
Error	90	675.1	7.5	

Asterik [*] denotes significance at $P \leq 0.05$ level .

B. The fungicide concentrations incidence table on : Shoot height per plant.

Concn / Fungicides	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	32.3 ±(0.81)	31.4 ±(0.40)	30.3 ±(0.34)	26.9 ±(0.85)	25.3 ±0.98)	2.44
Aspor	32.3 ±(0.82)	28.5 ±(1.31)	28.1 ±(1.34)	27.4 ±(0.69)	26.3 ±(0.50)	

Readings are means of ten replicates ± S.E.M.

Table 5.2
Effect of fungicide application on fresh weight per plant of mycorrhizal peanuts.

A. ANOVA table

Source	df	SS	MS	F-test
Fungicides (A)	1	17.64	17.64	2.01
Concentration (B)	4	323.46	80.865	9.2*
AXB	4	36.46	9.115	1.04
Error	90	791.4	8.783	

Asterik [*] denotes significance at $P \leq 0.05$ level .

B. The fungicide concentrations incidence table on : Fresh weight per plant.

Levels / Fungicides	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	**12.10** ±(0.69)	**12.10** ±(0.96)	**10.70** ±(1.51)	**12.20** ±(0.99)	**7.40** ±(0.99)	**2.54**
Aspor	**12.1** ±(0.69)	**10.8** ±(1.20)	**10.7** ±(0.76)	**9.3** ±(0.54)	**6.8** ±(0.39)	

Readings are means of ten replicates ± S.E.M.

Table 5.3
Effect of fungicide application on dry weight per plant of mycorrhizal peanuts.

A.ANOVA table

Source	df	SS	MS	F-test
Fungicides (A)	1	194657.44	194657.44	2.58
Concentration (B)	4	2521635.76	630408.94	8.36*
AXB	4	164119.76	41029.94	0.54
Error	90	6785806.4	75397.849	

Asterik [*] denotes significance at $P \leq 0.05$ level .

B. The fungicide concentrations incidence table on : Dry weight per plant.

Levels / Fungicides	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	**1164.3** ±(66.65)	**1008.8** ±(93.98)	**1171.7** ±(126.17)	**939.6** ±(101.14)	**791.6** ±(84.29)	**244.33**
Aspor	**1164.3** ±(66.65)	**1028** ±(35.24)	**1004.7** ±(107.58)	**777.8** ±(105.37)	**660** ±(22.57)	

Readings are means of ten replicates ± S.E.M.

Table 5.4
Effect of fungicide application on phosphorus content of mycorrhizal peanuts.

A.Statistical analysis

Source	df	SS	MS	F-test
Fungicides (A)	1	4.05E-03	4.08E-03	10.29*
Concentration (B)	4	0.35	0.034	85.35*
AXB	4	3.47E-03	8.66E-03	2.19
Error	90	7.93E-03	3.96E-04	

Asteriks [*] denote significance at $P \leq 0.05$ level .

B. The fungicide concentrations incidence table on : Phosphorus

Fungicides \ Levels	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	**0.29** ±(8.82E-3)	**0.27** ±(5.77E-3)	**0.26** ±(0.019)	**0.22** ±(0.015)	**0.13** ±(5.77E-3)	**0.02**
Aspor	**0.29** ±(8.82E-3)	**0.27** ±(8.82E-3)	**0.23** ±(0.02)	**0.19** ±(0.01)	**0.07** ±(8.82E-3)	

Readings are means of three replicates ± S.E.M.

Table 5.5
Effect of fungicide application on percent colonisation of mycorrhizal peanuts.

A.ANOVA table

Source	df	SS	MS	F-test
Fungicides (A)	1	81.675	81.675	3.97*
Concentration (B)	4	8552.133	2138.033	103.2*
AXB	4	693.867	173.467	8.44*
Error	90	411.167	20.558	

Asteriks [*] denote significance at $P \leq 0.05$ level .

B.The fungicide concentrations incidence table on : Percent colonisation.

Levels / Fungicides	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	**95** ±0.88	**82** ±1.45	**76** ±2.33	**68** ±1.53	**56** ±3.48	**4**
Aspor	**95** ±0.88	**85** ±0.44	**85** ±6.49	**57** ±1.53	**38** ±0.88	

Readings are means of three replicates ± S.E.M.

Table 5.6
Effect of fungicide application on sporulation of the AM fungus.

A. ANOVA table

Source	df	SS	MS	F-test
Fungicides (A)	1	48.164	48.164	16.45*
Concentration (B)	4	544.817	136.204	46.51*
AXB	4	31.23	7.807	2.67*
Error	90	58.571	2.929	

Asteriks [*] denote significance at $P \leq 0.05$ level .

B. The fungicide concentrations incidence table on : Sporulation (Number of spores per g soil).

Fungicides \ Levels	0 µg/g soil	50 µg/g soil	100 µg/g soil	150 µg/g soil	200 µg/g soil	Fisher's LSD Value
Plantvax	**16.00** ±(0.88)	**12.00** ±(2.08)	**10.00** ±(0.58)	**6.00** ±(0.58)	**6.00** ±(0.58)	**2**
Aspor	**16.00** ±(0.88)	**7.00** ±(0.58)	**7.00** ±(0.58)	**6.00** ±(1.20)	**2.00** ±(0.88)	

Readings are means of three replicates ± S.E.M.

APPENDIX E [PLATES]

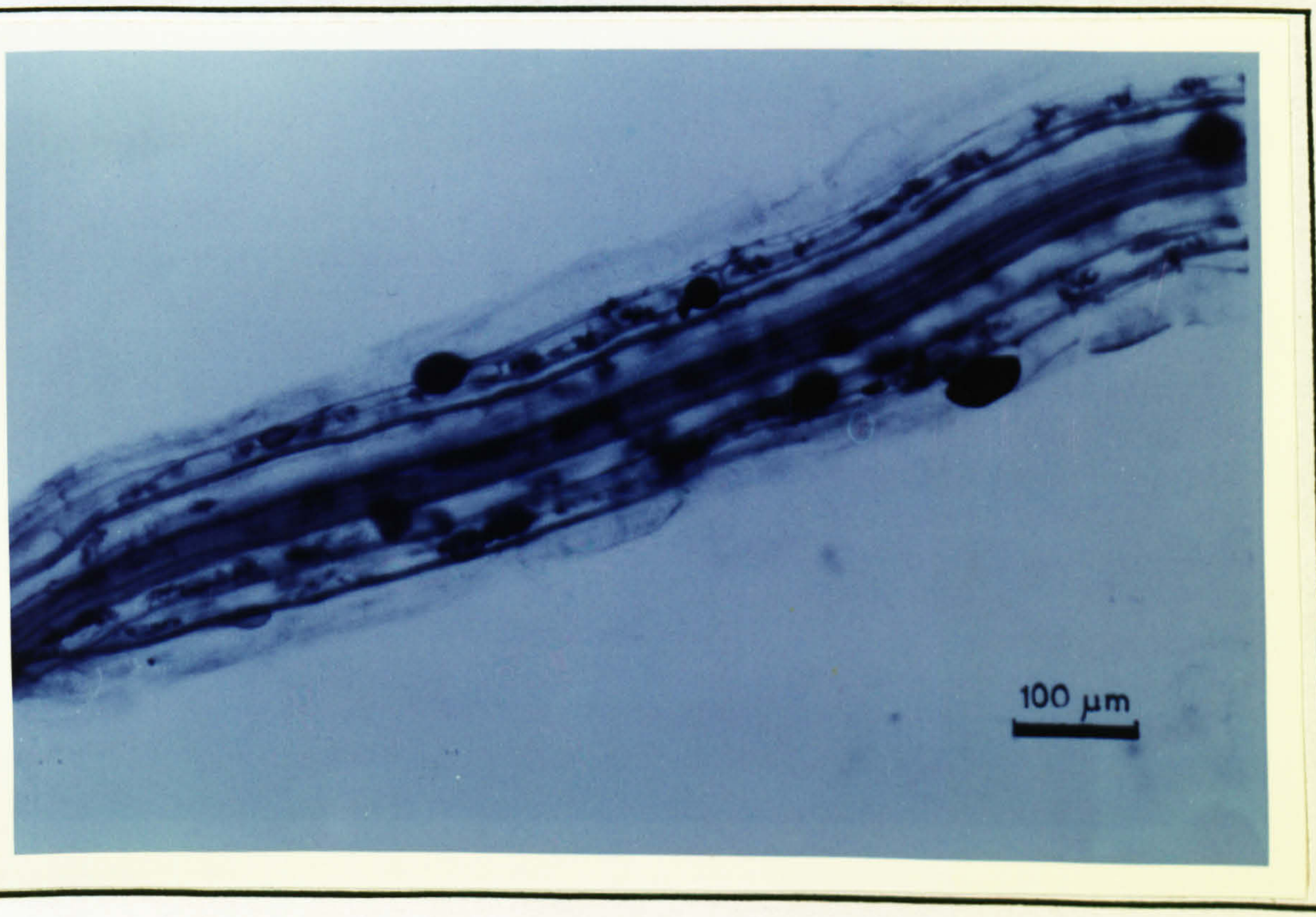

Plate 1
Root segment of *Arachis hypogaea* L. with typical AM fungal colonisation (*Glomus fasciculatum*).

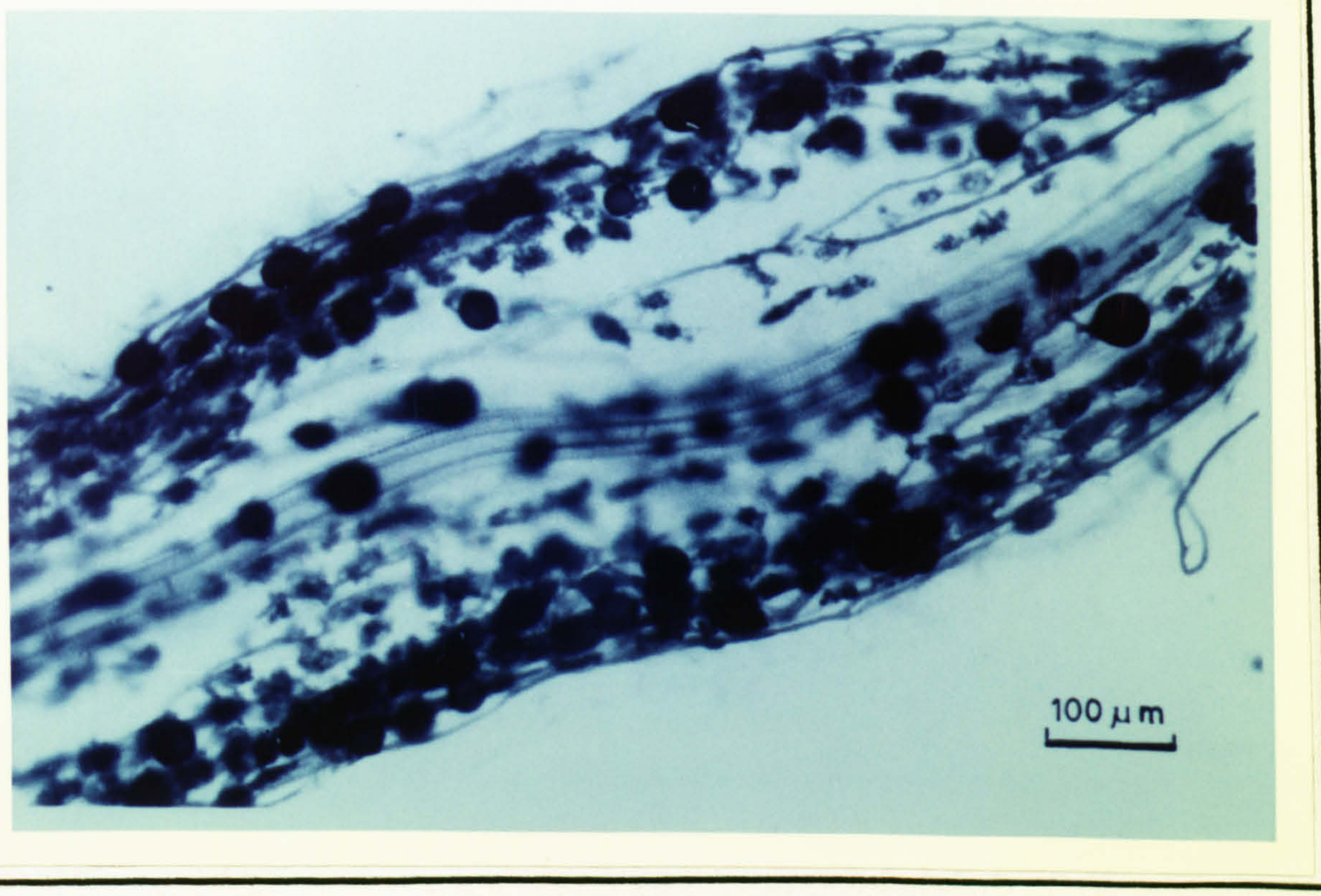

Plate 2
Young root segment of *Arachis hypogaea* L. showing heavy colonisation (vesicles, arbuscules and hyphae) caused by *Glomus mosseae*.

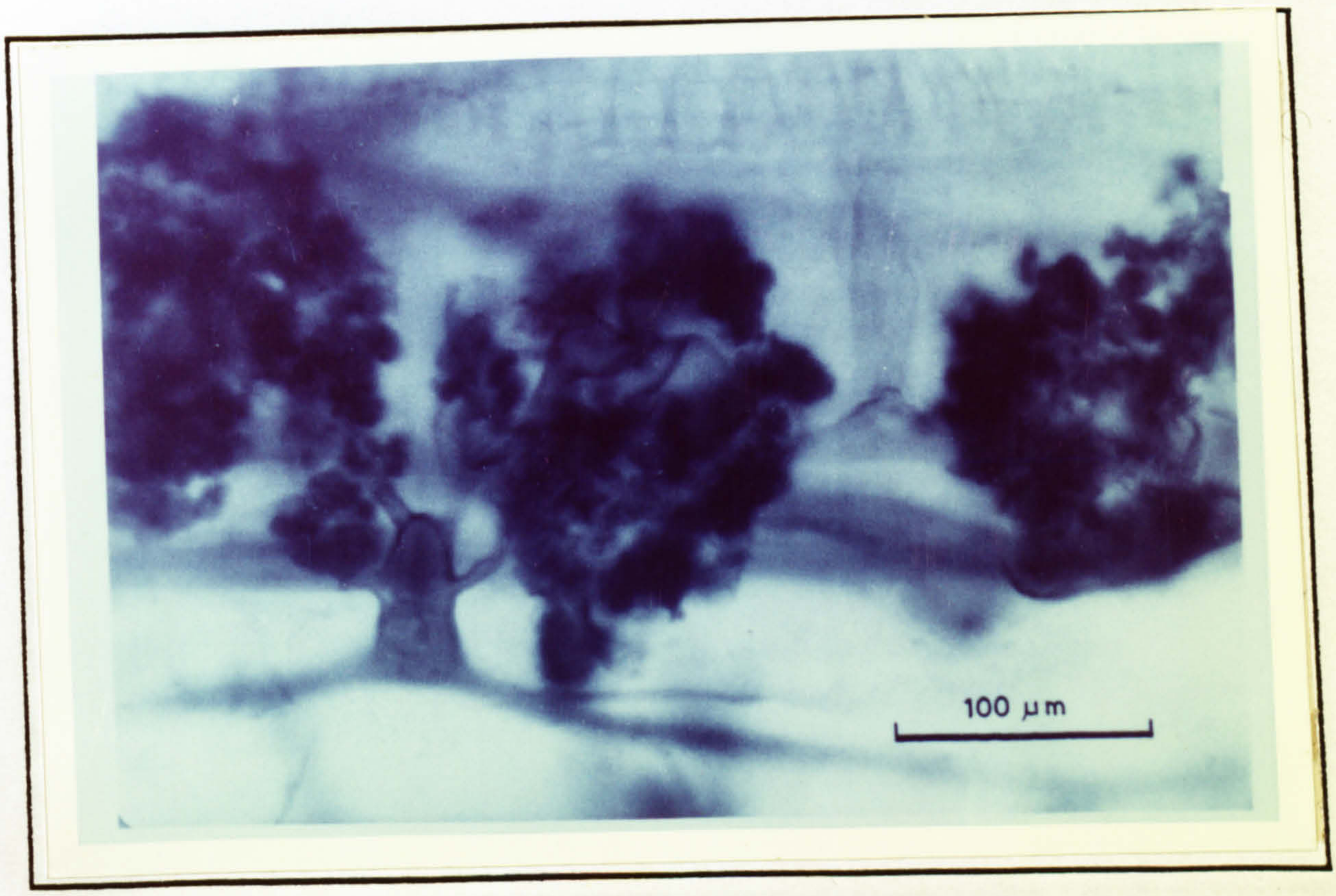

Plate 3
Abundant arbuscules produced in *Arachis hypogaea* L. by *Glomus mosseae*.

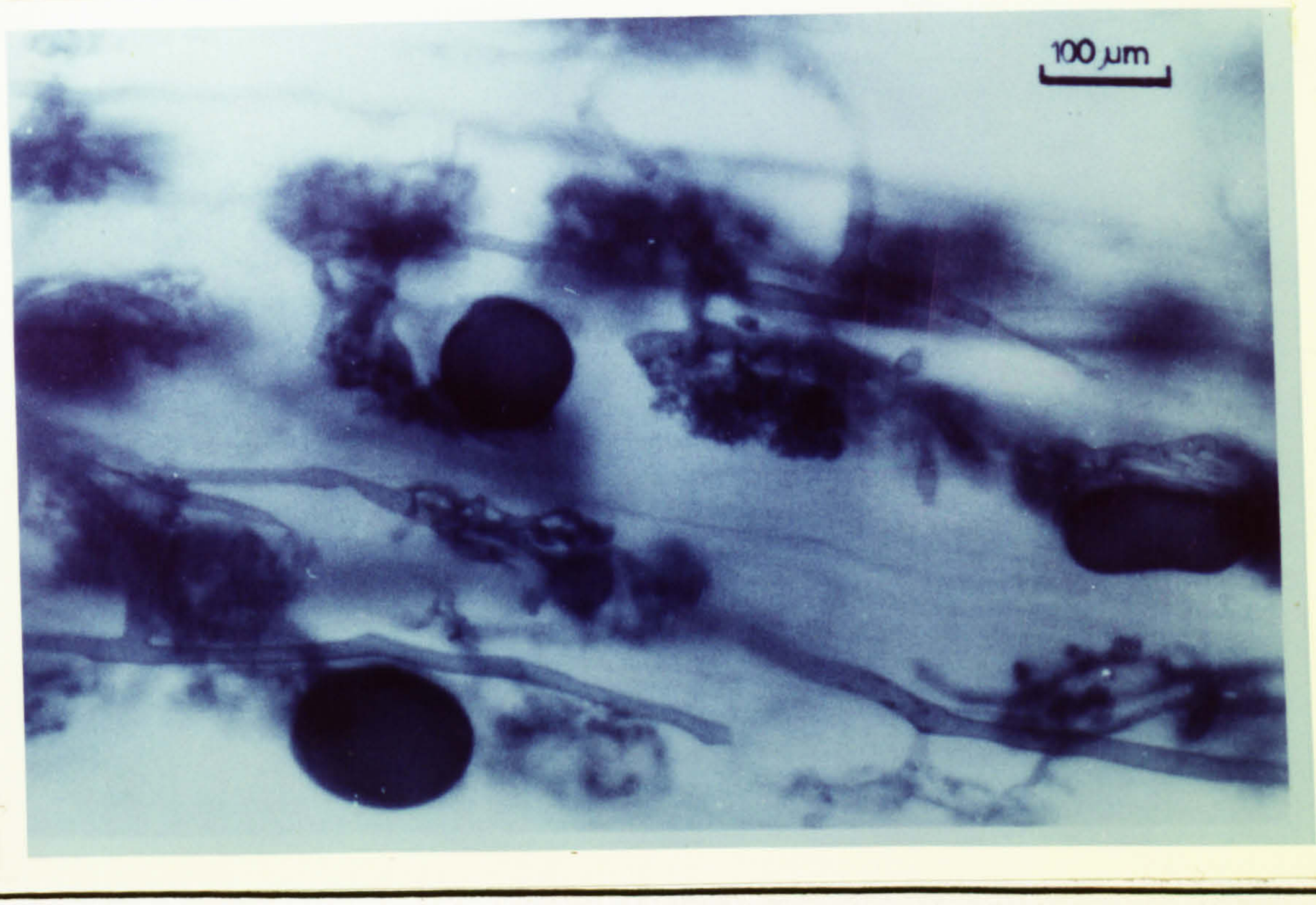

Plate 4
Glomus mosseae **arbuscule formation by branching of fungal hyphae within root of** ***Arachis hypogaea*** L.