INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHORUS ON EARLY GROWTH AND NODULATION OF INDIGENOUS *ALBIZIA* SPECIES.

A Thesis

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Technology, Kumasi.

In Partial Fulfilment of the Requirements for the Award of the Master of Science Degree

In

Agroforestry.

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JULY, 2001

DECLARATION

I do hereby declare that, except references to other people's work which have been duly cited, this work submitted as a thesis to the Department of Agroforestry, Institute of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, for the degree of Master of Science in Agroforestry is the result of my own investigations.

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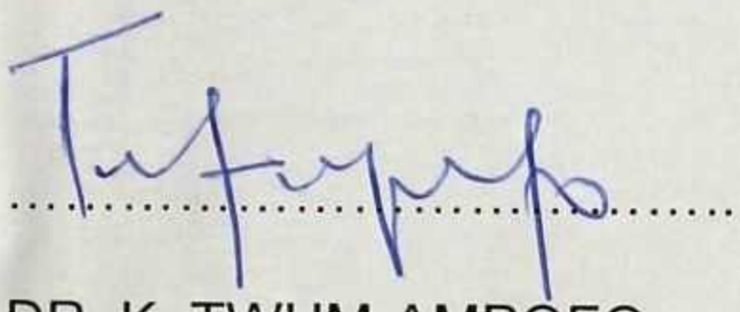

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DR. K. TWUM-AMPOFO

(HEAD OF DEPT./ SUPERVISOR)

DEDICATION

To My Late Parents and My Sister Diana Twumasi.

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ABSTRACT

This study was conducted to determine the response of four (4) species of arbuscular-mycorrhizal fungi (*Glomus clarum, Glomus intraradices, Glomus etunicatum, Gigaspora rosea*) on early growth and nodulation of *Albizia* species (*A. adianthifolia, A. ferruginea, and A. zygia*), and to evaluate the interaction of phosphorus-fertiliser in the form of tripple superphosphate (TSP) with arbuscular-mycorrhizal fungus (*Glomus clarum*). *Albizia* seedlings were grown in pots under nursery conditions and treatments applied to both sterilised and unsterilised soil conditions.

Arbuscular mycorrhizal fungi differed in their effectiveness in enhancing early growth, dry matter accumulation, nutrient (N, P, K, Ca, and Mg) uptake and root colonization in all the three *Albizia* species. *Glomus intraradices* was the most preferred fungus in *A. adianthifolia*, *Glomus clarum* in *A. ferruginea* and *Glomus etunicatum* in *A. zygia*. In general, *Glomus clarum* proved to be the most efficient fungus in almost all the parameters considered. Moreover, *A. ferruginea* gave the most promising initial growth characteristics as well as nutrient uptake and root colonization. However, sterilised uninoculated soil treatment showed superiority in almost all the three *Albizia* species in terms of the growth measurements taken which was contrary to earlier reports made by some authors but recorded significantly low root colonization.

Phosphorus addition in the form of TSP markedly stimulated nodulation (nodule number and nodule dry weight), increased plant growth, nutrient uptake and mycorrhizal infection in both uninoculated and inoculated seedlings of *A. ferruginea* but more pronounced in the inoculated seedlings. Phosphorus fertilizer application at 50kg/ha (100mgTSP) gave significantly higher plant growth and arbuscular mycorrhizal fungal

infection whilst these parameters declined above 100kg/ha(200mgTSP).

The outcome of this study indicates that arbuscular mycorrhizal fungi differ in their effectiveness on indigenous *Albizia* species. In P-deficient soils it is possible to reduce the phosphorus fertilizer application with AM fungal inoculation and still maintain high productivity. Therefore in most tropical soils AM fungi strains may help plant growth and establishment on degraded soil or plants that cannot adequately meet their nutrient requirements.

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CHAPTER ONE

1.0 Introduction

Sub-Saharan Africa has a critical developmental problem because the population is growing at an average rate of 2.5-3.0%, while food production is increasing only by about 1.5% per annum (IBRD, 1989). Of this food production, 90% comes from small holder farmers who generally crop less than 3ha (Spencer, 1991), practice shifting cultivation (Sanchez, 1976), and use few improved technologies or none. This rapid increase in human population has led to continuous cultivation and reduced fallow periods previously used to partially restore soil fertility. The result is deforestation, soil degradation and reduced crop yields (Tiessen *et al.*, 1989; Kang *et al*, 1990). Moreover, the demand for more forest products such as fuel wood gathering, timber harvesting, as well as agricultural lands has resulted in the destruction of the natural vegetation, thereby accelerating the process of desertification (Schofield, 1992; Hanson and Cassman, 1994).

To meet the ever increasing demand for food and wood resources in the tropical and sub-tropical developing countries, there has been a renewed interest in the development of viable and sustainable land use systems (Agroforestry) in which multipurpose trees and shrubs (MPTS) are incorporated. Some of these MPTS are Nitrogen-fixing trees (NFTs) which could play important roles in the development of such systems and in reforestation schemes due to their ability to fix atmospheric nitrogen in symbiosis with mycorrhizal fungi (Hogberg, 1986; Habte and Turk, 1991). Such associations are important for the absorption of relatively immobile nutrients from soil particularly phosphorus (Hayman, 1986) and other micro-nutrients essential for nitrogen fixation (O'Hara *et al.*, 1988).

These MPTS provide fuel wood, fodder for livestock, construction materials, poles among others (Burley, 1987; Hughes and Styles, 1989). They also improve soil fertility and protect soil from erosion (Young, 1987).

Biological nitrogen fixation and fungi stimulation for nutrient uptake have become the subject of scientific and practical importance (Sasson, 1993), because they minimise the use of fertiliser and other chemical inputs. One group of soil micro-organisms that is believed to have a potential role in the improvement of land productivity are mycorrhizae, in which fungi colonise and form intricate symbiotic relationship with plant roots of which they become an integral part and greatly form a mycorrhizal root system. Some of the fungi types that have been identified include, ectomycorrhiza, arbuscular mycorrhiza (AM), ericoid, arbutoid and orchidaceous mycorrhizae (Harley and Smith, 1983). The arbuscular mycorrhiza is by far the most widespread in nature (Harley and Smith, 1993) and the most commonly occurring on nodulated nitrogen - fixing plants (Barea *et al.*, 1992; Hayman, 1986).

Successful establishments of most tropical woody legumes depend on their ability to form symbiotic associations between their roots and beneficial microorganisms-*rhizobium* and mycorrhiza (Herrera *et al.*, 1993).

Inoculation with AM fungi enhances plant growth by supplying plants with nutrients that are lacking and also by enhancing nitrogen fixing process (Fitter and Garbaye, 1994). However, certain factors could hamper successful mycorrhiza establishment. High level of inorganic fertiliser such as Sulphate of ammonia, NPK among others, soil pH and soil temperature affect root infection, while the establishment is also delayed by high run-off and low infiltration among others (Lynch, 1995).

Plants that are most likely to benefit from AM fungi inoculation are those that cannot adequately meet their phosphorus demand; for instance, Nitrogen fixing trees (Habte and Aziz, 1985). This is because they have a restricted root system; hence they depend on AM fungi for their phosphorus supply which is a major element in plant nutrition. Considering the importance of these trees, particularly in the tropics, and the fact that many tropical soils are deficient in available phosphorus, utilisation of AM fungi has been proposed to improve their establishment in the tropics (Habte and Aziz, 1985: Mosse, 1986). However, very little research has been done to elucidate the detail of the symbiosis between AM fungi and nitrogen-fixing trees compared with the work done with other leguminous species.

AM fungi are known to be non-host specific (Barea and Azcon-Aquilla, 1983). Despite the non-specificity of these fungi with respect to host plant, certain fungus-plant associations are more efficient than others. Some species of AM fungi may enhance plant growth and nutrient uptake in some legume species but have little or no effect on others (Dela Cruz *et al,* 1988). There is however, limited data available on AM fungi interaction with nitrogen fixing trees (NFTs) and studies have shown that NFTs respond differently to inoculation with different strains of AM fungi (Aziz & Habte, 1989; Aziz and Sylvia, 1993).

In the tropical soils, production of NFTs is often limited by low phosphorus availability. The symbiotic nitrogen fixation is strongly influenced by P nutrition of the host (Isreal, 1987; Sanginga *et al*, 1988), and phosphorus deficiency is therefore a major nutritional limiting factor to N_2-fixation of legumes in the tropics. Nitrogen fixation is highly important for the nutrition of leguminous plants in natural and agricultural systems in the tropics (Högberg, 1989), and planting of Nitrogen-fixing tree legume is recommended for agroforestry projects. The Nitrogen-fixing

potential of tropical tree legumes has been estimated in a lot of laboratory experiments (Sanginga *et al.*, 1990). As field data is scarce, there is the need to extend it to field conditions. However, several factors may limit nodulation and fixation in the nursery and in the field (Högberg, 1989; Michelsen, 1992). Subsequently, knowledge of nitrogen fixation of seedlings of tree legumes grown under normal nursery conditions is thus, valuable for species selection and for evaluation of nursery practices.

In Ghana, most of the known studied leguminous woody tree species capable of improving the soil through nitrogen fixation are exotic. These include, *Gliricidia sepium*, *Leucena leucocephala* among others. However, one of the indigenous leguminous tree species that is gaining greater importance in agroforestry systems and reforestation schemes is the indigenous *Albizia* species. They are woody perennials and grow well in humid and sub-humid zones of West Africa (Irvine *et al.*, 1961). Moreover, they are fast growing, nodulate freely and hence nitrogen fixing. In addition, they are used as timber, fuel wood, shade, windbreaks, for medicinal purposes, as well as soil conservation (Ulzen – Appiah *et al.*, 1990).

For nitrogen-fixing trees, the derivation of their importance will depend on their infection with appropriate microorganisms. Recent studies indicate that, in certain soils the population of AM fungi are too low and/or ineffective to be of any benefit to the growing crop or plant (Latecon *et al*, 1989). Therefore, the introduction of appropriate AM fungi strain into such soils could help improve fertiliser recovery and lead to better production and establishment of the plant. Moreover the effectiveness of mycorrhiza in the phosphate nutrition of a plant depends on the plant's capacity to satisfy its phosphorus requirement (Letacon *et*

al., 1989), but there is inadequate information on the efficiency and effectiveness of AM fungi strains and phosphorus on *Albizia* species.

The purpose of this study, therefore, is to assess the influence of arbuscular mycorrhiza fungi and phosphorus on early growth and nodulation of indigenous *Albizia* species.

The specific objectives are to:

1. determine the influence of four (4) AM fungi strains on the early growth and nodulation and nutrient uptake *of Albizia zygia, Albizia ferruginea, and Albizia adianthifolia*;
2. find out the interactive effect of levels of Phosphorus and AM fungus on early growth and nodulation of *Albizia ferruginea*.

CHAPTER TWO

2.0 Literature Review

2.1. Leguminosae

The leguminoseae is an enormous plant family with world-wide distribution, survive on a wide range of climatic conditions and have multiplicity of uses with an estimated 16,000 to 19,000 species in about 750 genera. It is ranked second to Gramineae in economic importance, and in size only to the Orchidaceae and Compositae (Allen and Allen, 1981). Some members have the ability, not only to be self–sufficient in Nitrogen, but also providing high protein grain and herbage as well as maintaining and improving soil fertility (Faria *et al.*, 1989). The family is divided into three subfamilies; viz, Caesalpinoideae, Mimosoideae and Papilionoideae. Species of the subfamilies Caesalpinoideae and Mimosoideae are mainly woody. Most of the Papilonoid species are herbaceous although 4000 – 5000 are woody and of these 1000 are trees. Papilionoideae contain more tree species than Caesalpinoideae and Mimosoideae combined (Allen and Allen, 1981; Sutherland and Sprent, 1993).

Within the leguminoseae family about 50% of the plants have been studied for nodulation. Thus in the subfamily Caesalpinoideae and the genus Chamaecrista from the tribe Cassieae, nodulation has been found to be less common out of the 17 species examined, only 23% nodulated (Farie *et al,* 1989; Sprent and Sprent, 1990; Sutherland and Sprent, 1993). The authors however stated that nodulation is common with the members of the subfamily Mimosoideae except for four groups of the Mimoseae tribe and very few species of Acacia.

Approximately 17% of the Mimosoideae have been examined for nodulation with 90% nodulating. In Papiloinoideae, less than 20% of the species have been examined for nodulation out of which 97% nodulate. The only tribe from Papiloinoideae which appears not to nodulate is Dipterygeae (Farie *et al.*, 1986; Sprent *et al.*, 1989). According to Hawthorne (1990), three genera with seven species of the tribe Ingeae were observed among the species in the Ghanaian rainforest. Out of these, the genus *Albizia* (Durazz) recorded five species; *Albizia adianthifolia, A. coriara, A. ferruginea, A. glaberrima and A. zygia*. Although they are of great importance in both agricultural and ecological studies, little is known of their relevance to Agroforestry in the Ghanaian society, hence are being over exploited for timber, fuel wood, and constructional materials among others in most of our forest zones.

2.2 Albizia Species

Albizia species is a leguminous tree within the genus *Albizia* in the subfamily Mimosoideae of the leguminoseae. There are many African *Albizias*, and those in West Africa are mainly divided into "light" and "heavy" woods, the latter being the more durable (Irvine, 1961).

2.2.1. Albizia zygia (DC) J.F. Macbride

Albizia zygia, locally known as xkrx (Hall and Swaine, 1981; Abbiw, 1990; Hawthorne, 1990) is found scattered in secondary forests and high forests throughout tropical Africa (Irvine, 1961). In Ghana, they are mostly located in areas such as Kwahu, Tafo, Begoro, Kibi, Aburi Hills, Mampong Scarp, and Kumasi and throughout high forest zones. Swaine *et al.*, 1997 also reported that, *A. zygia* is a very ubiquitous plant because it is indifferent to soil and climate, hence widely

distributed in the wet evergreen, moist evergreen, moist semi-deciduous and dry semi-deciduous forest. Naturally, it regenerates abundantly in openings in the forest, along paths and in newly abandoned farms.

Albizia zygia grows to a maximum height of 31m when matured with a girth of 2.5m and develops buttress roots. The bark is pinkish-grey to dark-brown with prominent lenticels (Taylor, 1960).

The leaves of *A. zygia* are alternately arranged, bipinnate with 2 – 4 pairs of opposite pinnae and each pinna has 3 – 5 pairs of opposite leaflets. The leaflets are entire and vary in size from 1.3 – 5.0cm long and 1.3 – 3.8cm broad. The apex is obtuse and the base broadly cuneate and unequal. The midrib is not quite central and the lamina is green above and below. (Irvine, 1961).

Flowering and fruiting of *A. zygia* take place from February – March and pods ripen between December and February. Pods contain between 8 and 10 seeds, which are flat, round and dark in colour. The old leaves are shed from August to March and the tree may or may not bear fruit for a short period. However, it bears fruit annually (Irvine, 1961).

The pulverised bark of *A. zygia* is rubbed on the skin for eruptive fevers and also applied as a dressing for yaws, while in Yoruba, it is used for the treatment of certain stomach diseases such as ulcers, constipation etc. (National Academy of Science, 1979).

2.2.2 *Albizia adianthifolia* (Schumach) W.F.Wight

Albizia adianthifolia, locally called Pampena (Hall and Swaine, 1981;Abbiw, 1990; Hawthorne, 1990) does not have different distribution from that of *A. zygia* in terms of habitat, but is more common in secondary forests. The identified towns of

these species include, Kwahu, Tafo, Dunkwa, Axim, Prestea, Kumasi etc (Irvine, 1961).

The bole of this species is unbranched up to 7.5m and attains maximum height of 37m when mature with a spreading crown. It produces slightly buttressed roots. The bark is rough, scaly, grey to reddish and finely fissured with horizontal corrugations (Irvine, 1961).

It has 5 – 8 pairs of bipinnate leaves (20cm long). The petioles and rachis are brown and pubescent with 8 – 16 pairs of leaflets each measuring approximately 1.5 x 0.75cm (Irvine, 1961).

Flowering and fruiting take place between January and March. The flowers are greenish – white with long staminal tube and crimson anthers. The ports are linear, oblong, thin papery with yellow reticulate markings and contain 8 – 14 seeds which rattle when shaken (Irvine, 1961).

According to Irvine (1961) firewood produced from *A. adianthifolia* is good but tends to burn quickly while its charcoal is used mostly by Goldsmiths. Moreover, its medicinal values include the following, the bark pulp is proposed as an enema and the stem decoction are used as purgative to ease constipation, the root infusion is applied to conjunctivitis and sore eyes to cure eye problems, and the cracked bark with kaolin is used in Ivory Coast as a liniment for itching (Irvine, 1961).

2.2.3 Albizia ferruginea (Guill and Perr.) Benth

Albizia ferruginea is locally known as Awiemfox samina (Hall and Swaine, 1981; Abbiw, 1990Hawthorne, 1990). Although, it is found in evergreen forests, it is less common in the deciduous forest. It is located in the following agro-ecological zones; Aburi hills, Kumasi and Mampong scarp (Irvine, 1961).

It grows up to a height of 46m when fully mature with a girth of 2.8m and a small or no buttress root. It produces clear bole up to 1.2m with a thick reddish brown bark and flat branches. (Irvine, 1961).

The leaves are bipinnate of 7 pairs with leaflets nearly 2.5cm long, oblong with rounded apex. Hairy structures grow on the midrib as well as on laminal veins. Leaves are rachis and slightly grooved when young. (Irvine, 1961).

Flowering and fruiting take place from February to March with greenish-white flowers in small heads and long protruding stamens. It produces fruits up to 8" x 2" which are reddish brown and generally up to 10 seeds or more (Irvine, 1961).

According to Irvine (1961), the wood from *A. adianthifolia* is resistant to decay and so is suitable for the manufacture of tool handles as well as general interior and exterior decorations. It has a lot of medicinal values. The leaves lather well in water, hence, used in preparation of medicated soap.

2.3 RHIZOBIA

2.3.1 Characteristics of Rhizobia

Rhizobia or root nodule bacteria are medium-sized, rod-shaped cell, 0.5 - 0.9µm in width and 1.2 – 3.0µm in length, thus genetically diverse and physiologically heterogeneous. Moreover, they are facultative microsymbionts that live as normal compounds in the root nodules of the host legume. Outside the root nodule, rhizobia are mostly found on the root surface (rhizoplane), soil around and close to the root surface (rhizosphere), and to a lesser extent, non-rhizosphere soil (Somasegaran, 1990). In the soil, they can exchange genetic information with

other *rhizobium* strains (Schofield *et al*., 1987) and with other genera of soil bacteria (Jarvis *et al*, 1989).

Taxonomically, they have been classified into four genera, thus *Rhizobium*, *Bradyrhizobium* (Jordan, 1984) and *Azorhizobium* (Dreyfus *et al*, 1988) and *Sinorhizobium* (Lajudie *et al*, 1994).

2.3.2 *Rhizobium* – legume Symbiosis

Rhizobia are unique among soil microorganisms in their ability to form nitrogen – fixing symbiosis with legumes and, exceptionally, a non-legume (Parasponia) of a member of Ulmaceae (Trinick, 1979). This interaction results in the formation of nodules on the root of host plant (Bergersen, 1982; Sprent and Sprent, 1990). The process benefits the host plant by gaining constant supply of reduced nitrogen from the atmospheric through nitrogen fixing and the rhizobia in return are supplied with carbohydrate and other nutrient by the host plant (Sprent, *et al.*, 1989). Moreover, the host plant provides a niche for rhizobial growth and metabolism within the symbiotic Unit (Bergersen, 1982). In the non-symbiotic state rhizobia are common Gram-negative, non-spore-forming, living saprophytically on a wide range of organic carbon sources, but unable to fix- nitrogen in the absence of the appropriate legume root, they are able to overcome the plants defence mechanism, infect the root, form nodules, synthesize nitrogenase and other vital components and fix-nitrogen (Somasegaran *et al*, 1990).

2.3.3 Nodulation Process in Woody Legumes

Infection process by rhizobia in leguminous plants may either be

i) by root hair i.e. rhizobia enters host cell at the most curled region of the root hair

ii) by wound or crack i.e. rhizobia gain entry at the point where lateral root emerge or

iii) epidermal infection i.e. rhizobia penetration directly between undamaged epidermis of legume (Sprent & Sprent, 1990).

Nodule development in many legumes starts with rhizobial invasion of a root hair and produce deformation either by curling or branching of the root hair possibly due to the production of Indole Acetic Acid by rhizobia from tryptophan excreted by the root. Curling is due to the development of cell wall growth from top of a growing root or to the outside of the curl and results in curvatures up to or more than 360^0 which appears to be characteristic of a compatible interaction. Branching of root hairs also occurs frequently and seems to involve different mechanisms including wall degradation (Dart. 1977; Sprent *et al.*, 1989). When the rhizobia penetrate the inner cortical cells of the root they multiply and cause a proportion of the cells to start proliferating thereby forming a nodule. Once the bacteria have filled a proliferating cell they become enlarged and change into bacteroids. They lose most of their ribosomes and the ability to multiply, but synthesise nitrogenase and are surrounded by membranes formed by the host cell. The bacteroids are bathed in a solution containing leghaemoglobin, which transports O_2 for respiration at very low partial pressures thereby protecting the oxygen-sensitive nitrogenase. Leghaemoglobin, which is similar to haemoglobin, gives nodules a characteristic pink colour (Allen *et al.*, 1991).

Almost all young nodules are globose. Some remain so, but many produce a district apical meristem and continuous to grow for a very length of time, with or without branching, thus determinate and indeterminate growth. In the determinate or limited growth, nodules do not have persistent meristems, hence the vascular system becomes more or less closed and tends to be spherical in shaped (Sprent, 1980; Bergerson, 1982). But the indeterminate or indefinite growth, nodules have persistent meristems with open vascular system and are usually branched or Spherical in shape (Sprent, 1980; Bergersen, 1982). Nodule shape is a taxonomic character which may be linked to physiological properties (Sprent, 1981).

2.3.4. Factors Affecting Nodulation

Several factors contribute to the poor performance of *rhizobium* – legume symbiosis resulting to poor nodulation and nitrogen fixation. Among them are the environmental factors, which affect results obtained by inoculation under field conditions. These factors include:

2.3.4.1 Nitrogen Supply

A number of studies have been carried out on the effect of different sources of nitrogen and how these affect nodulation and nitrogen fixation. Fertilizer had a direct effect on nodule growth and mineral nitrogen is more likely to inhibit nodule formation than to stimulate nodule growth except at very low concentrations. e.g. Nodules on *Inga jinicuil* roots occurred mainly around the base of the coffee trees in the area in which NPK fertilizer had been applied (Abaidoo *et al*, 1990). The effect of nitrogen fertilizer addition has been evaluated in Kenya and the result shows that small doses of 20kg/ha had little effect on nodulation. However, higher doses increased seed yield over inoculated treatments which again indicates that

the amount of nitrogen fixed is insufficient for maximum yield in *Phaseoulus vulgaris* seeds (Ssali and Keya, 1982; Abaidoo *et al*, 1990). The mechanism with which low nitrogen inhibits nitrogen fixation in an already formed nodule is not known, however, the addition of nitrate to *P. Vulgaris* plants causes early nodule senescence and internal pH indicates a rapid drop in pH. This decay stimulates leghemoglobin hydrolysis by proteolytic enzymes (Pladys *et al*., 1988).

In crop legume, different forms and levels of combined N have been reported to inhibit and suppress nodulation and nitrogen fixation (Buttery *et al*, 1990; Hansen *et al*, 1992). They further explained that nitrate suppresses nodulation more than ammonium and this however depends on the amount applied. Atwell (1992) supported this observation when he observed a less severe effect on nodulation and nitrogen fixation than nitrate application in lupins. According to Ulmali-Garcia (1990), combined nitrogen in the form urea could promote or inhibit nodulation of legume trees and this depended on the host species and concentration of nitrogen.

Studies with tree legumes have revealed significant variations in their response to combined nitrogen. *Acacia* species exhibit specific and provenance differences in growth, nodulation and nitrogen fixation (Goi *et al*., 1992; and Gitonga, 1994). Ulmali-Garcia (1990) showed that combined nitrogen in the form of urea could promote or inhibit nodulation of legume trees and this depended on the host species and concentration of nitrogen.

2.3.4.2 Soil Acidity

In the tropics, the acid soil complex affect nodulation and nitrogen fixation, however, rhizobia strains varied in terms of their tolerance to acidity. In Brazil, Franco and Day (1980) found that liming a soil of pH 5 increased nodulation,

nitrogen fixation and plant growth. Buerkert *et al* (1990) showed that liming acid soils increased nodulation and nitrogen fixation, resulting in better establishment and increased pod number per plant, seed number per pod and seed weight; it also contributed to a significant yield increase. Franco and Munns (1982) also found on solution culture that a pH of 4.5 – 5.5 did not affect nodule growth and nitrogenase activity. However, a decrease in pH from 5.5 to 5.0 resulted in a decrease in number of nodules formed per plant.

2.3.4.3 Phosphorus Nutrient Deficiencies

Nodules are extremely strong sinks for phosphorus, indicating that nitrogen fixation is limited by phosphorus. A greenhouse experiment conducted by Bonetti *et al* (1984) showed that nodulation and plant weight increased under increasing rates of phosphorus when water tension was low. A similar experiment conducted by Morales and Ramirez, 1988) provided evidence that strains tolerant to low phosphorus can increase yields on certain soils and at specific phosphorus concentrations. Field experiment in Kenya (Ssali Keya, 1983) indicated that, compared with combined nitrogen, the application of phosphorus increases nodule mass, dry-matter yield, N. yield and nitrogen fixation.

2.3.4.4 High Soil temperature

Temperature is one of the most significant physical factors affecting survival, nodulation and nitrogen fixation. The greatest number of nodules per root and percentage of root nodulated were formed at 25^0C, and that no nodules were formed at 33^0C using *Phaseolus vulgaris* (Gitonga *et al*, 1989). Other observations

also indicate that the optimum temperature is 20^0C and that a drastic decline occurs when the temperature rises above 30^0C (Mulongoy *et al*, 1990). Experiments reported by Hernandez - Armeta *et al*, 1989) indicate that the critical temperature is 32^0C for nodule function and 38^0C for nodulation.

2.3.4.5 Strain Competition

In general, one of the major factors contributing to inoculation failure in the field is the competition from indigenous, adapted strain that have been shown to occupy most of the soils (Velazquez *et al.*, 1988). This has also been confirmed by Ahmed and Phelps (1990) where inoculation with a strain isolated from an acid infertile soil showed an increase in nodule weight, total N and nitrogenase activity in greenhouse experiments. The practice of inoculation with single, highly competitive strains may not be effective in all environmental conditions. It may lose effectiveness or become suppressed by more effective strains (Lal and Khanna, 1993)

The ability of a strain to compete is determined by several factors which include the following: superior performance by differences in the growth rates of competing strains, motility, chemotactic differences, the ability to colonise the rhizosphere and resistance to environmental factors (Mulongoy *et al.*, 1990).

2.4 Mycorrhizae Symbiosis

Mycorrhizae literally mean, "fungus – roots " are mutualistic beneficial associations between microscopic soil fungi and the plants whose roots they inhabit. They are critical components of the root-soil interface. This is because, they function as the absorptive organs of the plant, but they are also members of

the soil population. The spores and extra-metrical mycelia of mycorrhiza fungi thus constitute a fraction of the soil - borne fungal biomass (Harley & Smith, 1993).

Morphologically, different types of mycorrhizae are formed depending on the plant and fungal taxa involved in the association (Ganinazzi- Pearson, 1984). These are (i) Ectomycorrhizae, characterised by dense mycelia sheaths around the roots and intercellular fungal invasion of the roots cortex limited to about 3% of higher plants, and mostly confined to temperate forest trees; the fungi involved are mainly ascomycetes and basidiomycetes

(ii) Endomycorrhizae, in which the fungal partner forms a loose external hyphal network in the soil and grows extensively within cells of the root cortex, formed by nearly all other plants. The type of mycorrhizae which is by far the most ecologically and economically important is the vesicular-arbuscular endomycorrhizae formed by more than 80% of the plant species.

Mycorrhizal fungi are not a monolithic group, affecting plants only by their presence or absence, but highly variable organisms (Morton *et al*, 1990) that can elicit a variety of host responses (Stahl *et al,* 1990). One aspect of vesicular arbuscular mycorrhiza (VAM) which is of interest to both plant biology and agro-ecology is a broadening of the ecological niche of plants (Allen *et al*. 1984) through changes in the availability of nutrients. According to Morton and Benny (1990), VAM fungus is now classified as Arbuscular mycorrhiza (AM) fungi.

2.4.1. Arbuscular – Mycorrhizal (AM) Fungi

Arbuscular mycorrhiza symbiosis are the most widespread in the plant kingdom, commonly occuring in the bryophytes (mosses), the pteridophytes (ferns) and the gymnosperms as well as angiosperms and occurs in most plant species in most communities. (Suzonne *et al*., 1990, Torrey, 1992).

Currently, about 140 AM fungi species are recognised (Morton and Benny, 1990) in six zygomycetous genera. Many have a cosmopolitan distribution and they display little host specificity (McGonigle and Fitter, 1990).

AM species are also confined to the order Glomales, comprising two suborders (Glomineae and Gigasporineae). The Glomineae (including families of Acaulosporaceae and Glomaneae) are vesicular-arbuscular fungi while Gigasporineae with one family Gigasporoceae are Arbuscular mycorrhizal fungi as they do not form vesicles (Morton and Benny, 1990; Walker, 1992).

The extent to which a root system may be colonised by a fungal symbionts is modified by a variety of environmental factors, many of which apparently exert their influence through the effects on the physiology of the host plant, (Suzonne *et al*.., 1990). Most species or strains of AM fungi will infect any AM host, but the degree of infection and the physiological effect can vary in different host and endophyte combinations.

Although, AM fungi are not host specific, there are important quantitative differences in colonisation and host response among AM species and isolates. These differences in mycorrhizal effectiveness within a genus, species and isolates are dependent on the physiological characteristics of the fungus, the amount and distribution of mycelium in the soil and the interactions between the fungus and its environment (Mosse, 1977). When comparing the effectiveness of AM fungal strains, it is important to know their inoculum potential. Habte and Aziz (1985) compared the effectivity of *G. fasciculatum* and *G. mosseae* on *Sesbania grandiflora* in sterile and non-sterilise soils. They observed a 6-fold increase in nodule dry weight, a 9-fold increase in shoot, and a 15-fold increase in root dry weight due to the inoculation of the sterilised soil with *G. fasciculatum*.

Interestingly, mycorrhizal colonization of roots and growth of *S. grandiflora* were curtailed severely when the soil was infested with *G. mosseae*.

2.4.2 Infection Process of Arbuscular - Mycorrhizal (AM) Fungi.

The infection process of AM fungi originates from fungal propagules in the soil (Harley and Smith, 1993). The process is started by the germination and development of propagules of the fungi living in the proximity to the feeder roots of the host. It then releases certain substances that produce a remote and selective stimulation of the tentative micro-symbionts (Barea and Azcon-Aquilar, 1993). Physical and chemical properties of the soil are the two main factors that determine spore germination as well as the primary growth of the germ tube (Sieverding, 1991). Hyphae may enter roots via root hairs or more commonly between epidermal cells (Harley and Smith, 1983) resulting in a swollen structure called appresorium. Hyphae spread intracellularly along the cortical cells and become intracellular in the second layer of the cortical cells but without passing beyond the endodermis into the meristems. The growing hyphae develop into arbuscles within the inner cortex. This consists of a dense cluster of very fine dichotomously branched filaments, which may occupy the entire lumen of the cell. These finely branched hyphae are surrounded by the plasmalemma, which provides an extensive area of contact for nutrient exchange between fungus and the host cell protoplasm (Allen *et al.*, 1991).

At the time of Arbuscular formation, vesicles are developed, normally in the middle and outer cortex, and appear as terminal swellings either within cells or main intercellular position. Vesicles are generally regarded as temporary storage organs containing lipids, which supply the fungus with metabolites when the host

plant is stressed, and supply to the fungus is reduced (Harley and Smith, 1983; Barea and Azcon-Aquilar, 1983).

2.4.3 Physiology of Arbuscular-Mycorrhiza (AM) Fungi

Plants can greatly benefit from mycorrhizal associations, and their major role is to produce access to key growth limiting nutrients at crucial stages in plants development (Read, 1991). Moreover, the introduction of an appropriate mycorrhiza forming fungi usually alleviates stress conditions, which leads to enhanced plant growth (Ganinazzi, 1982). The presence of the fungi in the roots increases the volume of soil from which nutrients can be absorbed, by virtue of fungal structures such as mycelia. The amplitude of this phenomenon, called "mycorrhiza dependency" is based on the degree to which a plant relies upon to mycorrhizal conditions to enhance its maximum growth or yield at a given level of soil fertility. This can vary greatly between plant species and even cultivars.

According to Harley and Smith (1983), mycorrhizal plants can absorb and accumulate several times more phosphate than non-mycorrhizal plants from soils or solutions because of their greater uptake rates per unit amount of root. One consequence of this greater efficiency of mycorrhizal root system is an alteration in the partitioning of the plant biomass between root and shoot; thus in mycorrhizal plants, more shoot material per unit mass of root is produced, so that root / shoot ratios are often lower in mycorrhizal than in non-mycorrhizal plants.

It is now well established that, the mycorrhizal fungi play a central role in this greater efficiency of phosphate absorption by mycorrhizal plants. The ability of the fungus to absorb nutrients may be closely linked to the development of the external hyphae. The external hyphae of AM fungi are able to affect phosphate absorption

beyond the depletion zone up to approximately 10cm from the root (*Li et al..*, 1991a). Furthermore, the external fungal structures (hyphae, rhisomorphs) developing in the soil can absorb phosphate and transport it over large distances into the root tissues (Finley and Read, 1986). In this way, the external fungal medium increases the number of sites for absorption and allows the root to explore a much greater volume. Gianinazzi, (1986) also reported that, mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and then release it to plant cells of well defined sites in the root tissues.

AM fungi inoculation has been found to improve fertiliser utilisation in oil palm. On this basis, it has been suggested that, in an acid or phosphorus fixing soils, AM fungi can help improve the efficiency of the use of both superphosphate and rock phosphate (Li *et al.*, 1991).

The ability of *Senna siamea* to achieve such high leaf nitrogen contents on the same soil is due to its heavy VAM infection. The same applies to the soil-improving tree of West Africa, *Parkia biglobosa*, which was observed to have no root nodules but prolific endomycorrhiza infection (Tomlinson *et al.*, 1995). Trials conducted in Nigeria using *L. leucocephala* and *Gliricidia sepium* intercropped with cassava indicated a higher uptake of nitrogen and phosphorus in AM fungi inoculated trees than uninoculated trees, and plant growth strongly correlated with phosphorus uptake (Osonubi *et al.*, 1995).

Mycorrhiza infection may also decrease resistance to water transport, thus mycorrhizal plants recover faster from water stress than do non-mycorrhizal one's (Nelson, 1987). Mycorrhizal infection may help plants withstand root diseases either by protecting the root system against pathogen attack or compensating for root damage (Sieverding, 1991).

2.5 Factors Affecting AM fungi Symbiosis.

Some of the factors known to influence mycorrhizal symbiosis in Nitrogen – fixing trees include the following:

2.51 Soil Phosphorus

Soil Phosphorus (P) is one of the essential nutrients for plant growth (Brady, 1988). It is one of the important plant mineral nutrient after nitrogen found to be deficient in most tropical soils (Lynch, 1990). It functions in growth and metabolism of plants (Rufty *et al*, 1991), and its deficiency leads to general reduction of most metabolic processes, including cell division and expansion, respiration and photosynthesis (Marschner, 1986). According to Mengel and Kirkby (1982), the availability of Phosphorus to plants is determined by the ionic form of this element. The ionic form is also determined by the pH of the solution in which the ion is found. The maximum Phosphate available to plant is obtained when the soil pH is maintained in the range 6.0 – 7.0. The deficiency of the compound is often difficult to correct agronomically because Phosphorus binds to several soil constituents in forms that are limited to plants (Fixen and Grove, 1990).

Phosphorus absorption is usually related to the plants ability to acquire maximum amounts of phosphorus for minimum investment in root growth, and the efficiency of utilisation is the relative ability to produce biomass for each unit of P accumulated (Elliott and Lauchli, 1985). However, phosphorus absorption and assimilation vary among and within plant species (Sanginga, 1992, Gourley *et al*, 1993) but its uptake is influence by pH (Marschner, 1986) and nitrogen availability in the soil (Sylvia and Neal, 1990).

In general, leguminous plants have a naturally higher potential for assimilating P due to their high requirement of P for nodulation and nitrogen

fixation (Israel, 1987). Phosphorus supply has been found to influence both plant growth (Israel, 1987; Sanginga *et al.*,1991a) and nodule formation and functioning (Israel,1987). However, there are different reports on whether P increases symbiotic nitrogen fixation by stimulating host plant growth or by exerting a specific effect on nodule development and function. Jakobsen (1985), observed that nodule dry weight and specific C_2H_4 reduction in young pea plants responded to P additions before any growth response could be observed, indicating a direct effect of P on nodule growth and functioning. The higher P supply increased the number of leaves, leaf area and dry weight of all plant parts particularly nodule dry weight (Aranjo, 1996).

Although, Phosphorus status of soils has been shown to affect mycorrhizal symbiosis in plants, Stamford *et al,* (1996), reported that, P-fertilizer-mycorrhizal inoculation interactions are variable depending on species and soil conditions. For instance, high soil P has been reported to inhibit AM fungal infection (Miranda & Harris, 1994). Paulino *et al,* (1986) also observed limited effectiveness of mycorrhizal root infection on various tropical legumes when plants were growing in unsterilised soils, but Manjunath *et al*, (1989) described a positive effect of AM inoculation on *L. leucocephala*. Similarly, Copper (1984) suggested that, mycorrhiza enhance P uptake resulting in increased plant growth. This might result from a better greater length of absorbing surface such as greater length of root & fungal hyphae, higher P inflow rates among others (Jackobson, 1991).

Phosphorus limits AM formation by reducing the number of fungal entry points per root length (Amijee *et al.* 1989). Layman (1986), observed that, mycorrhizal infection decreased with increasing addition of P, but the rate of root colonization and the infection plateau varied with the endopyhtes. Similarly, Pearson *et al.* (1994), noted that the percentage and total colonisation by *Glomus*

spp was reduced to much greater extent than *Scutellospora calispora* when the soil P status was increased in subterranean Clover. Graham *et al*, (1981) also demonstrated that, there is an increased in membrane permeability and exudation of amino acids and sugars leading to an increase in mycorrhizal infection, thus the roots of P – deficient plants inoculated for 5 weeks with *G. intraradices* were 52% infected, compared with 31% in moderated P plants, indicating that, the intensity of the mycorrhizal colonization was relatively inhibited by moderate P- plants. Moreover, roots of P-deficient plants are often thinner and have a higher specific root length (Anghnoni and Barber, 1980) and greater mycorrhizal colonization (Tinker *et al.*, 1980). Miranda *et al.* (1989) suggested that soil P could be a controlling factor in arbuscular mycorrhizal formation and that, this control might occur through an effect of soil phosphate on the growth of mycelium during the early stages of fungal development. However, some authors have reported that root colonization is more likely to be replaced by P content of the plant tissue rather than by soil P (Menge *et al*, 1978, Jasper *et al*, 1979).

Mengel and Kirkby (1982), observed that most crops do not take up more than 10 – 15% of the Phosphorus added in fertiliser during the year of its application. This is not only due to the tendency of the soil to fix the added Phosphorus but, also to the slow rate of the movement of this element into plant roots in the soil. However, soil Phosphorus is a critical factor affecting mycorrhizal symbiosis (Rajapakse *et al.*, 1989).

Habte and Manjunath (1987) observed changes in mycorrhizal activity and root colonization in *Leuceana leucocephala* as the soil – solution phosphorus level was modified. Yost and Fox (1979) concluded that, the soil-solution phosphorus level for maximum colonisation of *L. leucocephala* ranged from 0.012 – 0.025mgl^{-1}, a level comparable to that determined by Habte and Mangunath (1987). Therefore,

optimum levels of soil-solution phosphorus need to be determined for mycorrhizal symbiosis in other nitrogen–fixing trees (NFTs).

Phosphorus interacts with soil Potassium and Calcium affecting the growth of mycorrhizal plants (Purcino *et al*.., 1986). Thus, nutrients other than Phosphorus may have important roles in determining mycorrhizal effectiveness in NFTs. According to Sylvia and Neal (1990), the ratio between soil phosphorus and nitrogen is important in determining mycorrhizal activity in some plants; however, no such information is available on NFTs.

Seedlings raised in nurseries are generally supplied with large quantities of N and P to high concentrations of available soil P have shown to decrease the proportion of root length colonised by AM fungi (Onguene and Habte, 1995)

2.5.2 Soil Nitrogen

According to Steven (1982), nitrogen is one of the most important nutrients for plant growth and yield. It is found in the atmosphere, in plant leaf and litter. Its concentration in a given soil depends on soil type and the amount of organic matter content present in the soil.

Baethgen and Alley (1987), reported that much of the nitrogen added to the soil undergoes much transformation before it is removed by the growing plants. Accordingly, the Nitrogen is subjected to simplification; first to simple amino compounds; then to ammonium ions; and finally to nitrate ions. Higher plants are able to use these forms of nitrogen for their growth and yield. Nitrogen in available form for plant uptake is subjected to leaching and volatilisation (Hauck, 1984) which result in the reduction of the amount of nitrogen uptake by plants.

However, mycorrhiza activity determined by the sub-leaflet-P technique increased in *L. leucocephala* when eroded soil with phosphorus level for AM

symbiosis was amended with 25um/g^{-1} Nitrogen (Aziz and Habte, 1989). The increase in mycorrhizal activity was also associated with increase in mycorrhizal colonisation of roots, dry weights of nodule, shoot, root and uptake of phosphorus and nitrogen. Moreover, the increases observed in the non-eroded soil, which had greater nitrogen content dramatic than those observed in the eroded soil, indicating nitrogen deficiency was limiting mycorrhizal effectiveness in the eroded soils. However, once the required amount of nitrogen has been added to the soil, to optimise AM fungi activity and nodulation, no additional benefit will be obtained with further addition of nitrogen. Moreover, root colonization by arbuscular mycorrhizal fungi is often suppressed by phosphorus additions, however nitrogen addition has been reported to both stimulate and suppressed root colonization (Sylvia and Neal, 1990), thus under N-limiting conditions, phosphorus additions had no effect on root colonization by *Glomus etunicatum* but increased root colonization by *Gigaspora margirata*.

2.5.3 Soil pH

Soil pH is an indicator of H_3O^+/H^+ ion activity present in a liquid phase of the soil (Van Ranst, 1989). Accordingly, a soil pH may vary from 2 (soil containing Sulphur) to 10 (soils containing alkaline salts). Between these extremes, the pH of most soils fall between 4 and 8. Brady (1988) reported that, the soil pH significantly influences other soil chemical properties as well as biological activities of organisms. Moreover, higher plants are known to respond significantly to soil pH, because it tends to control soil chemical environment. According to Nair (1984), soil pH significantly affects the availability of most of the chemical elements of importance to plants and microbes. For instance, the availability of Nitrogen is restricted at low pH values, whereas that of Phosphorus is best at intermediate pH

levels. He further reported that, under severe acidic soil conditions (pH less than 5.0), the aluminium ion becomes soluble in the soil, and is absorbed in preference to other cations by the soil colloids, and therefore becomes toxic to higher plants.

AM fungi are often adapted to a narrow range of soil pH. Both spore germination and root colonisation can be affected by soil pH. (Hayman and Tavares, 1985). Soil pH needs to be taken into consideration and adjusted, if necessary, before inoculating a soil with AM fungi. Haung *et al.*, (1983), studied the effect of three AM species on the growth of *L. leucocephala* at three pH levels and observed that, the best growth was attained when the soil was infested with *G. aggregatum* at a pH of 5.7. These results indicate some degree of pH endophyte specificity in NFTs, as has been observed in other plant species. Aziz and Habte (1989) reported that *L. leucocephala* grown in association with *G. aggregation* on a subsoil that was acidic had maximum root colonisation and dry matter yield when the soil pH was raised to 6.0. This isolate was sensitive to pH 6.5, as indicated by a decrease in root colonisation and plant growth. Fox *et al.*, (1985) also observed increased growth of *L. leucocephala* due to liming of two tropical soils which raised the pH from 4.8 to 7.0. An attempt to make *L. leucocephala* grow on a strongly acidic, aluminium rich soil through the agency of VAM was not successful, unless the soil was limed (Habte *et al.*, 1995).

Research to compare AM fungi infection of four MPTs (*Parkia roxburghii*, *Acacia farnesiana, A. nilotica and A. Senegal*), grown in soil of widely differing pH revealed that infection was greatest in high pH (8.5) soils and was absent from all species in soil from low pH (4.5) sites (Halliday, 1982).

2.5.4 Other Soil Chemical Factors

Organic matter can influence soil structure, pH nutrient, content and water holding capacity. All these may directly or indirectly affect AM fungi symbiosis. Hepper and Wanner (1983) noted an increase in mycorrhizal ineffectivity of soil and growth of mycorrhizal clover when the soil was amended with organic material. However, Aziz and Habte (1988), observed a depression in colonisation of roots by AM fungi when *L. leucocephala* was grown in a soil amended with high level of organic residue. The organic residue releases high ° level of manganese into the soil solution, which can be toxic to AM fungi (Hepper and Smith, 1976). Manganese toxicity could be important in tropical and sub-tropical soils where the rate of plant residue decomposition is high.

Since AM fungi have a role in increasing nodulation and nitrogen fixation in leguminous plants, Aziz and Habte (1988b) investigated the effect of molybdenum, an element essential for biological nitrogen fixation or AM fungi symbiosis. They reported that, when an eroded soil low in extractable molybdenum was amended with 4.4kg molybdenum ha^{-1}, there was an increase in AM colonisation in *L. leucocephala*. Nodulation and Nitrogen fixation of Nitrogen-fixing trees may be improved by adding molybdenum to a soil that is deficient in this nutrient.

2.5.5 Agricultural Chemicals / Pesticides

The influence of pesticides or agricultural chemicals on VAM symbiosis ranges from inhibitory to stimulatory, including some chemicals that have little or no effect on symbiosis (Dodd and Jeffriers, 1989). Aziz *et al.*, (1990) reported on inhibition of VAM symbiosis in *L. leucocephala* due to the application of the fungicide, chlorothalonil (tetrachloroisophthalo nitrile) to soil, i.e. at the level of 50mg/kg or above, the fungicide was deleterious to mycorrhizal symbiosis. An

experiment designed to investigate the residual effect of chlorothalonil reveals that toxicity persisted for 12½ weeks after application (Habte *et al.*, 1995).

2.5.6 Biological factors

2.5.6.1 Host Plant

Plants response to AM fungi colonisation depend greatly on the host species, cultivars or even genotypes with which the fungus is associated (Kristina *et al.*, 1985). Different plant species respond differently to mycorrhizal infection (Janos, 1980), for example, some show a high degree of "dependency" i.e. they do not grow in natural soil unless infected; others are highly responsive i.e. they can grow in the absence of infection but show much increased in growth when infected.

Mycorrhizal dependency of a plant is determined by its root morphology and internal Phosphorus requirement (St. John, 1980), thus roots with few or no root hairs) are more dependent on mycorrhiza than are plants with fibrous root system. The dependence of a plant on mycorrhiza is also determined by the symbiotic effectiveness of the fungus (Daniels *et al.*, 1981). This was supported by Borges and Chaney (1988), when they reported that mycorrhiza dependency of *Acacia scleroxyla* Tuss, varied from 505 to 938% when the plant was grown in association with different species of AM fungi.

2.6 Arbuscular-Mycorrhizal fungi and Nitrogen-Fixing Bacteria Association

Leguminous plant species of the sub family Papilionoideae and Mimosoideae, which usually develops nodule in association with *Rhizobium* are also characteristically colonised by AM fungi (Hayman, 1986). Both microsymbionts

are known to interact with one another and with the host legume (Barea and Azcon-Aquilar, 1983).

A range of soil bacteria possesses the ability to cycle nitrogen from the atmosphere to the biosphere by means of a nitrogen-fixation process. All of them (with the exception of *Sesbania* species, which also nodulate on stem) live either in the endorhizosphere in an intimate association with the root surface (rhizosphere). Therefore, most of them co-exist with AM fungi in the soil ecosystem (Azcon-Aquilar and Barea, 1991). Ecophysiological and biochemical approaches – have been develop to elucidate the basis of the AM fungus and *Rhizobium* interactions (Due *et al.*, 1989), which reveal that such interactions occurs (i) at the formation of the symbiosis (ii) at the level of the nutritional status of the plant and (iii) at the level of development and activity of the tripartite symbiosis, which is host-mediated. Experiment carried out in-vitro by Gonzatez (1988) showed that, *Rhizobium* species produced an enhancement of development of the mycelium emerging from surface sterilised spores of the AM fungi *G. mosseae* in co-culture. Furthermore, it has been shown that AM fungus and *Rhizobium* do not compete for infection sites and that both endophyte colonise simultaneously, except in cases where the photosynthetic rate is limiting (Bethlenfalvay *et al.*, 1985). However, under certain conditions AM fungi can change the pattern of distribution of nodules along the root system (Patterson *et al.*, 1990).

Because of the benefit of dual inoculation, researchers have often infested soil or seed with the appropriate *Rhizobium* spp prior to inoculation of nitrogen-fixing trees with AM fungi (Habte and Aziz, 1985; Manjunath, 1988). Ferrara–Cerrato and Villerias, (1984), reported that infestation of *Eysenthardtia polystachya* seeds with four strains of rhizobia (SLP – 1, CESAT – 35, CESAT – 39 and CP – 44) did not result in nodulation unless the seeds were also infested with AM

fungus. In another study, Aziz and Habte (1990a), inoculated seedlings of *S. grandiflora* with *G. aggregatum* and mixed a culture of *rhizobium* strains in rice cells and then transplanted the colonised seedlings to pots containing a phosphorus-fixing soils. They observed greatest plant growth when the soil was infested with both *rhizobium* and AM fungus and least when the soil received no inoculum. However, Gardezi *et al.*, (1988), found no response of *Acacia cyanophylla* Lindl to dual inoculation, which might be due to the incompatibility of the tested *Rhizobium* strain to the host plant. Moreover, Azcon-Aquilar *et al.* 1992 cited Powel that, only a fraction of a given AM fungus spore population inoculated into unsterilised soils is able to germinate. This is proof of the occurrence of a fungistasis against spore of AM fungi that can occasionally prevent germination.

CHAPTER THREE

3.0 MATERIALS & METHODS

3.1 Study Area

The study was located at the Kwame Nkrumah University of Science Technology, Institute of Renewable Natural Resources farm in Kumasi, Ghana at 06° 43° N in latitude, 01° 36° W longitude and 287.1m in altitude.

3.2 Soil Description

The soil in the area is locally classified under Bomso-Asuansi/Nta-Ofin compound associations developed over Cape Coast granites (Adu and Asiamah, 1992). Accordingly, the soil is classified as Ferric Acrisols developed over coarsely – quartzose biotite granodiorite (Adu and Asiamah, 1992). It is characterised by frequent to abundant content of large flakes of muscovites or white mica derived from veins of pegmatite. The soil is medium to coarse – texture, good structure and moderately gravely soils. Fairly high moisture holding capacity is associated with the soil series, although surface layers are subject to dryness during drought (Adu and Asiamah, 1992).

3.3 Climate

The study area falls within the equatorial climatic zone with rainfall regimes typical of the moist semi-deciduous forest zone of Ghana. The area has a bimodal rainfall, with an annual mean of 1298mm, and annual temperature of 26.6^{0}C. It is characterised by rainfall periods starting from mid March to July ending and a minor season from September to November. The peak fall is in June with dry Harmattan winds commencing from mid-November to mid-March.

The Kumasi area receives on the average 1488mm rainfall annually. Generally, temperatures are high during the main dry season and low during the wet seasons with the mean monthly values varying between 26.°C and 27°C.

3.4 Vegetation

The natural vegetation of Kumasi district falls within the semi-deciduous forest zone of Ghana characterised by *Celtis-Triplochiton* association. None of the original vegetation exists as a result of intensive cultivation farming practice. Large percentage of this vegetation has now turned into mosaic of fallow farmlands consisting of secondary forest.

The vegetation found in the experimental area is considered under forb regrowth category which is characterised by soft-stemmed leafy herbs (Ulzen – Appiah *et al.*, 1990).

3.5 Land Preparation

A relatively gentle slope land close to sources of water was selected at the Institute of Renewable Natural Resources research farm for the nursery, which covers about 132m^2. The land was cleared with cutlass and hoe and all the stumps uprooted using mattock and the soil levelled with the help of rake to ensure uniformity in the landscape. A shed was erected in an open space without any shading interference from trees and was at a height of approximately 2.5m and covered with palm fronds with optimum light penetration.

3.6. Nursery Conditions

Platforms were raised using bamboo under the shed for the polythene bags to rest on. This served as a preventive measure to prevent any contaminant moving from the soil into the polythene bags. Moreover, within each experimental set-up, enough spaces were kept between the rows in each block and from one block to the other to reduce the possibility of contamination by different inoculant strains. Weeds in the nursery were continuously and carefully removed with hoes, because it can also aid in the transfer of strains from one polythene bags to another.

3.7. Seed and Seed Germination

Seeds of *Albizia* species (*A. zygia, A. ferruginea and A. adianthifolia*) were collected from Kubeasi and Bobri forest reserves. Prior to germination, uniform seeds were nipped with nail cutter and surface sterilised with 3% sodium hypochlorite (NaOCl) solution for 3 minutes and rinsed several times in sterilised distilled water. Seeds were then pre-germinated on moist sterilised filter paper in petri-dishes, placed in an incubator at a temperature of 25°C. Viable seeds germinated within 48hours. Germinated seeds with radical length of 2 – 3 cm were planted in polythene bags containing the growth medium (sandy loam soil).

3.8. Soil Sample Analysis

Before preparation of the growth medium, soil samples were collected for soil analysis. Samples were bulked and a representative sample of about 1kg was taken using the method described by Anderson and Ingram (1993), (at soil depth of 0 – 15cm). The representative soil samples were analysed for soil pH, Organic Carbon, total N, extractable cations (Ca, Mg, K, Na, Al, H in Cmol/kg), base

saturation, effective cations exchange capacity (ECEC), and available phosphorus. Organic matter and C/N ratio were computed from the result of the analysis.

The Properties of the soil sample for the experiment analysed are presented in Table 3.1

Chemical Properties of Soil Sample Analysed

Soil Properties		Soil Depth(cm) 0-15cm
pH (1:2.5) (H_2O)		5.9
Organic Carbon (%)		0.70
Organic Matter (%)		1.21
Total Nitrogen (%)		0.070
Available P (mg/kg)		13.4
Exchangeable Cations (cmol/kg)	Ca	2.6
	Mg	1.0
	K	0.17
	Na	0.13
	Al	0.2
	H	0.4
Effective CEC (cmol/kg)		4.50

3.9 Growth Medium and Container

Pre-germinated seeds were planted in top sandy loam soil (0 – 15cm) from IRNR research farm. The soil was sieved to remove all stones and plant parts before filling into black polythene bags. The polythene bags were filled to about 5cm from the brim for easy watering and to avoid spilling over. Moreover, perforations were made on the polythene bag up to half-length from below to enhance drainage.

3.10 Arbuscular Mycorrhizal (AM) Fungi Inoculum and Inoculation

Arbuscular mycorrhizal fungi used consisted of the following species; *Glomus etunicatum* (BR 149 – 3), *Glomus clarum* (BR 148 – 1), *Glomus intraradices* (UT 1148 – 2) and *Gigaspora rosea* (FL 105 – 5). Pure single isolate cultures in the form of whole inoculum (consisting of growth medium, roots and fungi propagules, spores, hyphae), were obtained from the International Collection of Arbuscular and Vesicular-Arbuscular mycorrhizal fungi (INVAM) centre, Division of Plant Sciences, West Virginia University, Morgan Town, 26505-6057, USA.

About 20g of the whole inoculum was spread halfway down each of the pot, and covered with soil before the pre-germinated seeds were sowed in the growth medium.

3.11 Plant Harvest

The inoculated seedlings were grown for 16 weeks. The harvested plants were separated into shoot, root and nodules using destructive method. The shoot portion was cut off, weighed and put in a paper enveloped for drying. The root system were gently shaken and washed under running tap water. Nodules were removed, counted, weighed and put in a separate small envelope. Some portion of the root required for mycorrhizal assessment were randomly sampled and stored in 50% alcohol at 4°C for later assessment. The remaining roots were also weighed. Plant parts (shoots, roots, and nodules) were dried to a constant weight at 70°C for 3 – 4 days. The dry weight of the total root system were estimated from the fresh and dry weights of the "remainder" roots and the fresh weight of the sample for AM fungi assessment.

3.12 Plant Chemical Analysis

Plant samples for chemical analysis were oven dried at 70°C for 72 hours, ground in a Thomas grinding mill and used for chemical analysis.

3.12.1 Total Nitrogen Content

The total N content was determined by the Macro- Kjeldahl digestion method (Thomas, 1989). In this method, 2g of soil sample were digested by boiling with concentrated sulphuric acid and catalyst. The nitrogen in the sample was converted into ammonium, which was treated with excess caustic soda. The ammonia liberated was distilled over and collected in boric acid. The nitrogen in the boric acid was determined by titration with standard HCl.

3.12.2 Total P, K, Ca, and Mg Contents

The ground sample was ashed at 450°C in a furnace for about 6 hours. The ash was taken up in 2M HCl, digested, filtered and the content of P, K, Ca, and Mg determined.

Available Phosphorus was determined using the Bray P 1 extractant method (Thomas, 1982). Calorimetric determination of phosphorus based on the measurement of Percent transmission at 520nm wavelength was done using a Bausch and Lomb spectronic – 20 spectrophotometer.

Exchangeable cations were determined after extraction with 1.0N ammonium acetate solution at pH 7.0 (Thomas, 1982). Potassium and Sodium were also determined using Flame Photometer.

Effective Cation Exchange Capacity (ECEC): This was calculated from the sum of all the exchangeable bases (Ca, Mg, K, Na).

3.13 Mycorrhizal Assessment

Mycorrhizal fungal infection was assessed by clearing and staining methods described by Phillips & Hayman, (1970) incorporating modifications from Koske and Gemma, (1989). Percentage colonisation was estimated using the gridline intersect method (Giovanneti & Mosse, 1980).

3.13.1 Root Sampling, Clearing and Staining

Root stored in 50% alcohol were cut into 1cm segments and spread in a petri-dish whose base has 50 dots randomly marked. One hundred pieces were selected, rinsed several times in tap water to remove alcohol bases and then covered with 2.5% potassium hydroxide (KOH). Roots in KOH were autoclaved at 121°C at 101.3kPa for 3 minutes. After cooling, the roots were rinsed thoroughly under running tap water until no more brown colouring appears in the rinsing water. Roots were bleached with freshly prepared alkaline hydrogen peroxide ($3cm^3$ of ammonia in $30cm^3$ of 3% hydrogen peroxide) for 30 minutes at room temperature and then the roots were rinsed well under running tap water. Roots were acidified by soaking in 1% HCl for an hour after which they were drained stained in acidic glycerol ($500cm^3$ glycerol, $50cm^3$ 1% HCl and $450cm^3$ H_2O) containing 0.05% trypan blue and autoclaved at 121°C at 15psi for 3 minutes. The stained roots were drained of trypan blue solution roots stored in acidic glycerol till assessment.

3.13.2 Infection Assessment

Stained roots were spread over the base of a Petri-dish so that no root overlap another and placed in another Petri-dish marked with a 1.2 x 1.2 cm grid underneath. Using a stereo microscope vertical & horizontal grid lines were scanned and the presence & absence of infections (fungal structures – vesicles,

arbuscles or hyphae) were recorded at each point when the roots intersect a line. The percentage infections was calculated by dividing the number of infected points by total number of points (infected and uninfected) and by multiplying by 100.

3.14 Experimental Design

The objective of experiment 1 was to examine the effect of four (4) different AM fungi on the early growth and nodulation of *Albizia zygia*, A. *adianthifolia and A. ferruginea* (in unsterilised and sterilised soil*).*

The design was randomised complete block with six (6) treatments. Four (4) AM fungi strains were imposed on each of the three (3) *Albizia* species with two controls (sterilised uninoculated and unsterilised uninoculated soils). Treatments were replicated ten (10) times.

The details of the treatments and symbols used were as follows:

Su – Sterilised soil, uninoculated with AM fungus

Un - Unsterilised soil, uninoculated with AM fungus

Gc - Unsterilised soil inoculated with *Glomus Clarum*

Ge – Unsterilised soil inoculated with *Glomus etunicatum*

Gi – Unsterilised soil inoculated with *Glomus intraradices*

Gr – Unsterilised soil inoculated with *Gigaspora rosea*.

The *Albizia* species that will come out to be the most responsive to the fungal strains as well as the most efficient AM fungus will be used in the second experiment.

The objective of experiment 2 was to compare the interactive effect of levels of phosphorus (P) and Arbuscular Mycorrhiza fungus inoculation on early growth and nodulation of *Albizia ferruginea*.

The experimental design was 2 x 4 factorial design in four randomised blocks. For each block there was four (4) levels of phosphorus at 0, 50, 100 and 150kg/ha per pot (0, 100, 200, & 300mg of tripple-superphosphate) with inoculated and uninoculated AM fungus application. In each of the block, the treatments were replicated 10 times. There was a control treatment with no phosphorus.

Details of the treatments and symbols used were as follows:

U_0 – uninoculated soil with Okg/ha

U_{50} – uninoculated soil with 50kg/ha (100mgTSP)

U_{100} – uninoculated soil with 100kg/ha (200mgTSP)

U_{150} – uninoculated soil with 150kg/ha (300mgTSP)

I_0 – inoculated soil with Okg/ha

I_{50} – inoculated soil with 50kg/ha (100mgTSP)

I_{100} – inoculated soil with 100kg/ha (200mgTSP)

I_{150} – uninoculated soil with 150kg/ha (300mgTSP)

3.15 Data collection

Plants were harvested at 16 weeks after sowing and the following parameters were assessed for both experiments:

Shoot height

Shoot diameter

Number of nodules

Nodule dry weight

Shoot and Root biomass

Total plant biomass

Percentage mycorrhiza root infection

Plant chemical analysis (N, P, K, Ca, and Mg)

3.16 Data Analysis

Data were subjected to ANOVA using stagraphics statistical package, Version 5. Sigma plot was used in the calculations and graphics. Significant treatments were separated by Duncan's multiple range test.

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment 1

The experiment provided inter- and intraspecific comparisons for plant responses to different AM fungi strain inoculations.

4.1.1 Height

Plant growth assessed by height at 16 weeks after planting (WAS) indicated a general increase in height in all the three *Albizia* species (figure 1), with sterilised uninoculated treatment consistently showing superiority throughout the trial period.

In *A. adianthifolia*, plants inoculated with *Gigaspora rosea* recorded the highest growth in height (10.40cm/plant), but was not significantly different from those with *Glomus intraradices*. However, there was a significant difference between these two species and the other treatments, with *Glomus etunicatum* seedlings *having* the least mean growth in height (8.20cm) (figure 1a). Significant differences were also observed among treatments in *A. ferruginea* where plants inoculated with *Glomus clarum* achieved higher growth in height (16.7cm/plant), with unsterilised uninoculated treatment having the least mean height growth of 12.67cm (figure 1b). In *A. zygia*, plants inoculated with *Glomus etunicatum* recorded the highest mean growth in height (9.67cm), but differed significantly from the other treatments which were statistically similar. Plants inoculated with *Glomus intraradices* recorded the lowest mean growth in height (8.10cm) (figure 1c).

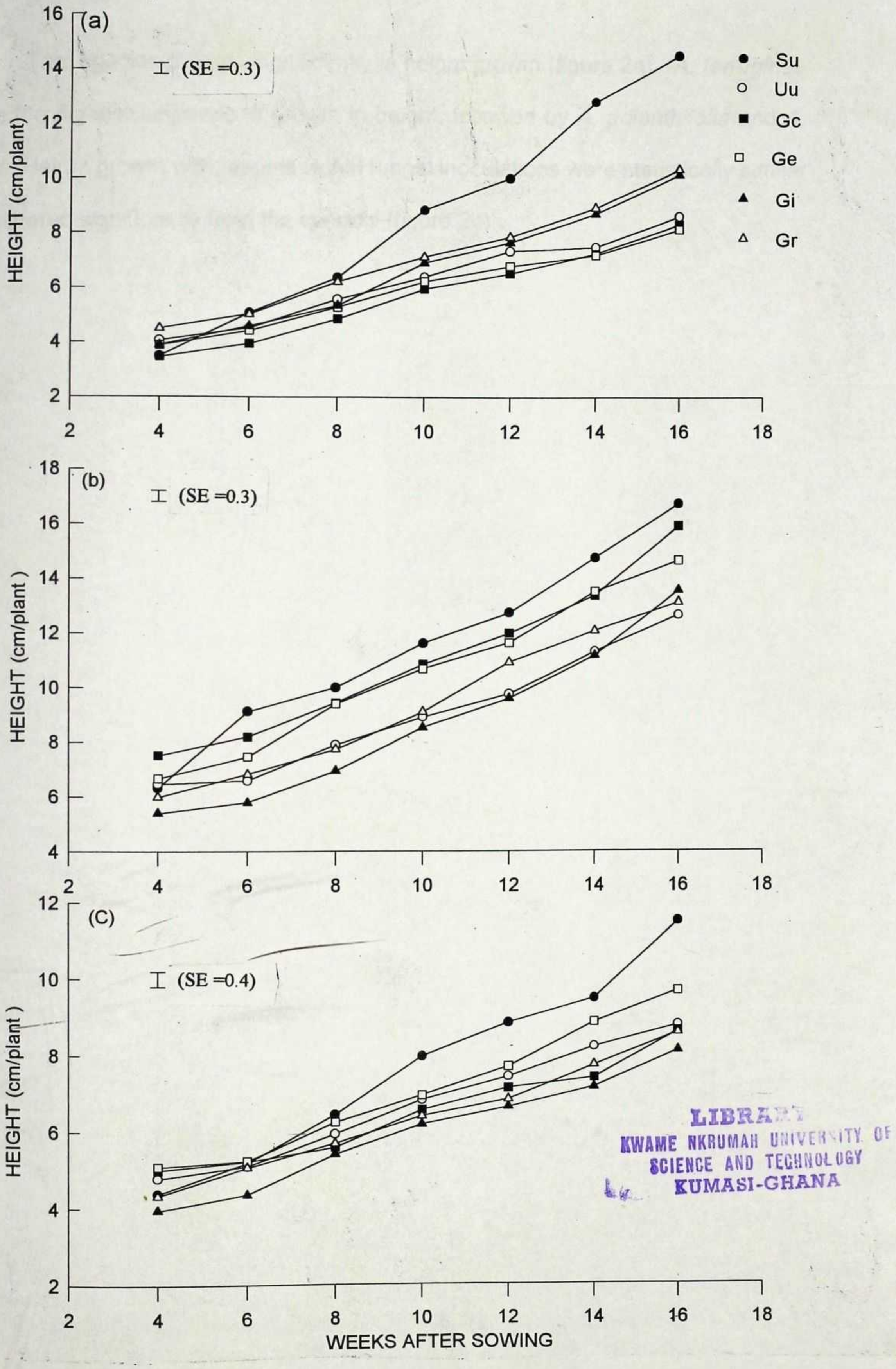


Figure 1. Height growth (cm) of three *Albizia* species (a) *A. adianthifolia* (b) *A. ferruginea* (c) *A. zygia* Data are means, and bar indicates standard errors, (n=7) and $p \leq 0.05$. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

Tree species differed significantly in height growth (figure 2a). *A. ferruginea* gave the highest response to growth in height, followed by *A. adianthifolia* and *A. zygia*. Height growth with respect to AM fungal inoculations were statistically similar but differed significantly from the controls (figure 2b).

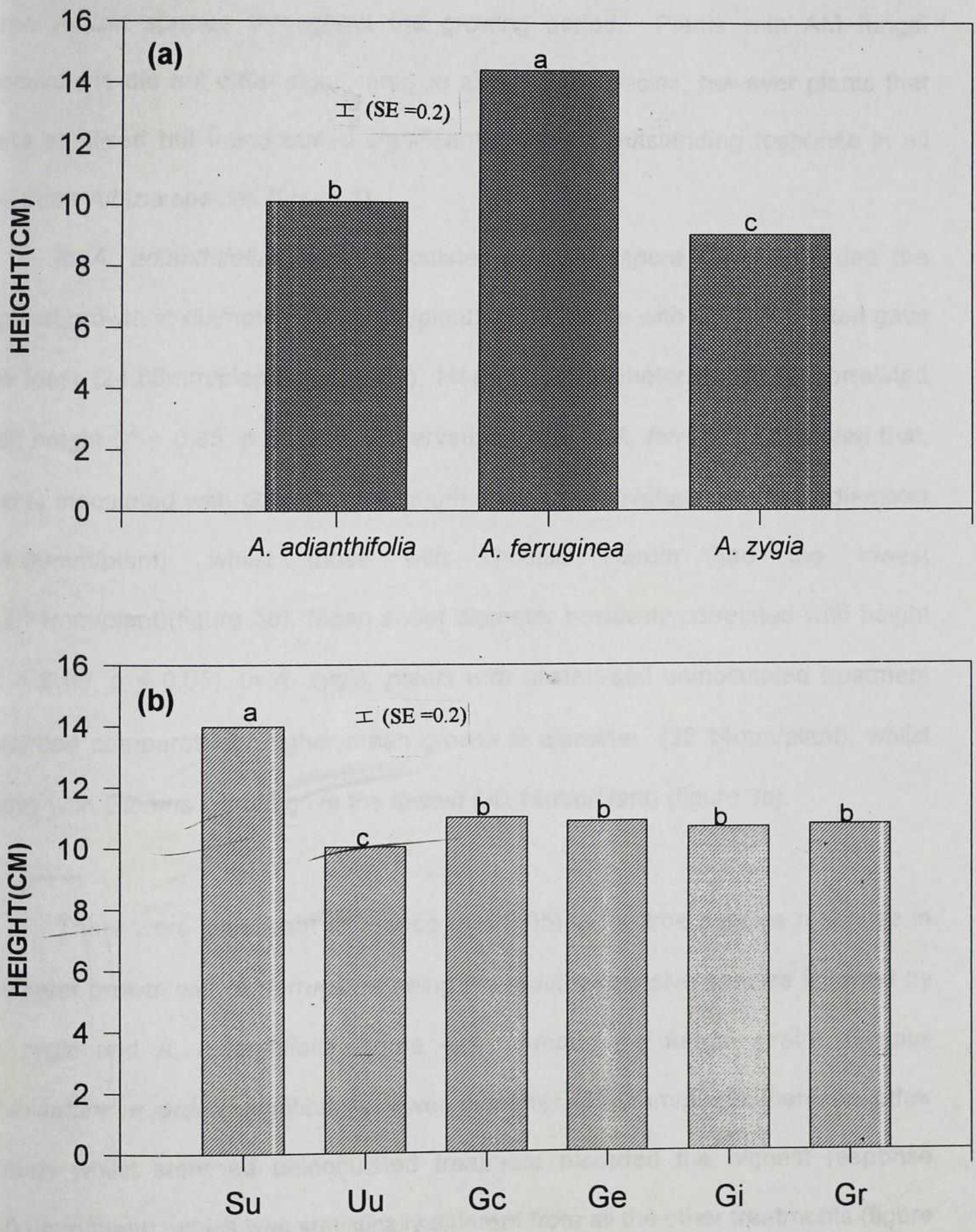


Figure 2. Height growth (cm) of three *Albizia* species in terms of (a) Species performance and (b) AM fungal inoculations. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

4.1.2 Diameter

Generally, there was a gradual increase in diameter growth of each of the three *Albizia* species throughout the growing period. · Plants with AM fungal · inoculations did not differ significantly in all the tree species, however plants that were sterilised but uninoculated significantly showed outstanding response in all the three *Albizia* species (figure 3).

In *A. adianthifolia*, plants inoculated with *Gigaspora rosea* recorded the highest growth in diameter (40.86mm/plant), whilst those with *Glomus clarum* gave the least (24.86mm/plant) (figure 3a). Mean shoot diameter positively correlated with height ($r^2 = 0.85$; $p \leq 0.05$). Observation made in *A. ferruginea* revealed that, plants inoculated with *Glomus etunicatum* attained the highest growth in diameter (44.00mm/plant), whilst those with *Glomus clarum* had the lowest (33.71mm/plant)(figure 3b). Mean shoot diameter positively correlated with height ($r^2 = 0.89$; $p \leq 0.05$). In *A. zygia*, *plants with* unsterilised uninoculated treatment recorded comparatively higher mean growth in diameter (32.14mm/plant), whilst those with *Glomus clarum* gave the lowest (30.14mm/plant) (figure 3c).

There were significant difference ($p \leq 0.05$) in the tree species response in diameter growth with *A. ferruginea* being the most responsive species followed by *A. zygia* and *A. adianthifolia* (figure 4a). Among the fungal strains *Glomus etunicatum recorded* significantly lower diameter (30.0mm/plant) than the other strains whilst sterilised uninoculated treatment recorded the highest response (40.0mm/plant), which was statistically different from all the other treatments (figure 4b).

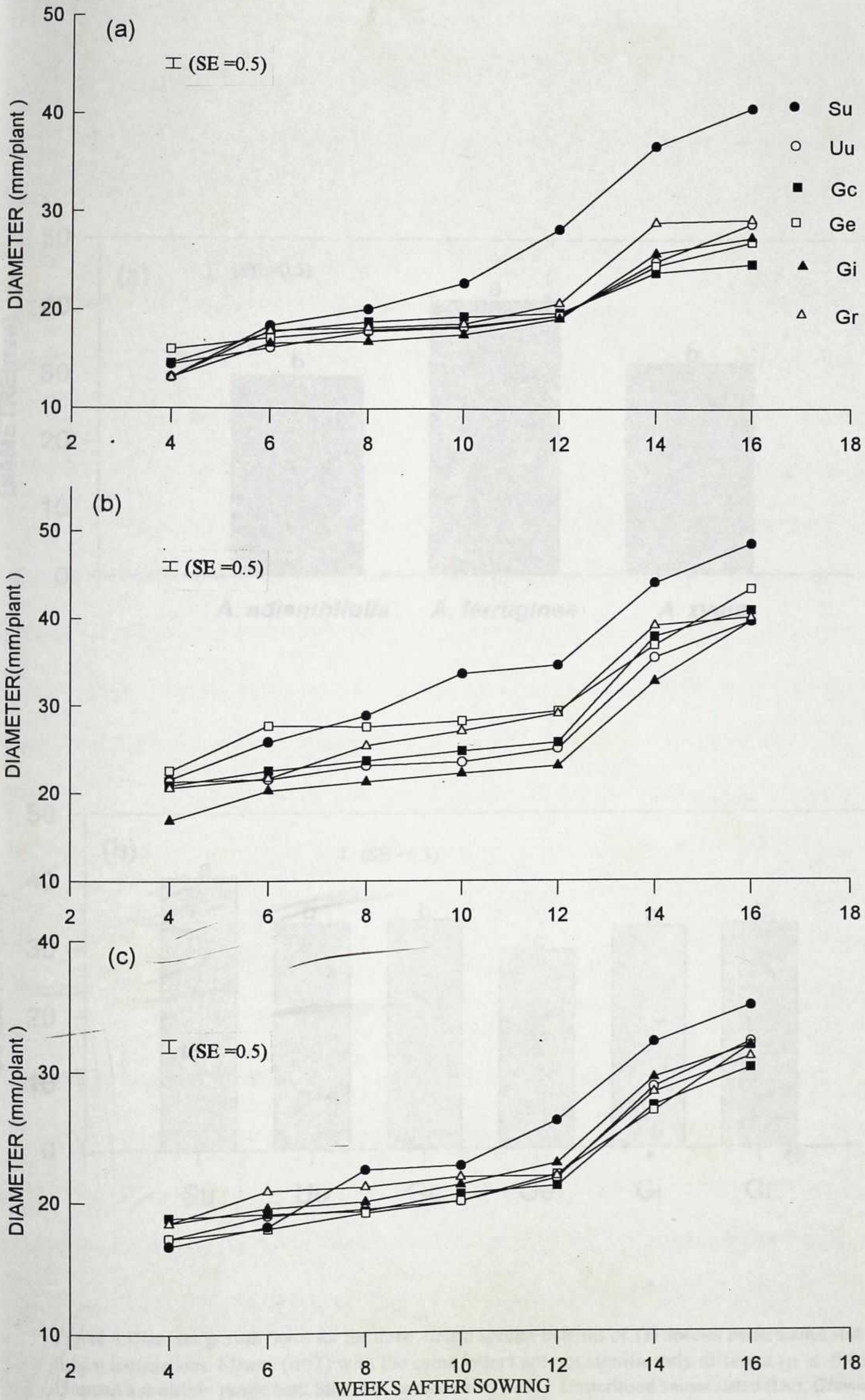
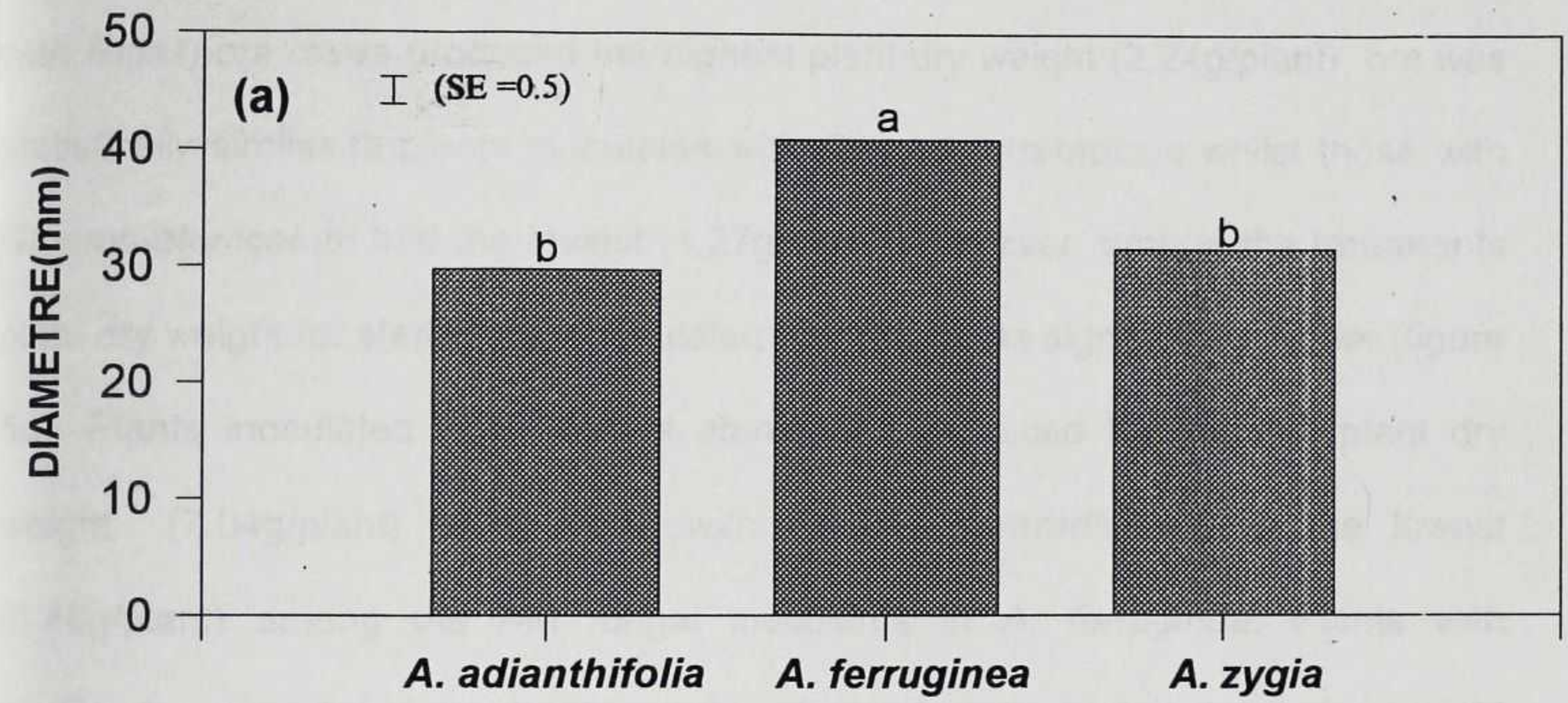


Figure 3. Diameter growth (mm) of three *Albizia* species (a) *A. adianthifolia* (b) *A.. ferruginea* (c) *A.. zygia.* Data are means, and bar indicates standard errors, (n=7) and $p \leq 0.05$. Sterilised ` uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).



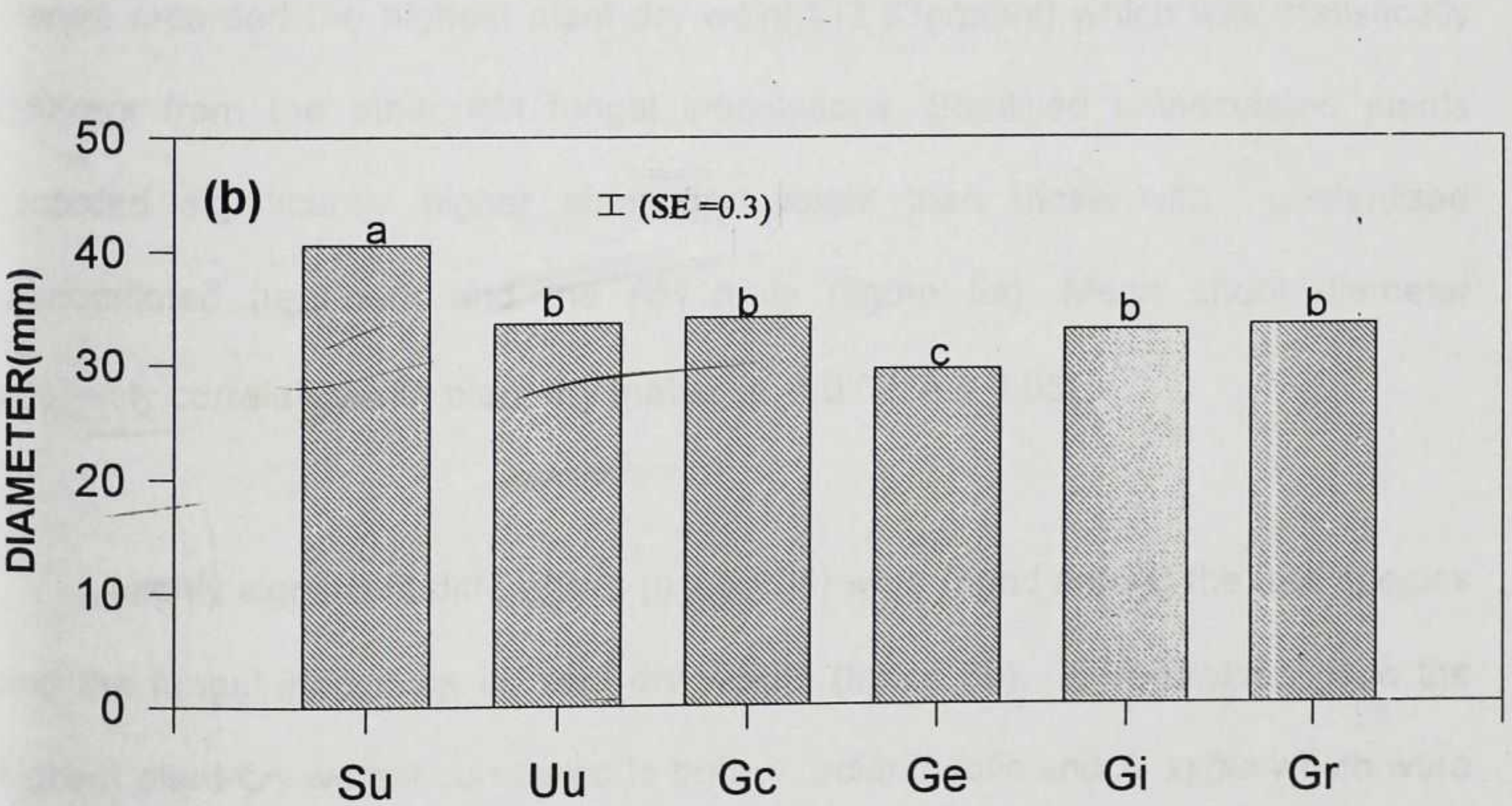


Figure 4.Diameter growth (mm) for the three *Albizia* species in terms of (a) Species performance and (b) AM fungal inoculations. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

4.1.3 Dry Weight

There was a highly significant difference ($p \leq 0.001$) between the treatments in each of the three *Albizia* species (figure 5a). In *A. adianthifolia*, plants inoculated with *Gigaspora rosea* produced the highest plant dry weight (2.24g/plant), but was statistically similar to plants inoculated with *Glomus intraradices* whilst those with *Glomus etunicatum* had the lowest (1.27g/plant). However, among the treatments plant dry weight for sterilised uninoculated treatment was significantly higher (figure 5a). Plants inoculated with *Glomus etunicatum* produced the highest plant dry weight (7.04g/plant) whilst those with *Glomus intraradices gave* the lowest (5.49g/plant) among the AM fungal inoculants in *A. ferruginea*. Plants with sterilised uninoculated treatment produced significantly higher plant dry weight than the other treatments (figure 5a). In *A. zygia*, plants inoculated with *Glomus clarum* recorded the highest plant dry weight (2.83g/plant) which was statistically different from the other AM fungal inoculations. Sterilised uninoculated plants recorded significantly higher plant dry weight than those with unsterilised uninoculated treatment and the AM fungi (figure 5a). Mean shoot diameter positively correlated with plant dry matter ($r^2 = 0.64$; $p \leq 0.05$).

Highly significant differences ($p \leq 0.001$) were found among the tree species and the fungal inoculants in plant dry weight (figure 5b). *A. ferruginea* gave the highest plant dry weight compared to both *A. adianthifolia* and *A. zygia* which were statistically similar (figure 5b). *Glomus clarum* recorded the highest plant dry weight (4.9g/plant), whilst *Glomus intraradices* the lowest (3.06)(figure 5b).

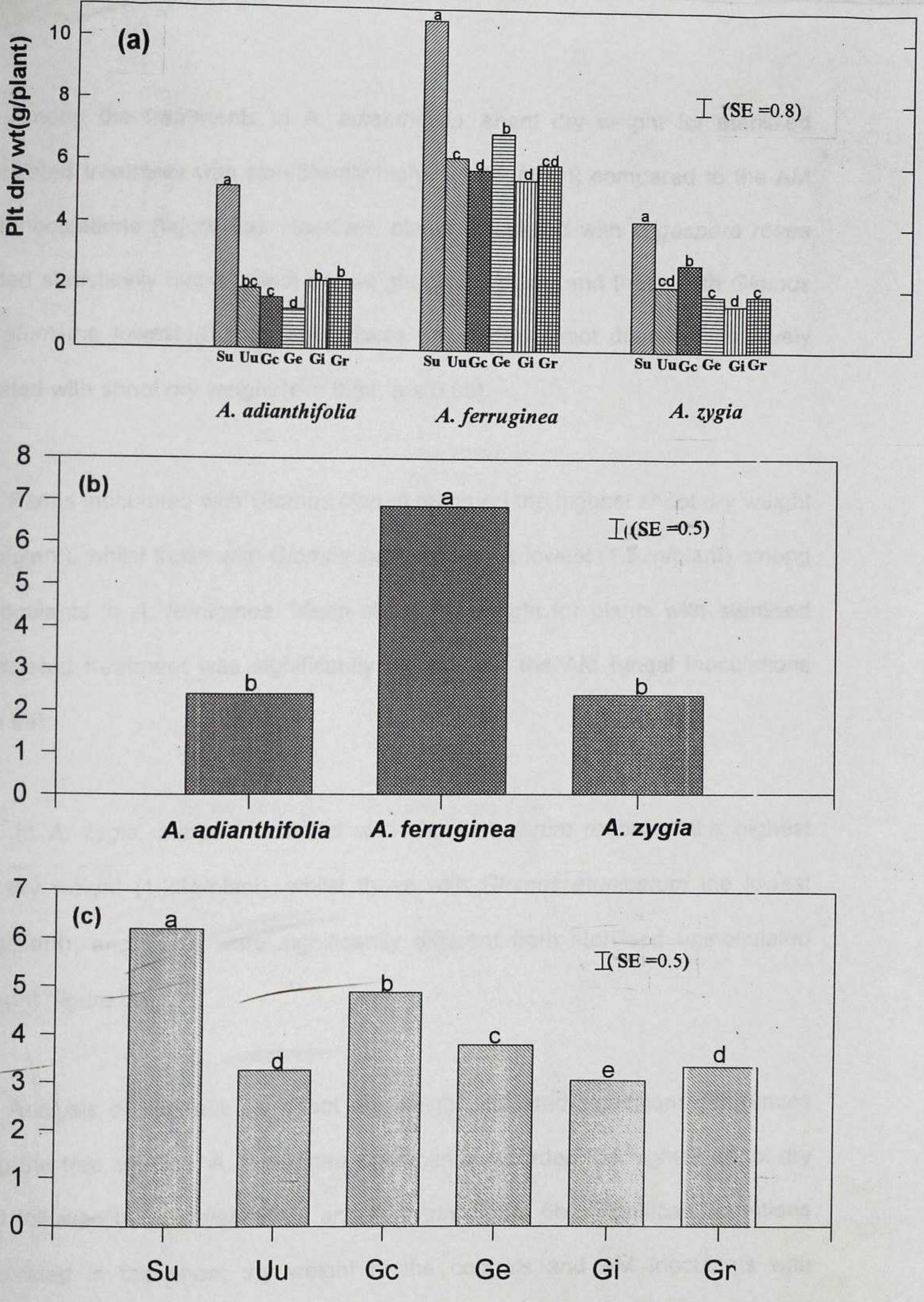


Figure 5. Plant dry weight (g/plant) of three *Albizia* species (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

Among the treatments in *A. adianthifolia*, shoot dry weight for sterilised uninoculated treatment was significantly higher (1.64g/plant) compared to the AM fungal inoculations (figure 6a). However, plants inoculated with *Gigaspora rosea* recorded statistically higher shoot dry weight (0.99g/plant) and those with *Glomus etunicatum* the lowest (0.38g/plant) (figure 6a). Mean shoot diameter positively correlated with shoot dry weight ($r^2 = 0.91$; $p \leq 0.05$).

Plants inoculated with *Glomus clarum* recorded the highest shoot dry weight (1.96g/plant), whilst those with *Glomus intraradices* the lowest (1.52g/plant) among the inoculants in *A. ferruginea*. Mean shoot dry weight for plants with sterilised uninoculated treatment was significantly higher than the AM fungal inoculations (figure 6a).

In *A. zygia*, plants inoculated with *Glomus clarum* recorded the highest shoot dry weight (1.23g/plant), whilst those with *Glomus etunicatum* the lowest (0.48g/plant), and these were significantly different from sterilised uninoculated treatment (figure 6a).

Analysis of variance for shoot dry weight indicated significant differences among the tree species. A. *ferruginea* significantly recorded the highest shoot dry weight followed by A. *adianthifolia* and *A. zygia* (figure 6b). Significant variations also existed in the shoot dry weight of the controls and AM inoculants with sterilised uninoculated treatment showing its superiority (figure 6c). In the inoculated plants, *Glomus clarum* recorded the highest (1.27g/plant) and *Glomus intraradices* the lowest (0.93g/plant).

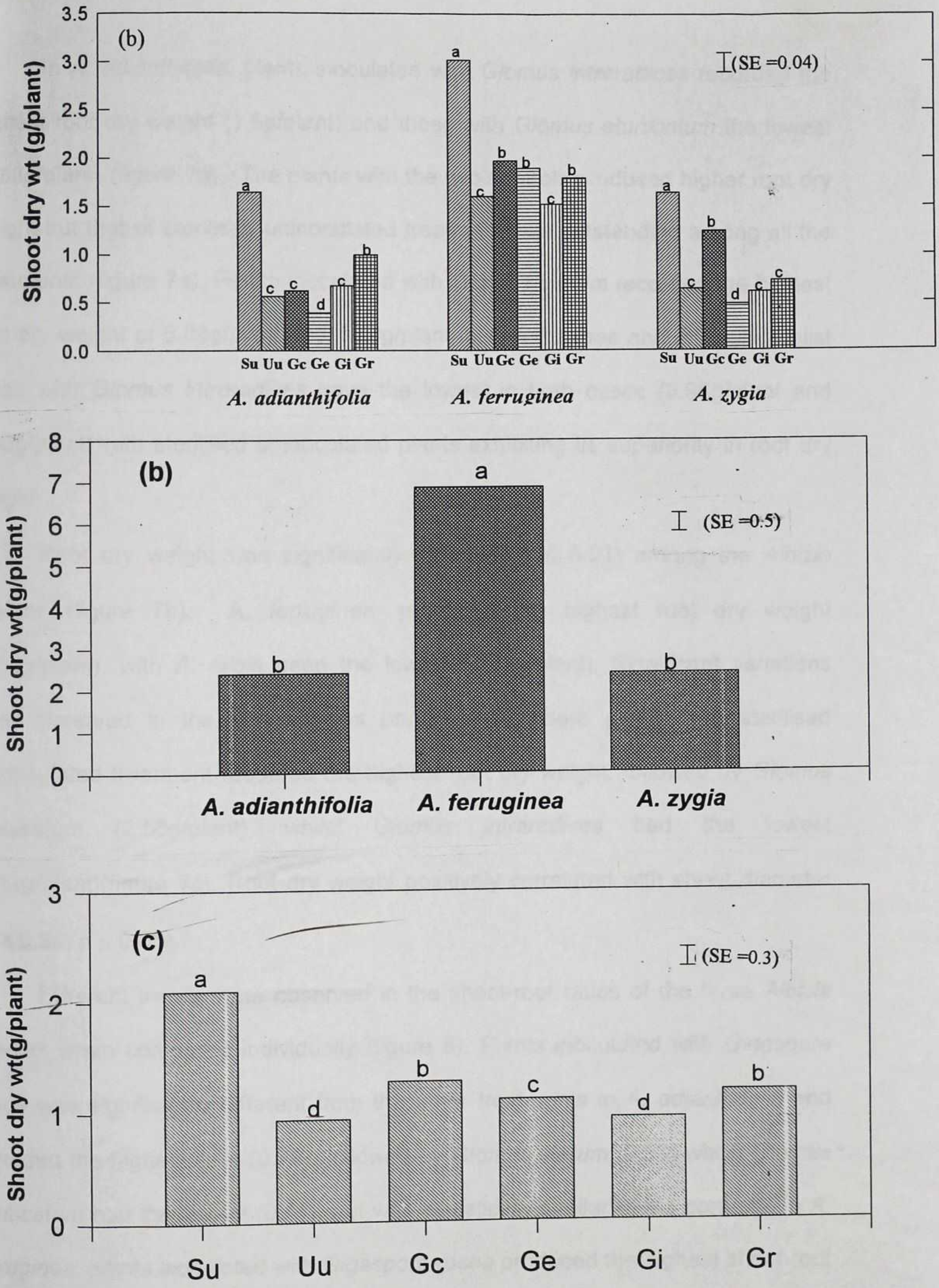


Figure 6. Shoot dry weight (g/plant) of three *Albizia* species (a) Inoculation with different AM fungus (b) Tree species performance c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

In *A. adianthifolia*, plants inoculated with *Glomus intraradices* recorded the highest root dry weight (1.5g/plant) and those with *Glomus etunicatum* the lowest (0.88g/plant) (figure 7a). The plants with the two controls produced higher root dry weight but that of sterilised uninoculated treatment was outstanding among all the treatments (figure 7a). Plants inoculated with *Glomus clarum* recorded the highest root dry weight of 5.08g/plant and 1.59g/plant in *A. ferruginea* and *A. zygia*, whilst those with *Glomus intraradices* gave the lowest in both cases (3.98g/plant and 0.89g/plant) with sterilised uninoculated plants exhibiting its superiority in root dry weight.

Root dry weight was significantly different ($p \leq 0.01$) among the *Albizia* species (figure 7b). A. *ferruginea*, produced the highest root dry weight (4.89g/plant), with *A. zygia* been the lowest (1.42g/plant). Significant variations were observed in the tree species performance where plants with sterilised uninoculated treatment recorded the highest root dry weight, followed by *Glomus etunicatum* (2.56g/plant), whilst *Glomus intraradices* had the lowest (2.12g/plant)(figure 7c). Root dry weight positively correlated with shoot diameter ($r^2 = 0.94$; $p \leq 0.05$).

Different trends were observed in the shoot-root ratios of the three *Albizia* species when compared individually (figure 8). Plants inoculated with *Gigaspora rosea* was significantly different from the other treatments in *A. adianthifolia* and recorded the highest ratio (0.79), followed by *Glomus clarum* (0.59) whilst *Glomus etunicatum* had the lowest (0.41), but was statistically similar to the controls. In *A. ferruginea*, plants inoculated with *Gigaspora rosea* produced the highest shoot-root ratio (0.43) which was statistically similar to those with *Glomus etunicatum* whist that of *Glomus intraradices* been the lowest (0.38), but similar to the controls statistically. In *A. zygia*, plants inoculated with *Glomus clarum* recorded the highest

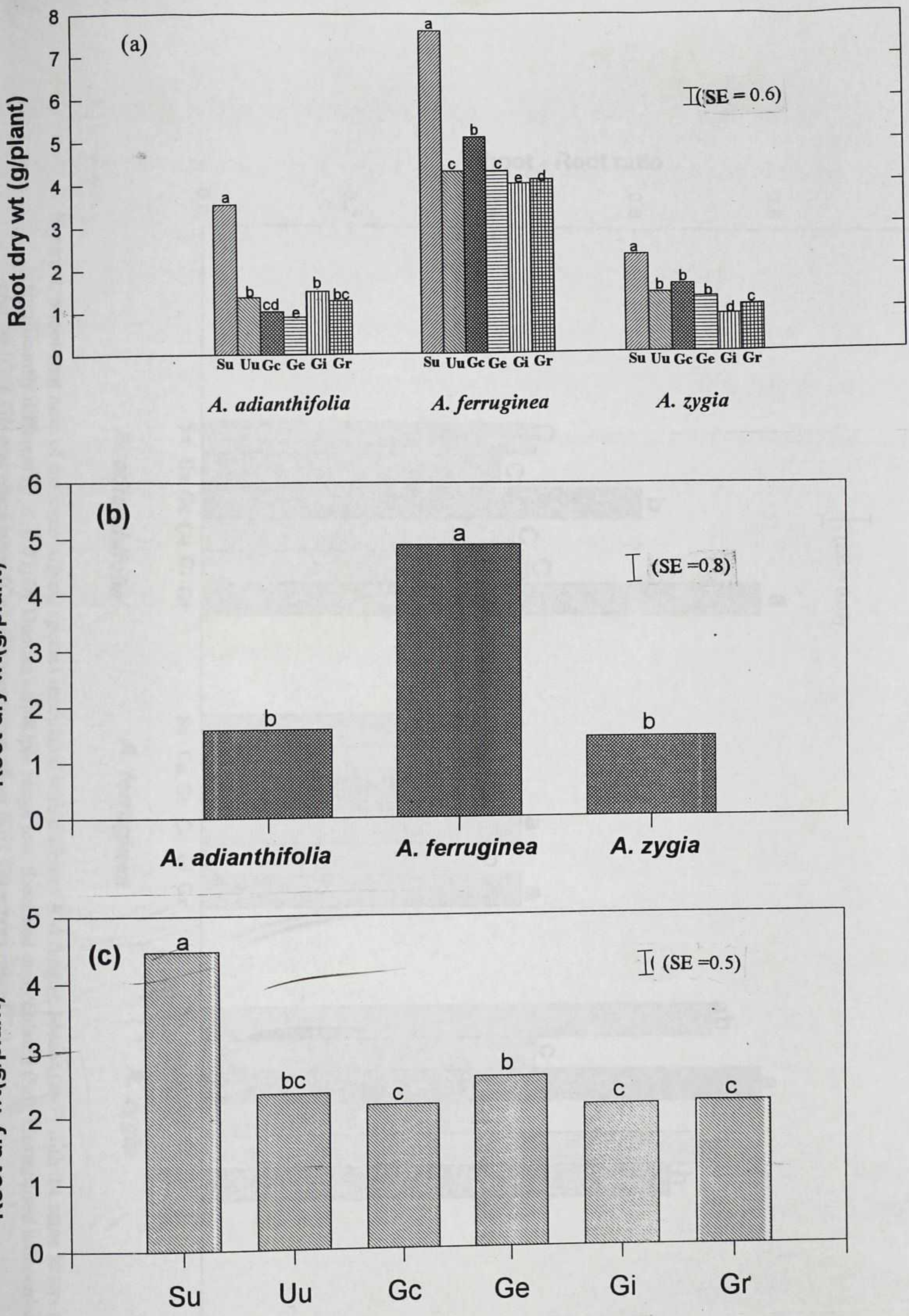


Figure 7. Root dry weight (g/plant) of three *Albizia* species (a) Inoculation with different AM fungus (b) Tree species performance c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

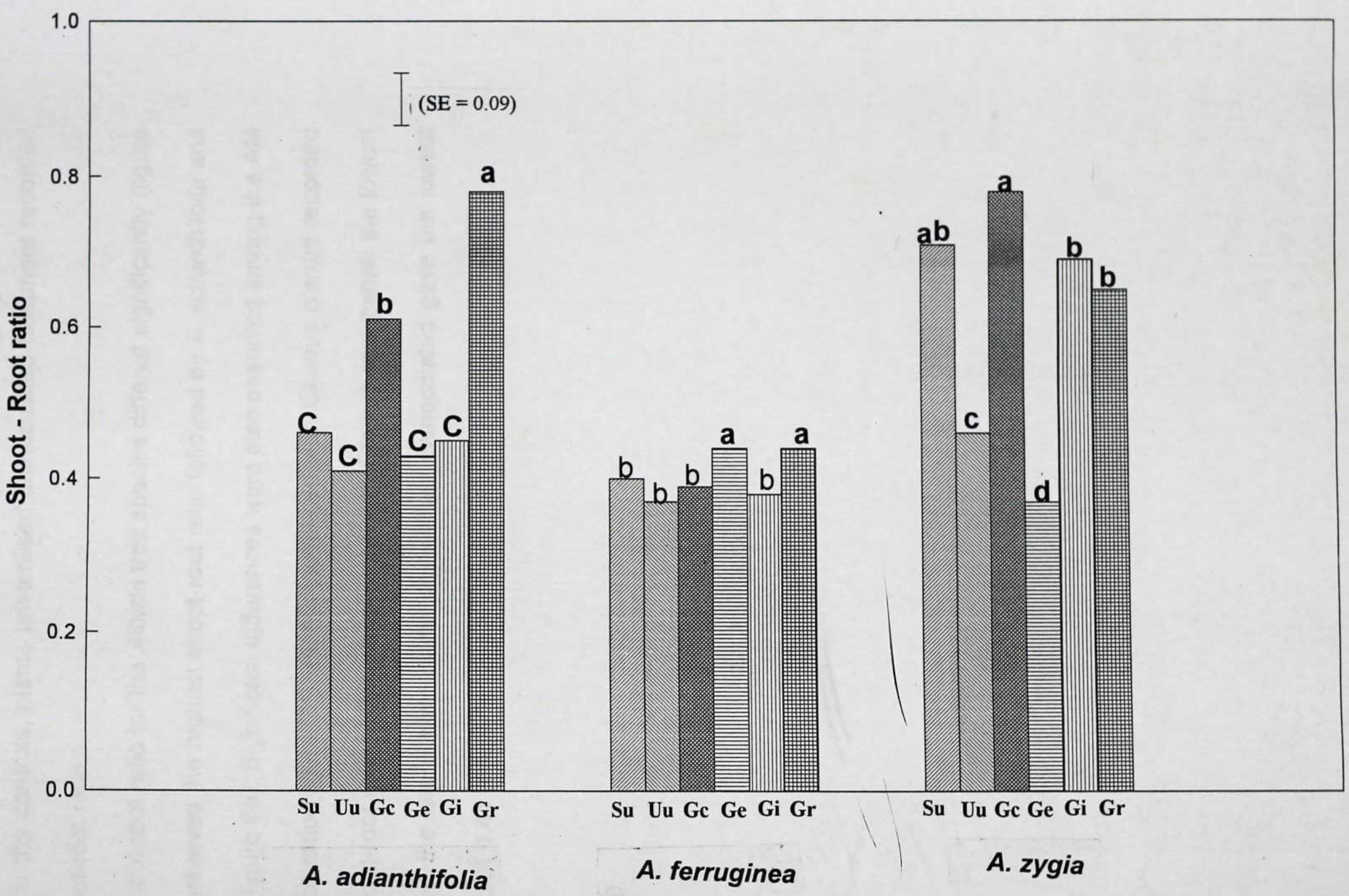
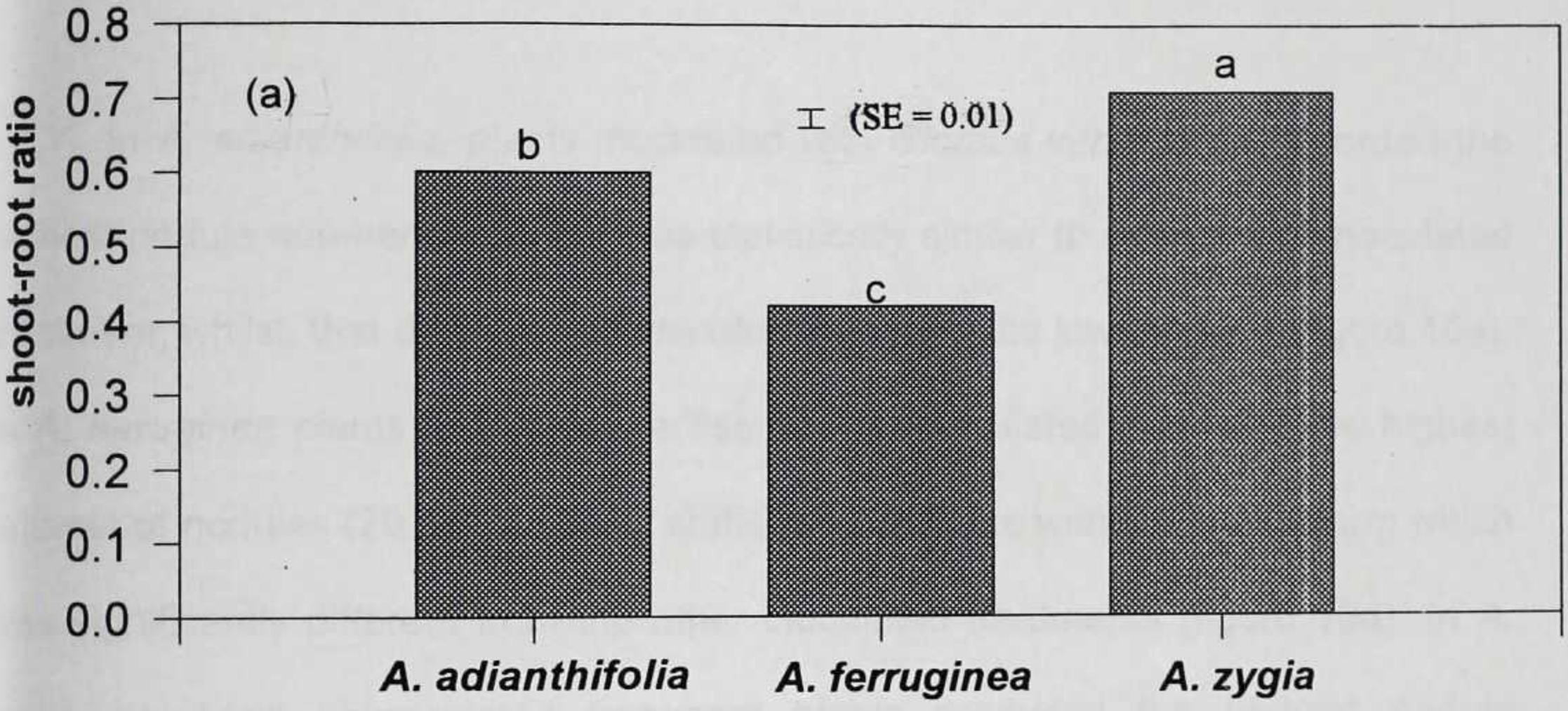


Figure 8. Shoot-root ratio of the three *Albizia* species inoculated with different AM fungus. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

shoot-root ratio (0.78) and those with *Glomus etunicatum* the lowest (0.37). However, within the controls, plants unsterilised uninoculated treatment recorded the lowest shoot-root ratio.

Plant shoot-root ratio for the *Albizia* tree species differed significantly (figure 9a). *A. zygia* achieved the highest shoot-root ratio followed by *A. adianthifolia* and A. *ferruginea* (figure 9a). Significant differences were also observed among the AM fungi shoot-root ratio (figure 9b). Plants inoculated with *Glomus clarum* recorded the highest shoot-root ratio (0.59) whilst those with *Glomus etunicatum* the lowest (0.41). Between the two control plants unsterilised uninoculated gave the lowest shoot-root ratio of 0.41 (figure 9b).



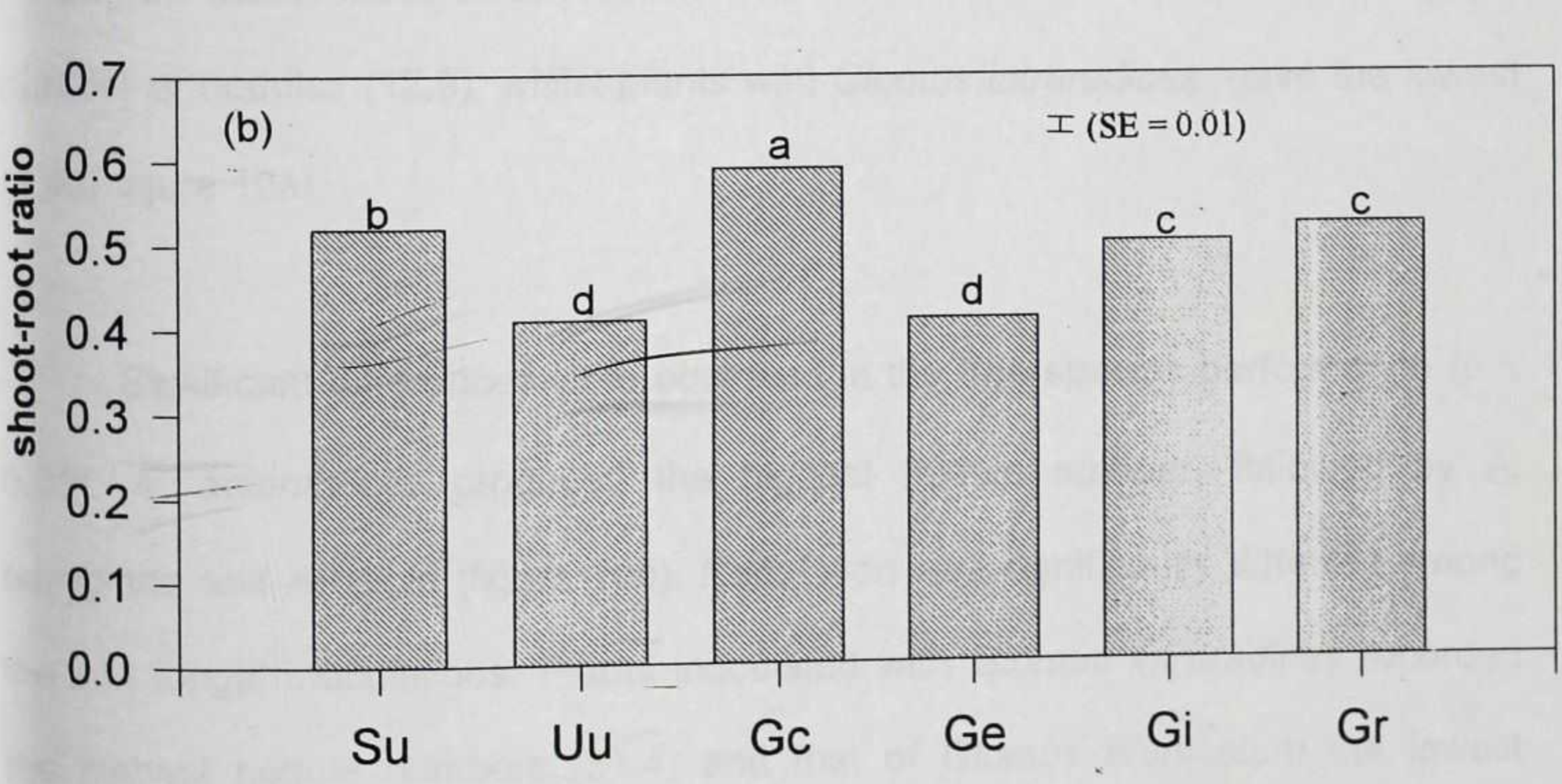


Figure 9. Shoot-root ratio (a) *Albizia* species (b) AM fungi. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

4.1.4 Nodulation

The patterns of nodulation varied markedly among the three *Albizia* species and significantly differed between the fungal treatment ($p \leq 0.05$)(figure 10).

In *A. adianthifolia*, plants inoculated with *Glomus intraradices* recorded the highest nodule number (77.9) but was statistically similar to sterilised uninoculated treatment, whilst, that of *Glomus etunicatum* recorded the lowest (28.0)(figure 10a). In *A. ferruginea* plants that were sterilised but uninoculated produced the highest number of nodules (20.2) but similar statistically to those with *Glomus clarum* which was significantly different from the other inoculated treatments (figure 10a). In *A. zygia*, sterilised uninoculated treatment plants produced the highest nodule numbers (31.4), and were significantly different from all the other treatments. Among the plants inoculated, those with *Glomus etunicatum* produced the highest number of nodules (12.9), whilst plants with *Glomus intraradices* gave the lowest (3.43) figure 10a).

Significant differences were observed in the tree species performance ($p \leq 0.05$). *A. adianthifolia* produced the highest nodule number, followed by *A. ferruginea* and *A. zygia* (figure 10b). Nodulation was significantly different among the AM fungal inoculations. Plants inoculated with *Glomus intraradices* recorded the highest nodule numbers (31.4) and that of *Glomus etunicatum* the lowest (17.9). However, plants that were sterilised but uninoculated recorded the highest nodule number (41.3)(figure 10c).

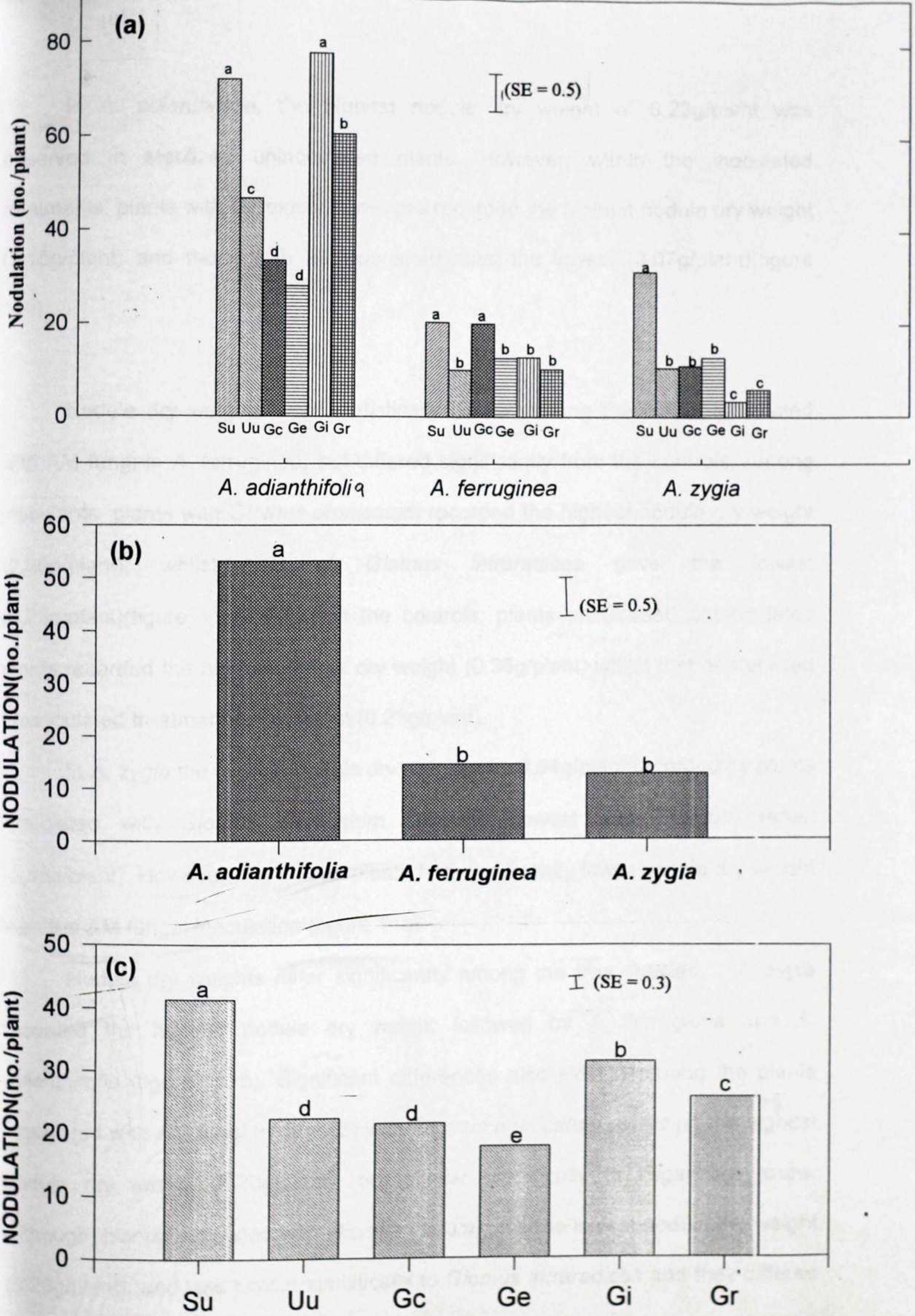


Figure 10. Nodulation (no/plant) of three *Albizia* species (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr).

In *A. adianthifolia,* the highest nodule dry weight of 0.23g/plant was observed in sterilised uninoculated plants. However, within the inoculated treatments, plants with *Glomus intraradices* recorded the highest nodule dry weight (0.15g/plant) and those with *Glomus etunicatum* the lowest (0.07g/plant)(figure 11a).

Nodule dry weights were statistically similar among the plants inoculated with AM fungi in *A.* ferruginea, but differed significantly from the controls. Among inoculants, plants with *Glomus etunicatum* recorded the highest nodule dry weight (0.26g/plant), whilst that of *Glomus intraradices* gave the lowest (0.23g/plant)(figure 11a). Between the controls, plants unsterilised uninoculated plants recorded the highest nodule dry weight (0.36g/plant) whilst that of sterilised uninoculated treatment gave lowest (0.23g/plant).

In *A. zygia* the highest nodule dry weight was 0.54g/plant recorded by plants inoculated with *Glomus etunicatum,* and the lowest was *Glomus clarum* (0.45g/plant). However, the control plants had significantly lower nodule dry weight than the AM fungal inoculation (figure 11a).

Nodule dry weights differ significantly among the tree species. *A. zygia* recorded the highest nodule dry weight followed by *A. ferruginea* and *A. adianthifolia* (figure 11b). Significant differences also existed among the plants inoculated with AM fungi treatments with *Glomus etunicatum* recording the highest nodule dry weight (0.30g/plant), but similar statistically to *Gigaspora rosea.* Although, plants inoculated with *Glomus clarum* gave the lowest nodule dry weight (0.26g/plant), and was similar statistically to *Glomus intraradices* and they differed from the control treatments where sterilised uninoculated plants recorded the lowest (0.16g/plant)(figure 11c).

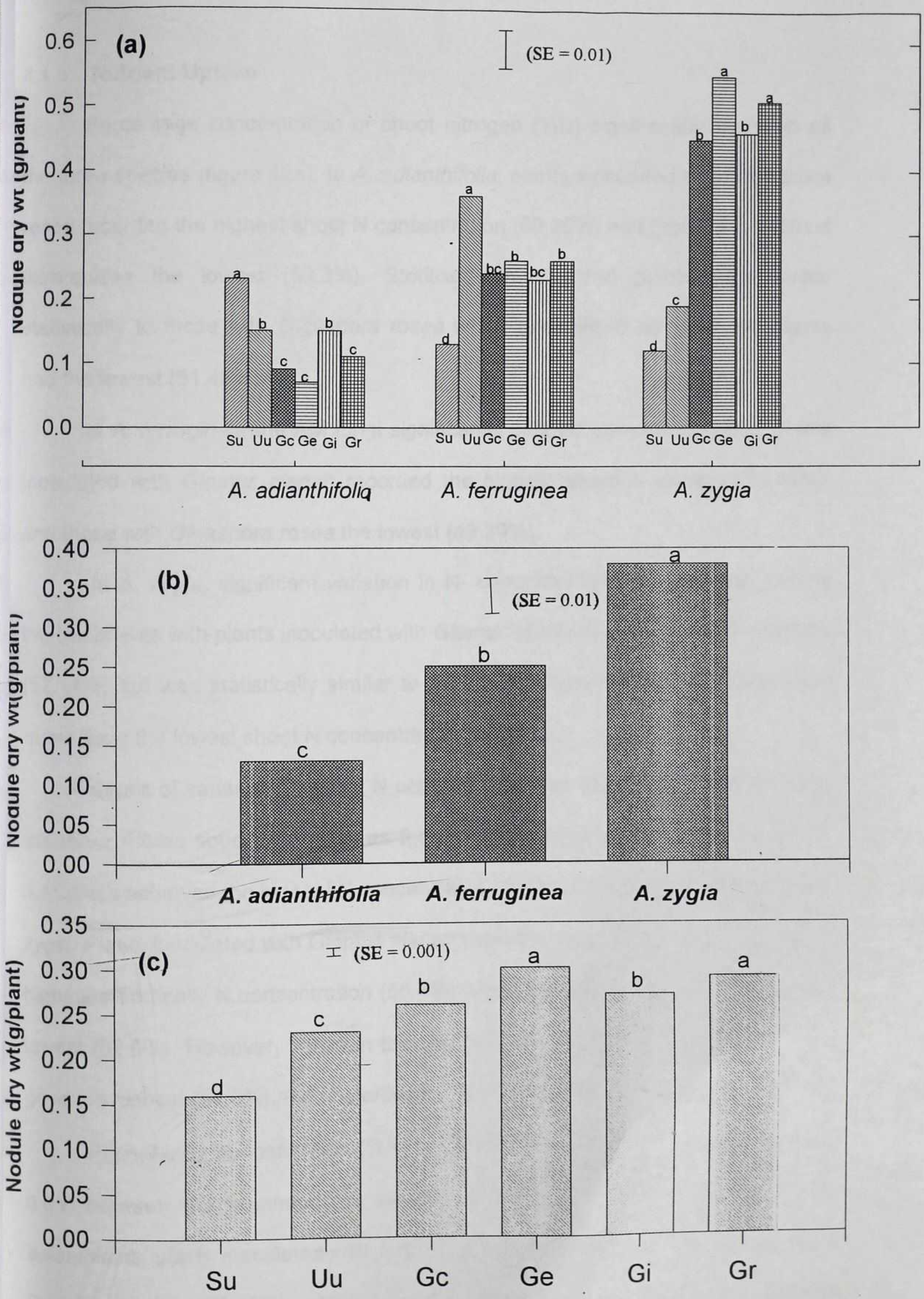


Figure 11. Nodule dry weight (g/plant) of three *Albizia* species (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

4.1.5 Nutrient Uptake

Percentage concentration of shoot nitrogen (%N) significantly varied in all the three species (figure 12a). In *A. adianthifolia*, plants inoculated with *Gigaspora rosea* recorded the highest shoot N concentration (60.86%) and those with *Glomus intraradices* the lowest (52.3%). Sterilised uninoculated plants was similar statistically to those with *Gigaspora rosea* whilst unsterilised uninoculated plants had the lowest (51.43%).

In *A. ferruginea*, the AM fungi significantly differed from the controls. Plants inoculated with *Glomus clarum* recorded the highest shoot N content (58.86%), and those with *Gigaspora rosea* the lowest (49.29%).

In *A. zygia*, significant variation in N- concentration was observed among the treatments with plants inoculated with *Glomus etunicatum* recording the highest (57.14%) but was statistically similar to the controls whilst those with *Gigaspora rosea* gave the lowest shoot N concentration (44.14%).

Analysis of variance for shoot N concentration was significantly different in all the three *Albizia* species, as well as the AM fungi inoculations (figure 12a,b). *A. ferruginea* achieved the highest N concentration, followed by *A. adianthifolia and A. zygia*. Plants inoculated with *Glomus clarum* proved to be superior by recording the highest statistically N concentration (56.4%) whilst those with *Gigaspora rosea* the lowest (52.0%). However, between the two controls sterilised uninoculated plants gave the highest (54.0%) and unsterilised uninoculated the lowest (50.0%)(fig 12c).

Phosphorus concentration (%P) in the shoot was significantly different ($p \leq 0.05$) between the treatments in each of the tree species (figure 13a). In *A. adianthifolia*, plants inoculated with *Glomus etunicatum* recorded the highest shoot P concentration (57.43%), which was statistically similar to those with *Glomus intraradices* whilst *Gigaspora rosea* the lowest (47.1%).

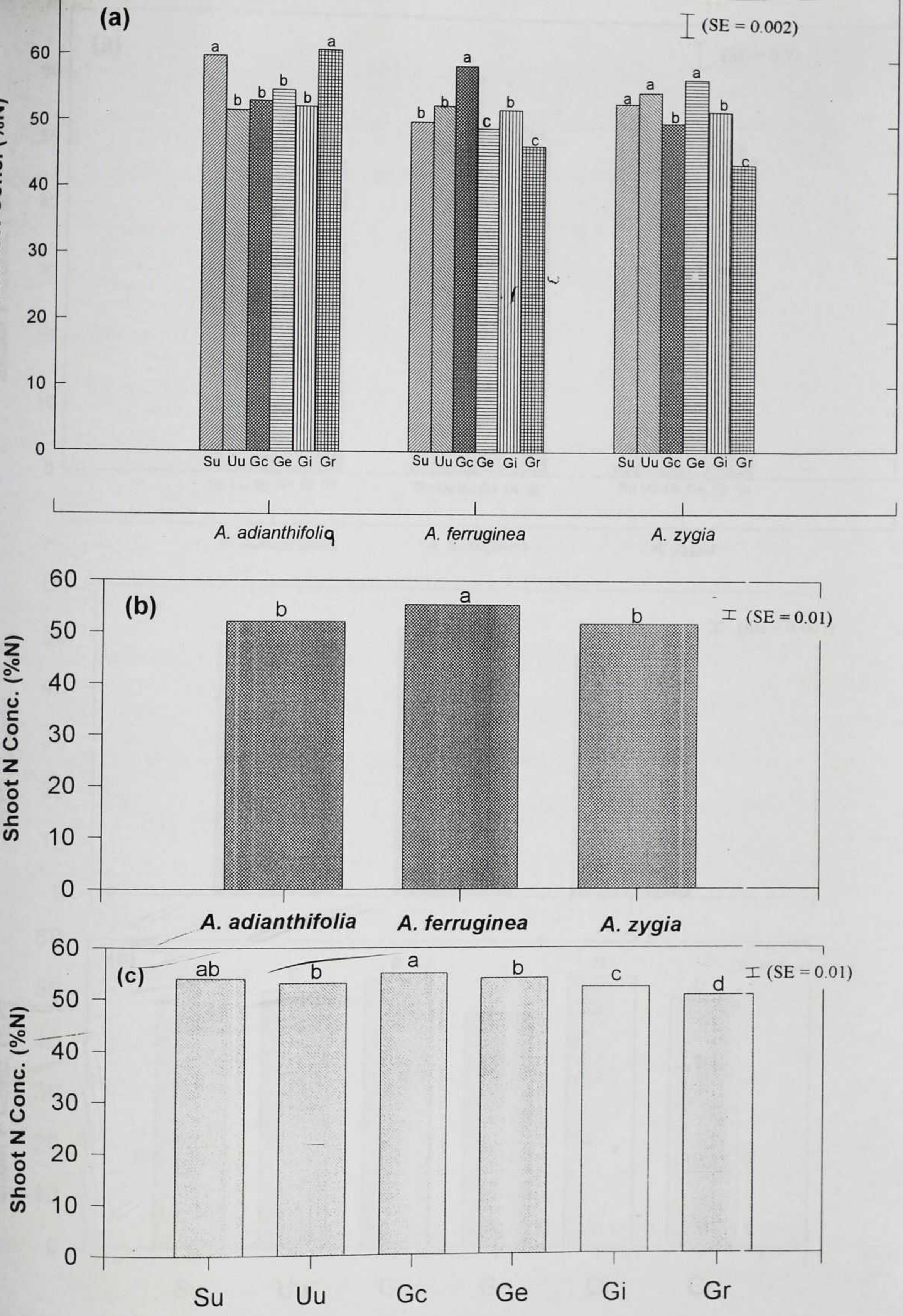


Figure 12. Shoot N concentration (%N) of three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

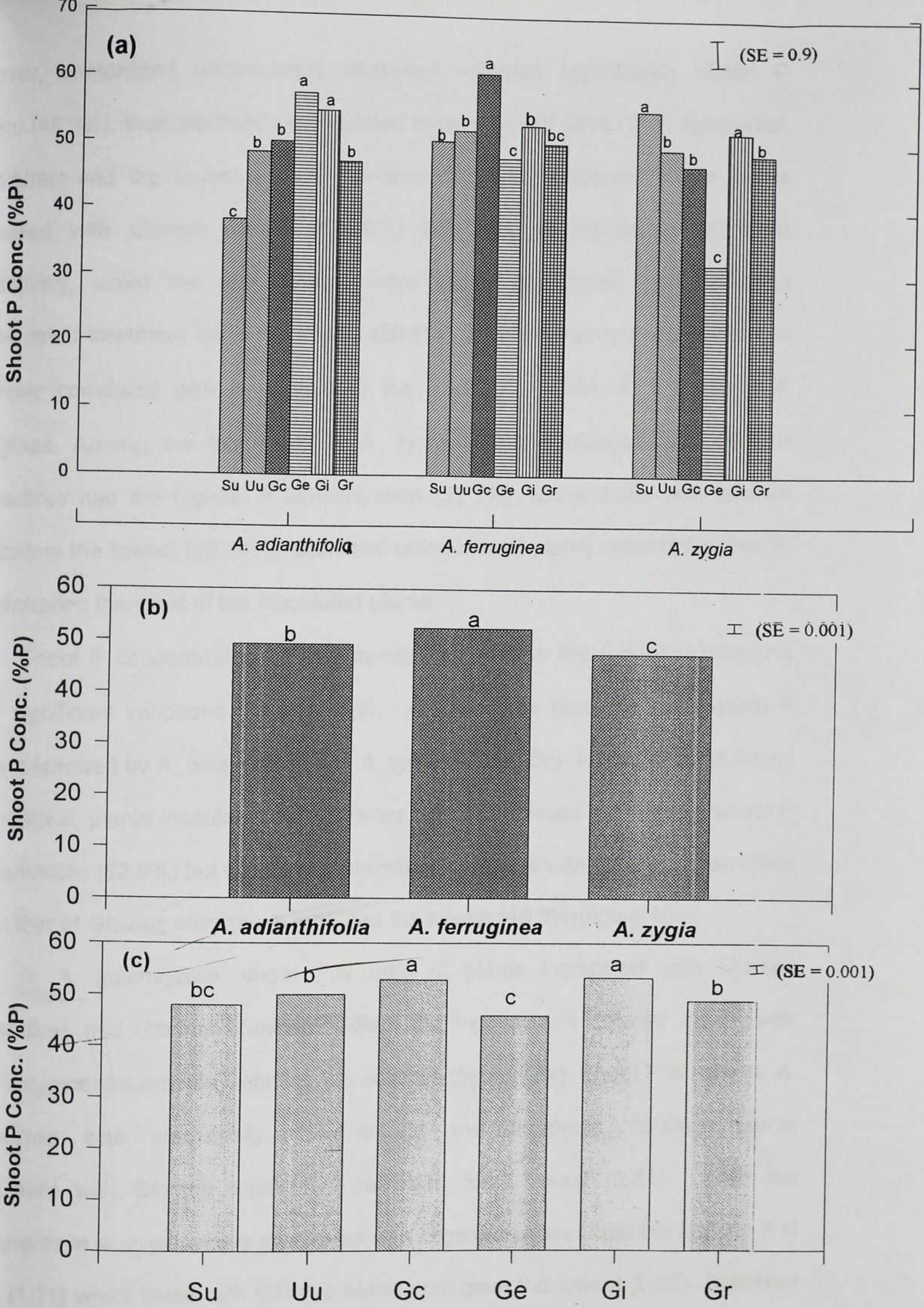


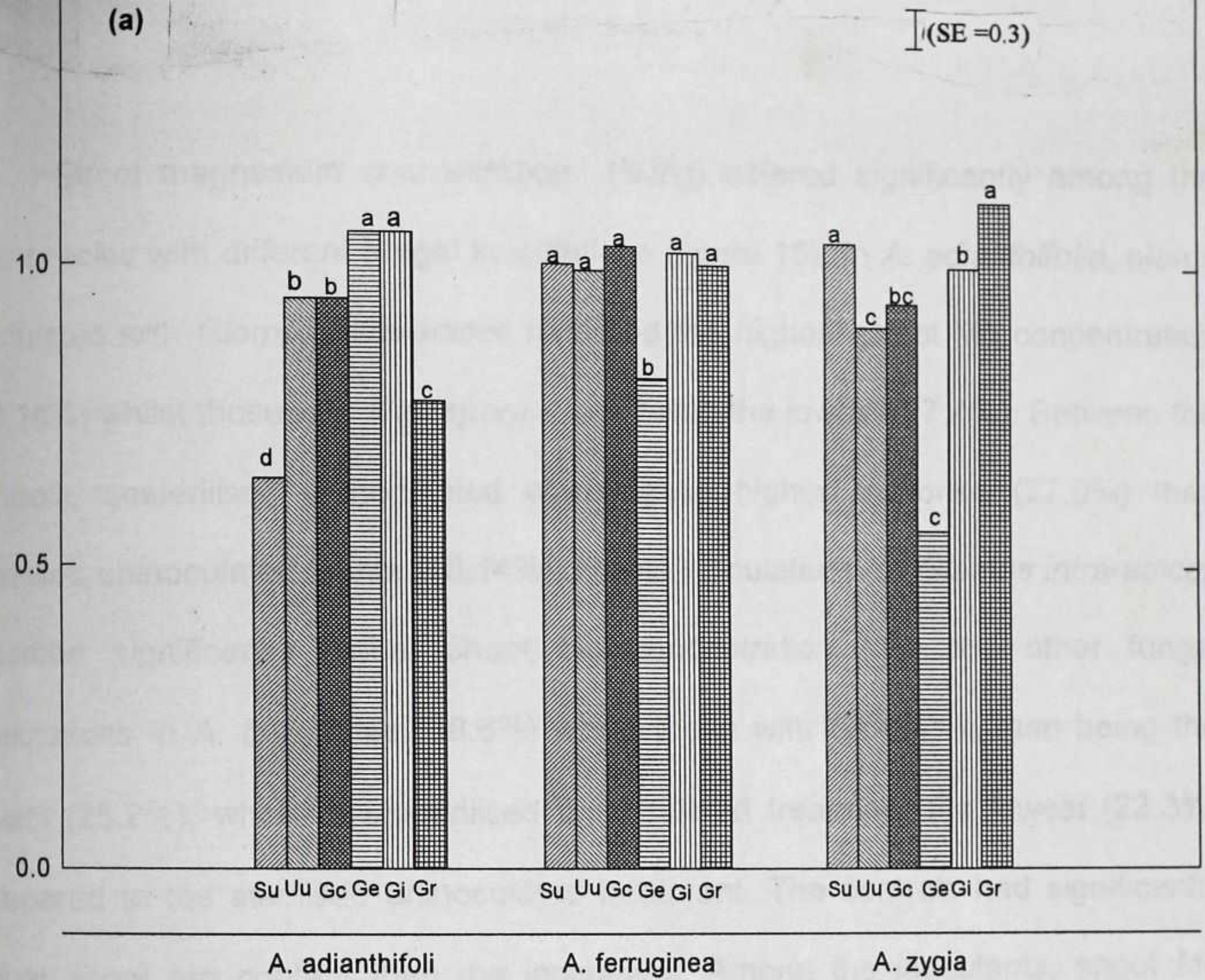
Figure 13. Shoot P concentration (% P) of three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

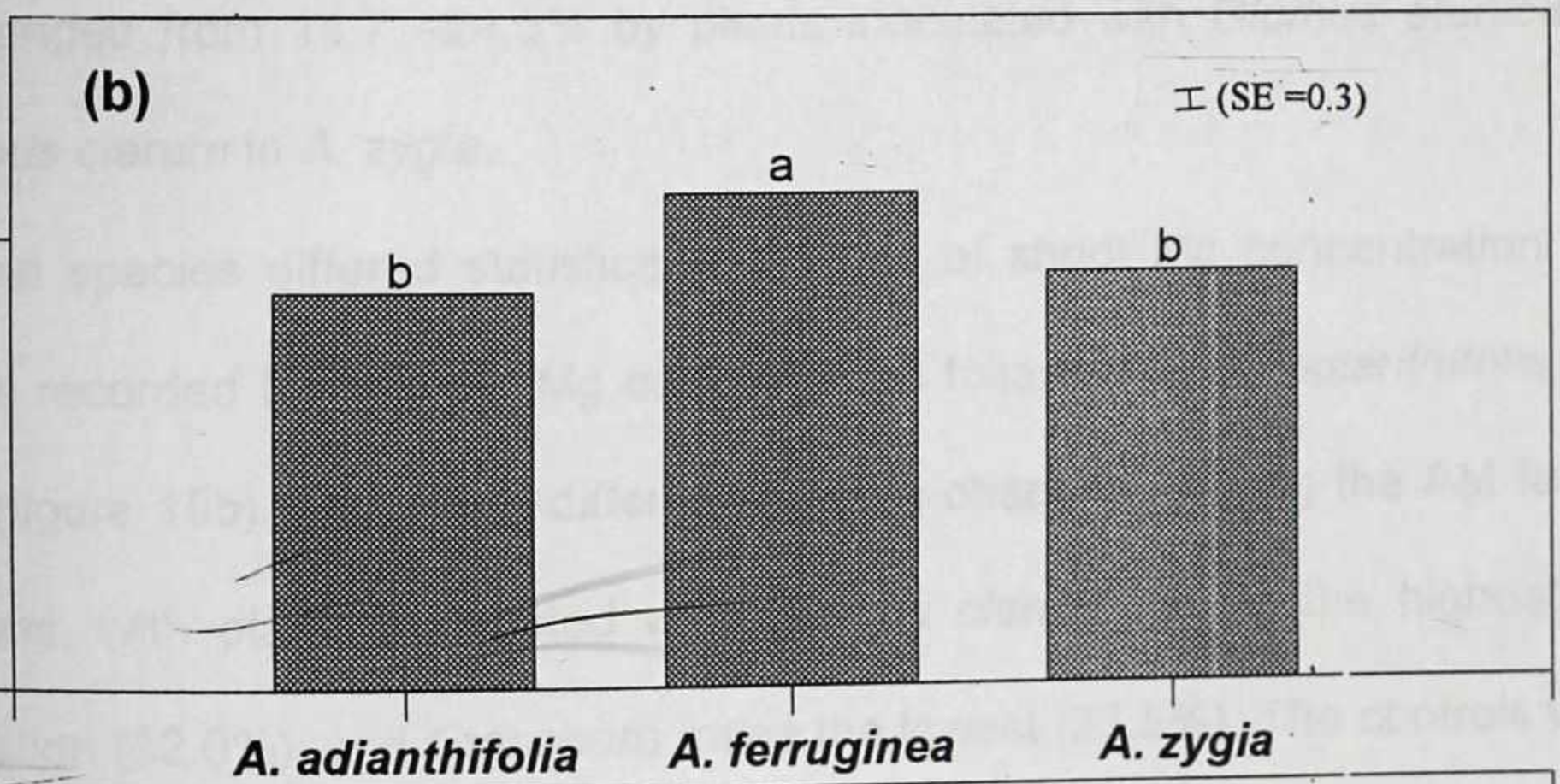
However, unsterilised uninoculated treatment recorded significantly higher P content (48.3%), than sterilised uninoculated treatment (38.14%). In *A. ferruginea*, the highest and the lowest shoot-P concentration were observed in the plants inoculated with *Glomus clarum* (58.86%) and *Glomus etunicatum* (48.00%) respectively, whilst the two controls were similar statistically with sterilised uninoculated treatment been the lowest (50.5%). Plant phosphorus concentration positively correlated with N content of the plant (r^2 = 0.84; P ≤ 0.05) in *A. ferruginea*. Among the inoculants in *A. zygia*, plants inoculated with *Glomus intraradices* had the highest P concentration (52.1%), whilst those with *Glomus etunicatum* the lowest (32.14%). Sterilised uninoculated plants recorded higher P-concentration than that of the inoculated plants.

Shoot P concentration for tree species as well as AM fungal inoculations gave significant variations (figure 13b,c). *A. ferruginea* recorded the highest P content followed by *A. adianthifolia* and *A. zygia* (figure 13b). Among the AM fungal inoculations, plants inoculated with *Glomus clarum* achieved the highest shoot P concentration (52.9%) but was similar statistically to those with *Glomus intraradices* whilst that of *Glomus etunicatum* recorded the lowest (45.9%)(figure 13c).

In *A. adianthifolia*, shoot P:N ratio of plants inoculated with *Glomus intraradices* and *Glomus etunicatum* were the highest and differed significantly from the other treatments including the controls (figure 14a). Shoot P:N ratio in *A. ferruginea* was statistically similar among the treatments however, plants inoculated with *Glomus etunicatum* recorded the lowest (0.81). With the treatments in *A. zygia*, plants inoculated with *Gigaspora rosea* had the highest P:N ratio (1.11) whilst those with *Glomus etunicatum* gave the lowest (0.56). Sterilised uninoculated plants recorded higher shoot P concentration (1.04) than the unsterilised uninoculated (0.94).

Generally, the highest shoot P:N ratio of tree species was observed in *A. ferruginea* which was significantly different from *A. adianthifolia* and *A. zygia* (figure 14b). Among the fungal species plants inoculated with *Glomus intraradices* recorded the highest shoot P:N ratio (1.04), whilst that of *Gigaspora rosea* gave the lowest (0.96). Between the controls, unsterilised uninoculated plants recorded statistically higher ratio (0.96) than unsterilised uninoculated plants (0.89) (figure 14c).





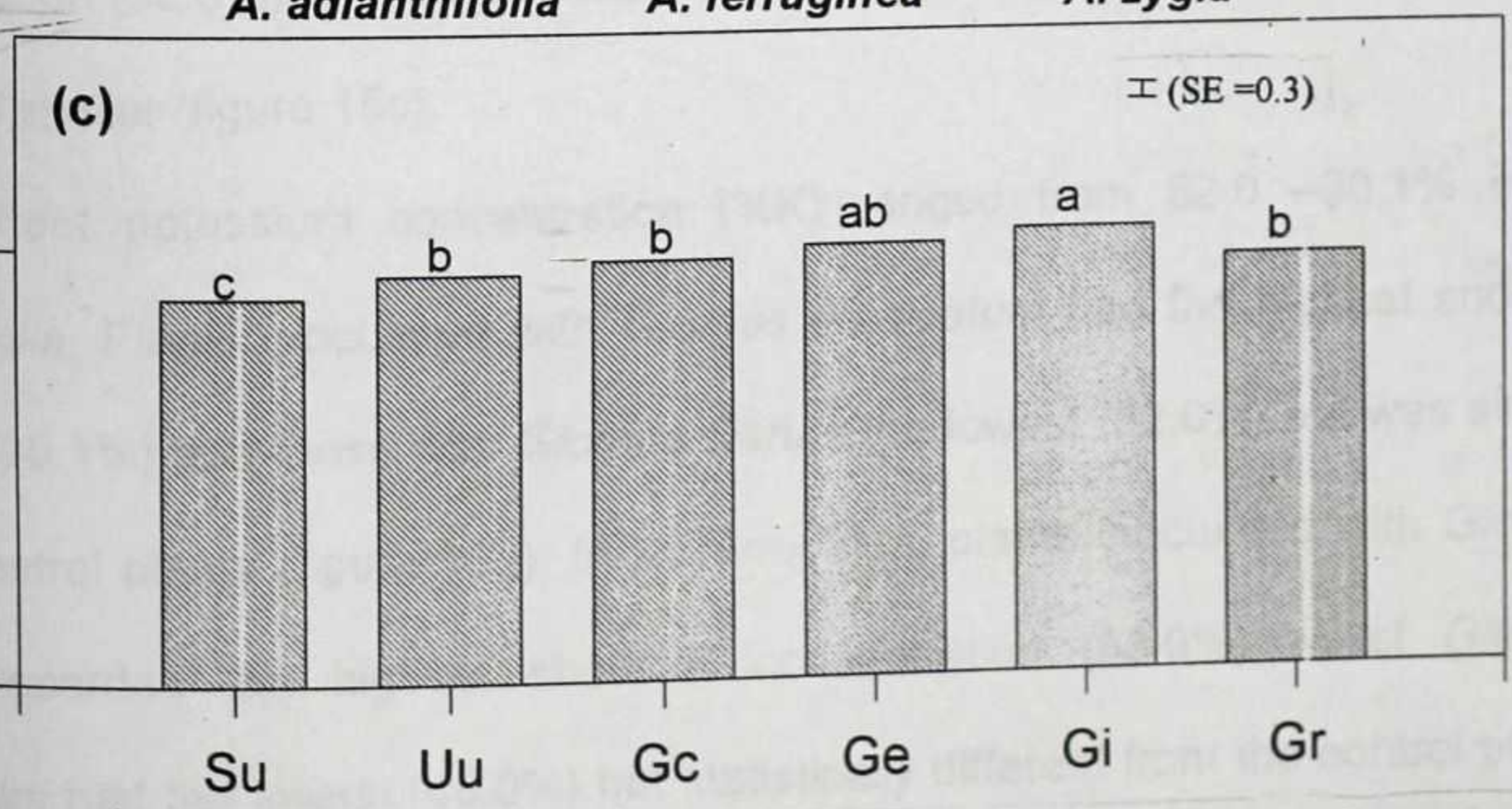


Figure 14. Shoot P: N ratio for three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

Shoot magnesium concentration (%Mg) differed significantly among the tree species with different fungal inoculations (figure 15). In *A. adianthifolia*, plants inoculated with *Glomus intraradices* recorded the highest shoot Mg concentration (43.14%) whilst those with *Gigaspora rosea* being the lowest (27.4%). Between the controls, unsterilised uninoculated plants gave higher response (27.0%) than sterilised uninoculated plants (16.14%). Plants inoculated with *Glomus intraradices* recorded significantly higher shoot Mg concentration than the other fungal inoculations in *A. ferruginea* (28.6%) whilst those with *Glomus clarum* being the lowest (25.2%), whereas unsterilised uninoculated treatment the lowest (22.3%) compared to the sterilised uninoculated treatment. The controls had significantly higher shoot Mg content than the inoculants. Among the inoculants, shoot Mg content ranged from 14.7 –24.5% by plants inoculated with *Glomus etunicatum* and *Glomus clarum* in *A. zygia*.

Tree species differed statistically in terms of shoot Mg concentration. *A. ferruginea* recorded the highest Mg concentration followed by *A. adianthifolia* and *A. zygia* (figure 15b). Significant differences were observed among the AM fungal inoculations, with plants inoculated with *Glomus clarum* having the highest Mg concentration (32.0%), and *Gigaspora rosea* the lowest (23.5%). The controls were statistical similar (figure 15c).

Shoot potassium concentration (%K) ranged from 82.0 –90.1% in *A. adianthifolia*. Plants inoculated with *Glomus etunicatum* had the highest shoot K content (90.1%) and those with *Glomus clarum* the lowest (82.0%) but was similar to the control plants (figure 16a). In *A. ferruginea*, plants inoculated with *Glomus clarum* recorded the highest shoot K concentration (60.9%) whilst *Glomus etunicatum* had the lowest (48.0%) but statistically different from the control plants (figure 16a). The range for K concentration in *A. zygia* was between 67.0 –84.1

where plants inoculated with *Glomus intraradices* recorded the highest K concentration which is statistically different from the other fungal inoculations but similar to the controls (figure 16a).

Analysis of variance for the tree species in terms of shoot K concentration indicated significant differences with *A. ferruginea* and *A. adianthifolia* giving the highest response and both significantly different from the *A. zygia* (figure 16b). There were some significant variations among the AM fungal inoculations where plants inoculated with *Glomus clarum* recorded the highest K concentration (85.0%) and *Glomus etunicatum* the lowest (77.0%). However, the controls were statistically similar (figure 16c).

Shoot calcium concentration (%Ca) was very high in *A. adianthifolia* with plants inoculated with *Glomus intraradices* recording the highest (40.1%) whilst *Gigaspora rosea* the lowest (31.1%)(figure 17a). However, between the controls sterilised uninoculated plants gave the highest response (29.7%) and were statistically similar to unsterilised uninoculated treatment (figure 17a). In *A. ferruginea*, Ca concentration ranged from 19.1 – 25.9% where plants inoculated with *Glomus clarum* recorded significantly lower Ca content compared with the other fungal treatments and the controls. In *A. zygia*, plants inoculated with *Glomus etunicatum* recorded significantly higher shoot Ca concentration (28.1%), than the other fungal inoculants but statistically similar to the controls.

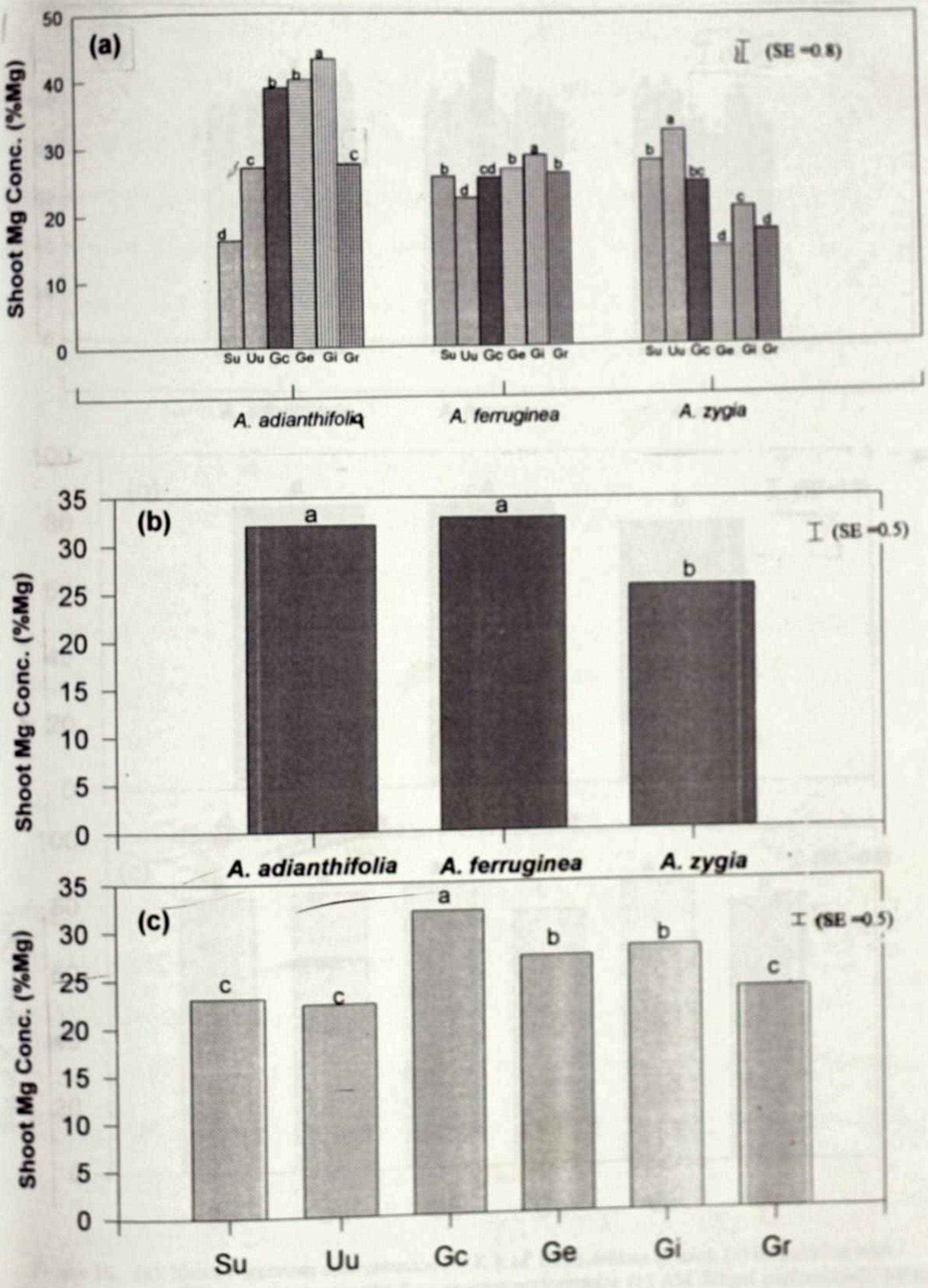


Figure 15. Shoot Magnesium concentration (% Mg) of three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

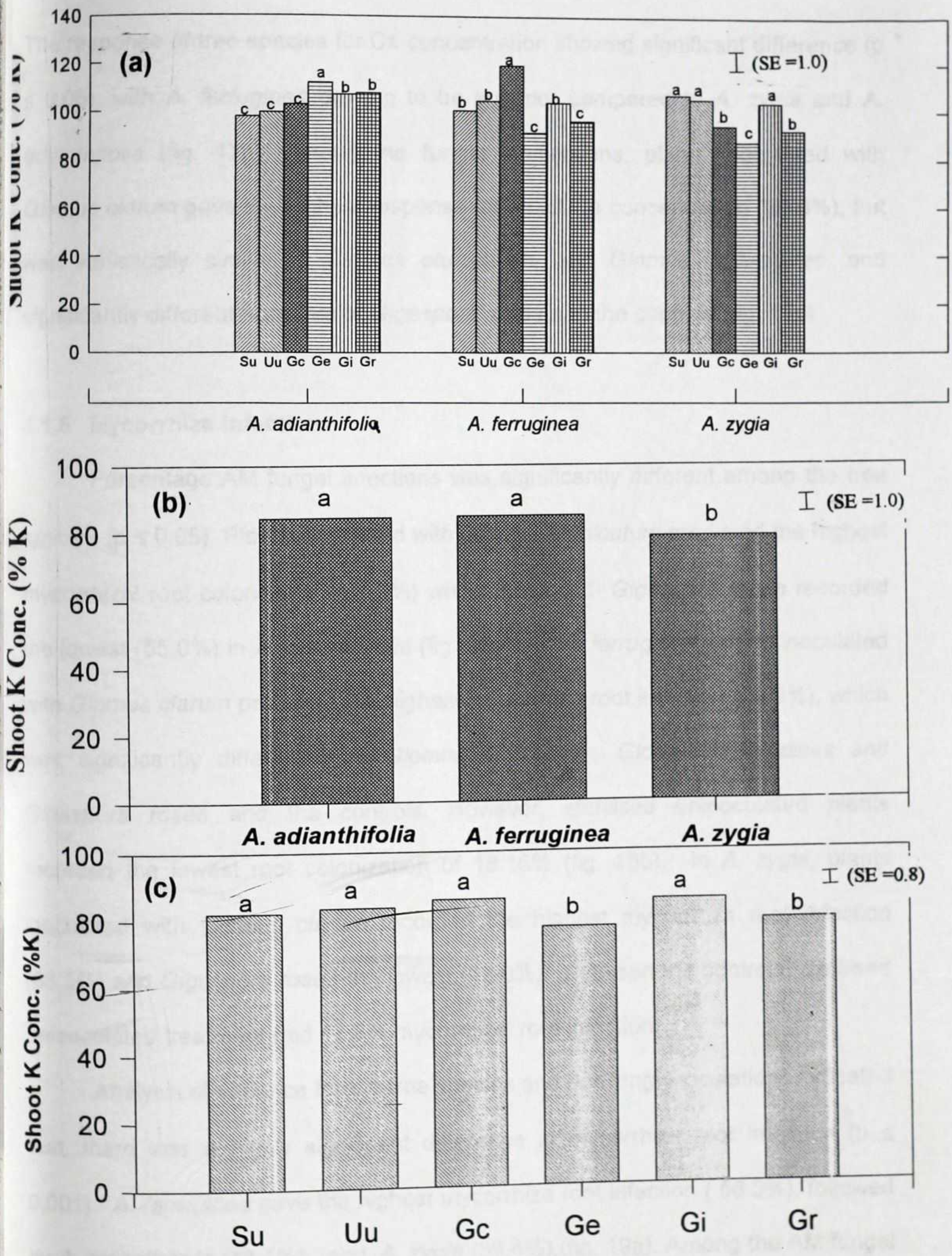


Figure 16. (a) Shoot Potassium concentration (% K) of three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr)

The response of tree species for Ca concentration showed significant difference ($p \leq 0.05$), with *A. ferruginea* proving to be superior compared to *A. zygia* and *A. adianthifolia* (fig. 17b). Among the fungal inoculations, plants inoculated with *Glomus clarum* gave the highest response for shoot Ca concentration (29.0%), but was statistically similar to *Glomus etunicatum*, and *Glomus intraradices*, and significantly different from that of *Gigaspora rosea* and the controls (fig. 17c).

4.1.6 Mycorrhiza Infection

Percentage AM fungal infections was significantly different among the tree species ($p \leq 0.05$). Plants inoculated with *Glomus etunicatum* produced the highest mycorrhizal root colonization (67.9%) whilst those with *Gigaspora rosea* recorded the lowest (55.0%) in *A. adianthifolia* (fig. 18a). In *A. ferruginea*, plants inoculated with *Glomus clarum* produced the highest mycorrhiza root infection (79.9%), which was significantly different from *Glomus etunicatum*, *Glomus intraradices* and *Gigaspora rosea* and the controls. However, sterilised uninoculated plants recorded the lowest root colonization of 18.16% (fig. 18b). In *A. zygia*, plants inoculated with *Glomus clarum* recorded the highest mycorrhiza root infection (63.5%) and *Gigaspora rosea* the lowest (42.0%). Between the controls, sterilised uninoculated treatment had 11.9% mycorrhizal root infection.

Analysis of variance for the tree species and AM fungi inoculations indicated that, there was a highly significant difference in mycorrhiza root infection ($p \leq 0.001$). *A. ferruginea* gave the highest mycorrhiza root infection (56.0%), followed by *A. adianthifolia* (48.1%) , *and A. zygia* (39.4%),(fig. 19a). Among the AM fungal inoculations, plants with *Glomus clarum* had the highest root infection (68.55%) and *Gigaspora rosea* the lowest (47.1%). Between the controls sterilised uninoculated recorded the lowest (18.0%)(fig. 19b).

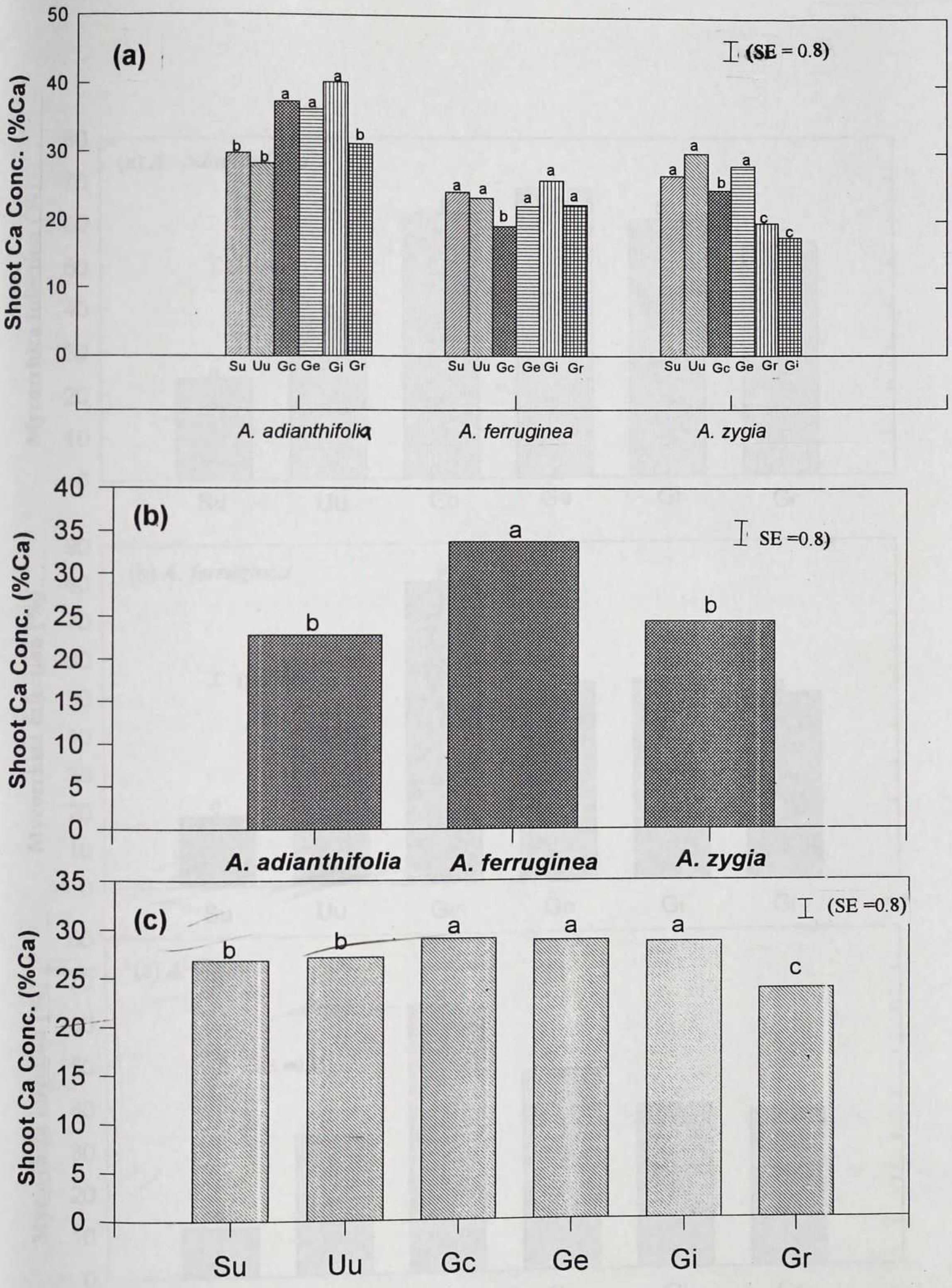


Figure 17. Shoot Calcium concentration (% Ca) of three *Albizia* species. (a) Inoculation with different AM fungus (b) Tree species performance (c) AM fungal performance. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr).

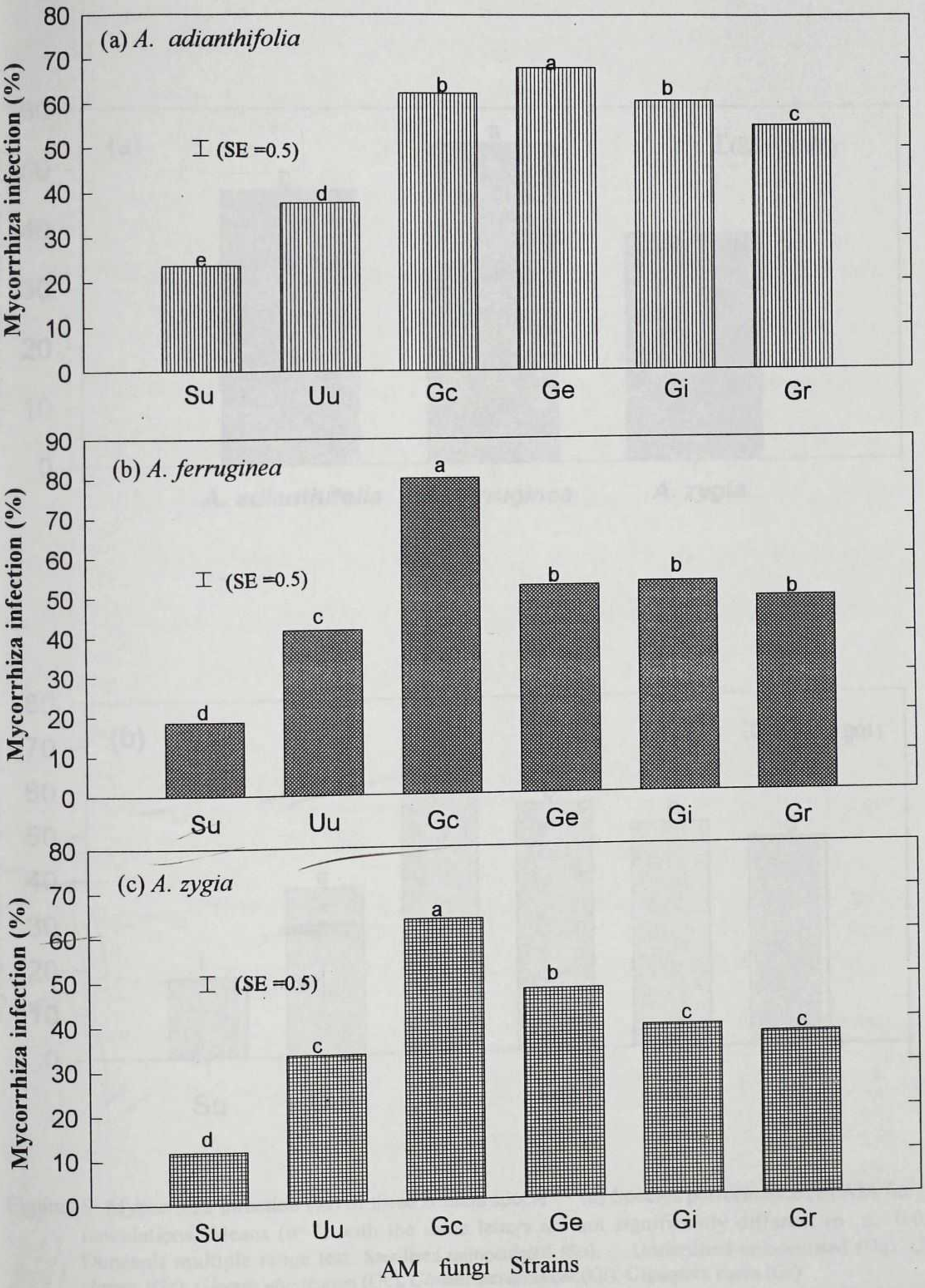
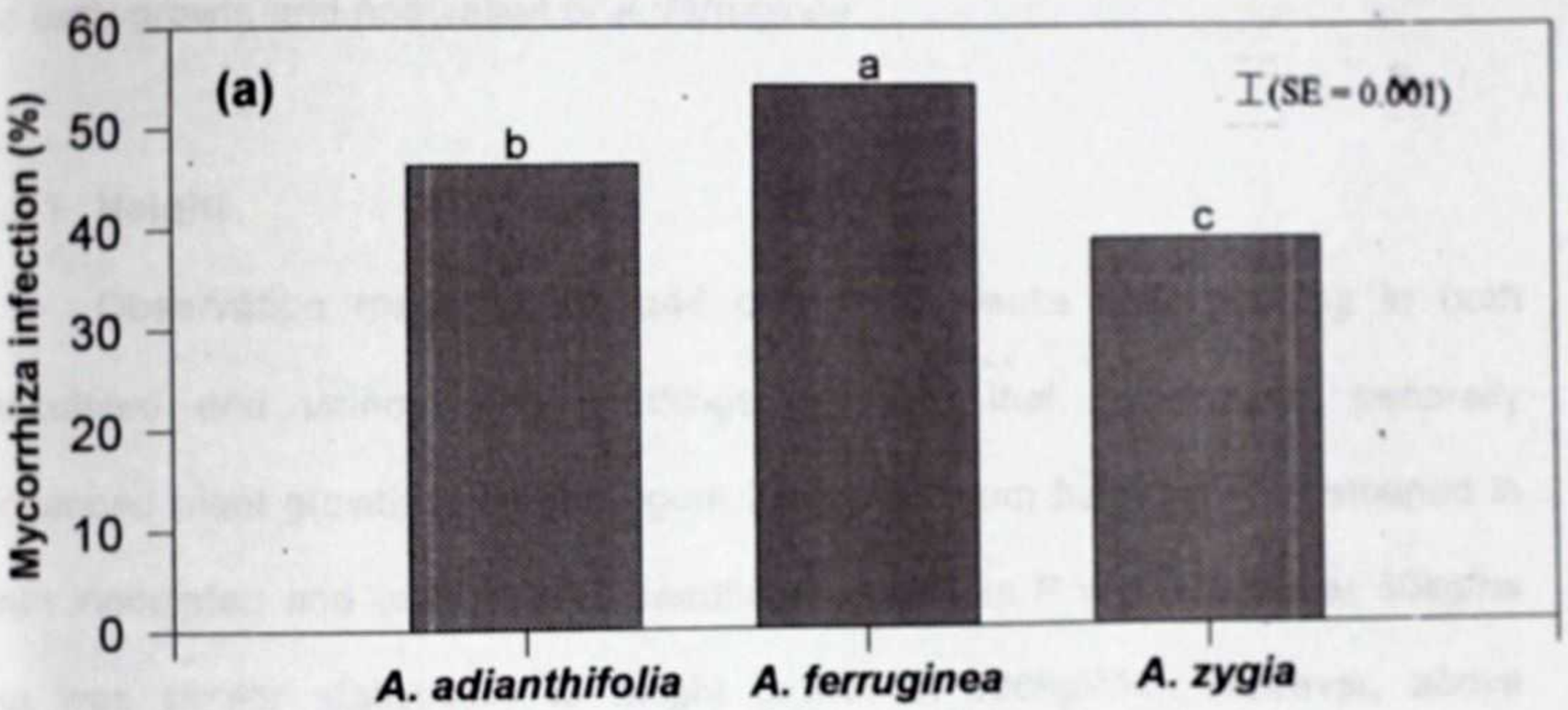


Figure 18. Mycorrhiza infection (%) of three *Albizia* species (a) *A.. adianthifolia* (b) *A. ferruginea* (c) *A. zygia*. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), *Gigaspora rosea* (Gr



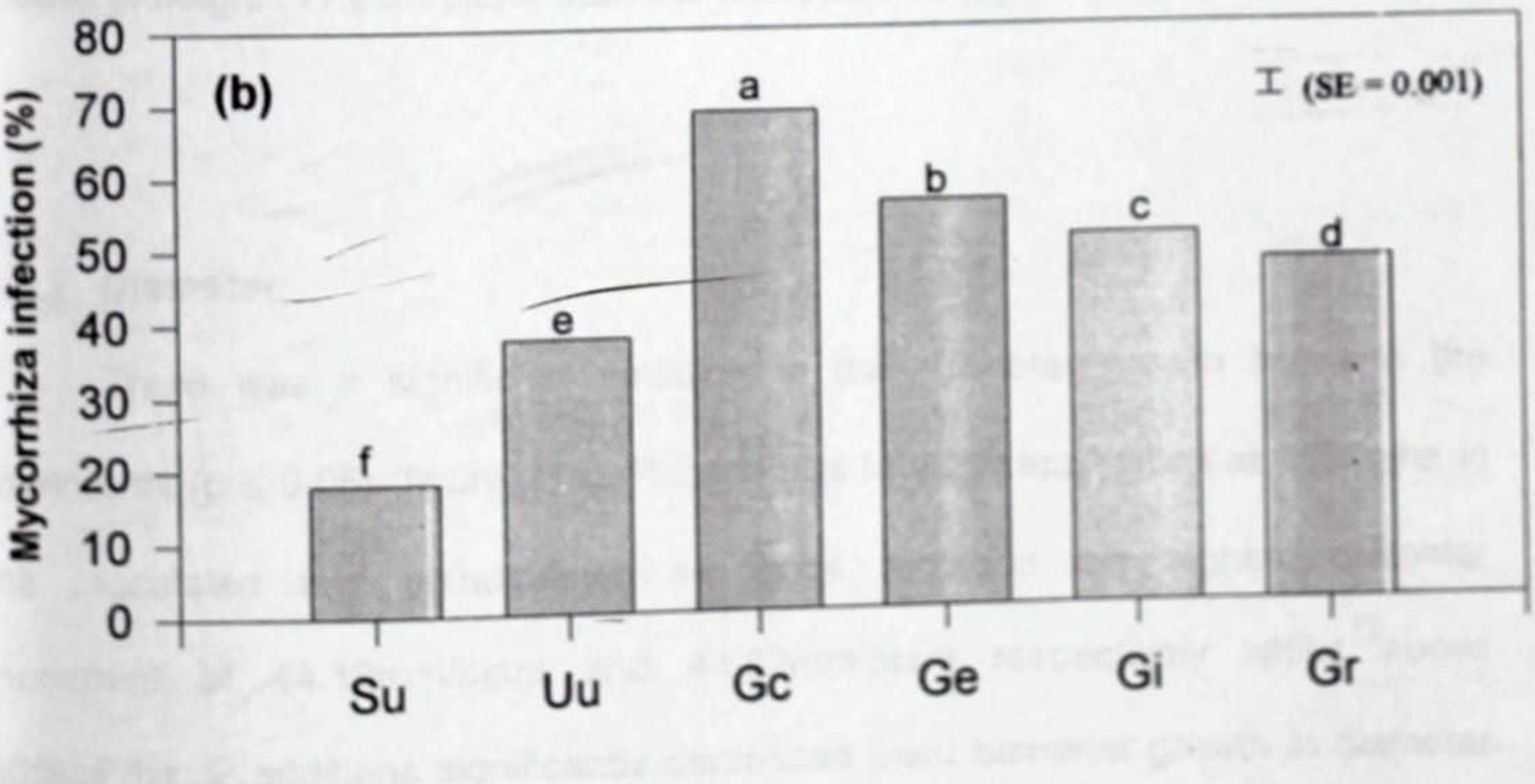


Figure 19. Mycorrhiza infection (%) of three Albizia specie (a) Species performance (b) AM fungal inoculations. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test. Sterilised uninoculated (Su), Unsterilised uninoculated (Uu), *Glomus clarum* (Gc), *Glomus etunicatum* (Ge), *Glomus intraradices* (Gi), Gigaspora rosea (Gr)

4.2 Experiment 2

This experiment was conducted to assess the effect of different levels of phosphorus in the form of tripple superphosphate and *Glomus clarum* inoculation on early growth and nodulation of *A. ferruginea.*

4.2.1 Height

Observation made at the end of the 16 weeks after planting in both inoculated and uninoculated seedlings indicated that, phosphorus generally enhanced plant growth in height (figure 20a). Maximum height growth attained in both inoculated and uninoculated seedlings was when P was applied at 50kg/ha but was similar statistically to height growth at 100kgP/ha. However, above 100kgP/ha growth in height declines gradually in both treatments. Main effect and interactions were not significant but the inoculated treatments recorded higher growth in height (17.2cm/plant) than the uninoculated (figure 20b).

4.2.2 Diameter

There was a significant variation in the diameter growth between the treatments ($p \leq 0.05$) (figure 21a). Phosphorus fertilizer application at 100kg/ha in the inoculated and uninoculated seedlings recorded the highest diameter increment of 44.19mm/plant and 44.22mm/plant respectively whilst above 100kgP/ha, P additions significantly decreased plant diameter growth in diameter (figure 21a). Interactions were not significant but main effect for growth in diameter revealed significant differences between the treatments. Inoculated treatments recorded the highest growth in diameter (44.2mm/plant), compared to the uninoculated treatment (42.0mm/plant)(figure 21b).

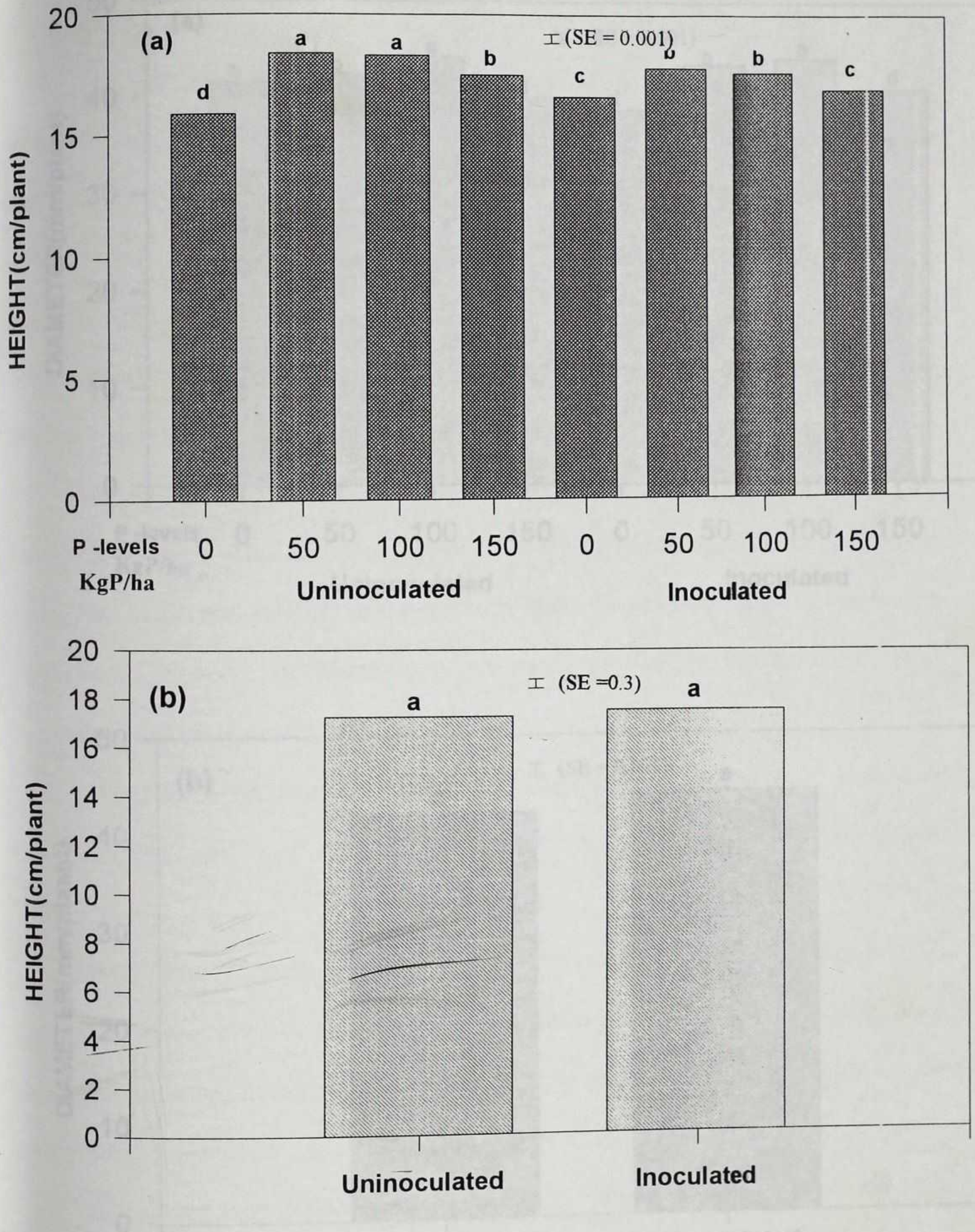


Figure 20. Effect of phosphorus and *Glomus clarum* on height growth of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

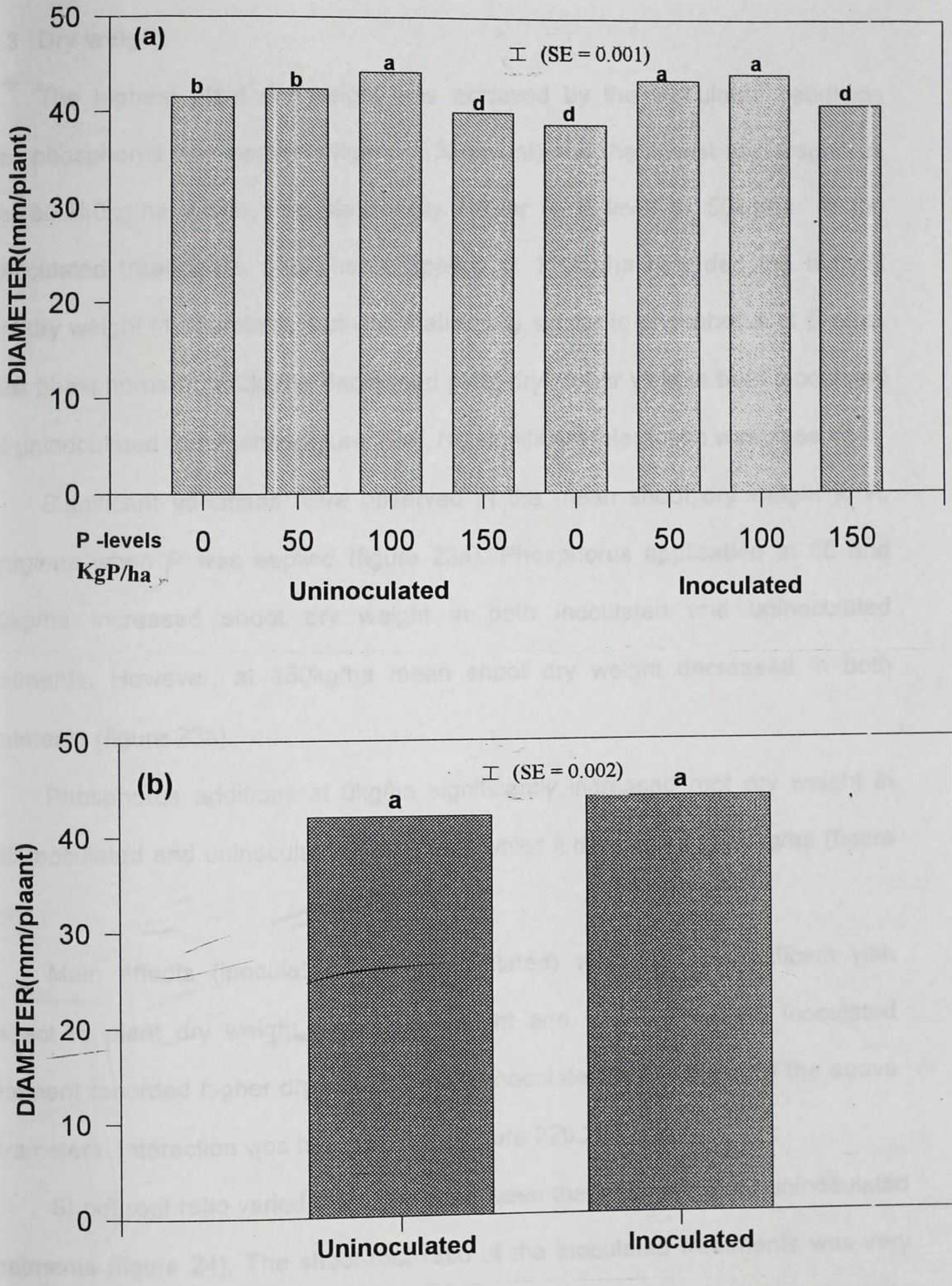


Figure 21. Effect of phosphorus and *Glomus clarum* on diameter growth of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

4.2.3 Dry weight

The highest plant dry weight was achieved by the inoculated seedlings when phosphorus was applied 0kg/ha (2.32g/plant) and the lowest at phosphorus level of 150kg/ha which was statistically similar to P level at 50kg/ha. In the uninoculated treatments, phosphorus applied at 100kg/ha recorded the highest plant dry weight (1.85g/plant), but was statistically similar to phosphorus at 0kg/ha, whilst phosphorus at 150kg/ha decreased plant dry matter yield in both inoculated and uninoculated treatments (figure 22a). No significant interaction was observed.

Significant variations were observed in the mean shoot dry weight in *A. ferruginea* when P was applied (figure 23a). Phosphorus application at 50 and 100kg/ha increased shoot dry weight in both inoculated and uninoculated treatments. However, at 150kg/ha mean shoot dry weight decreased in both treatments (figure 23a).

Phosphorus additions at 0kg/ha significantly increased root dry weight in both inoculated and uninoculated treatments whilst it decreased at 50kg/ha (figure 24a).

Main effects (inoculated and uninoculated) were highly significant with respect to plant dry weight, shoot dry weight and root dry weight. Inoculated treatment recorded higher dry weight than uninoculated treatment in all the above parameters. Interaction was not significant (figure 22b,23b & 24b).

Shoot-root ratio varied significantly between the inoculated and uninoculated treatments (figure 24). The shoot-root ratio of the inoculated treatments was very high when phosphorus was applied at 50kg/ha (1.8) and low when at 0kg/ha (1.2). In the uninoculated seedlings phosphorus at 150kg/ha recorded the highest value (1.6), whilst at 0kg/ha gave the lowest (1.4).

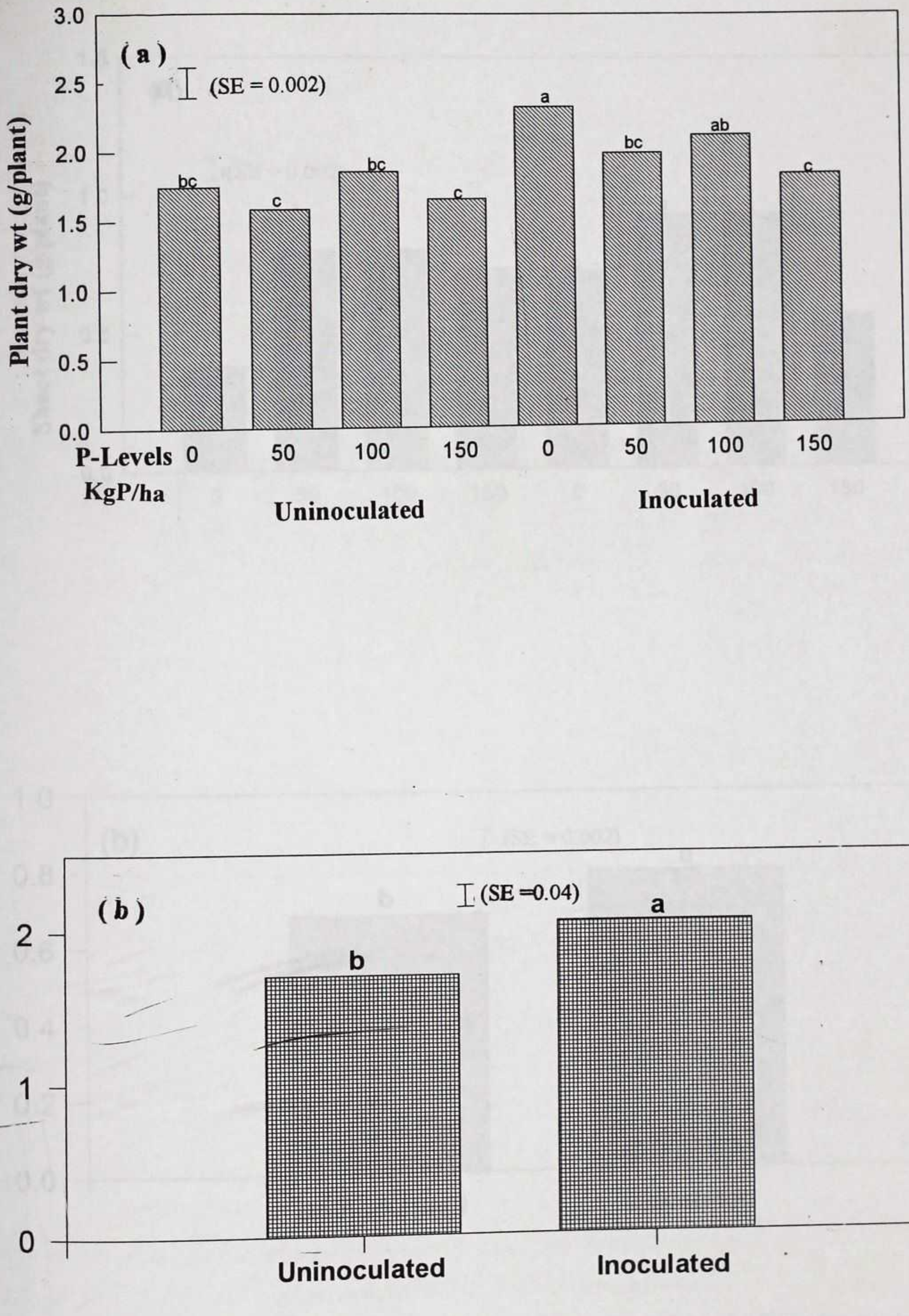
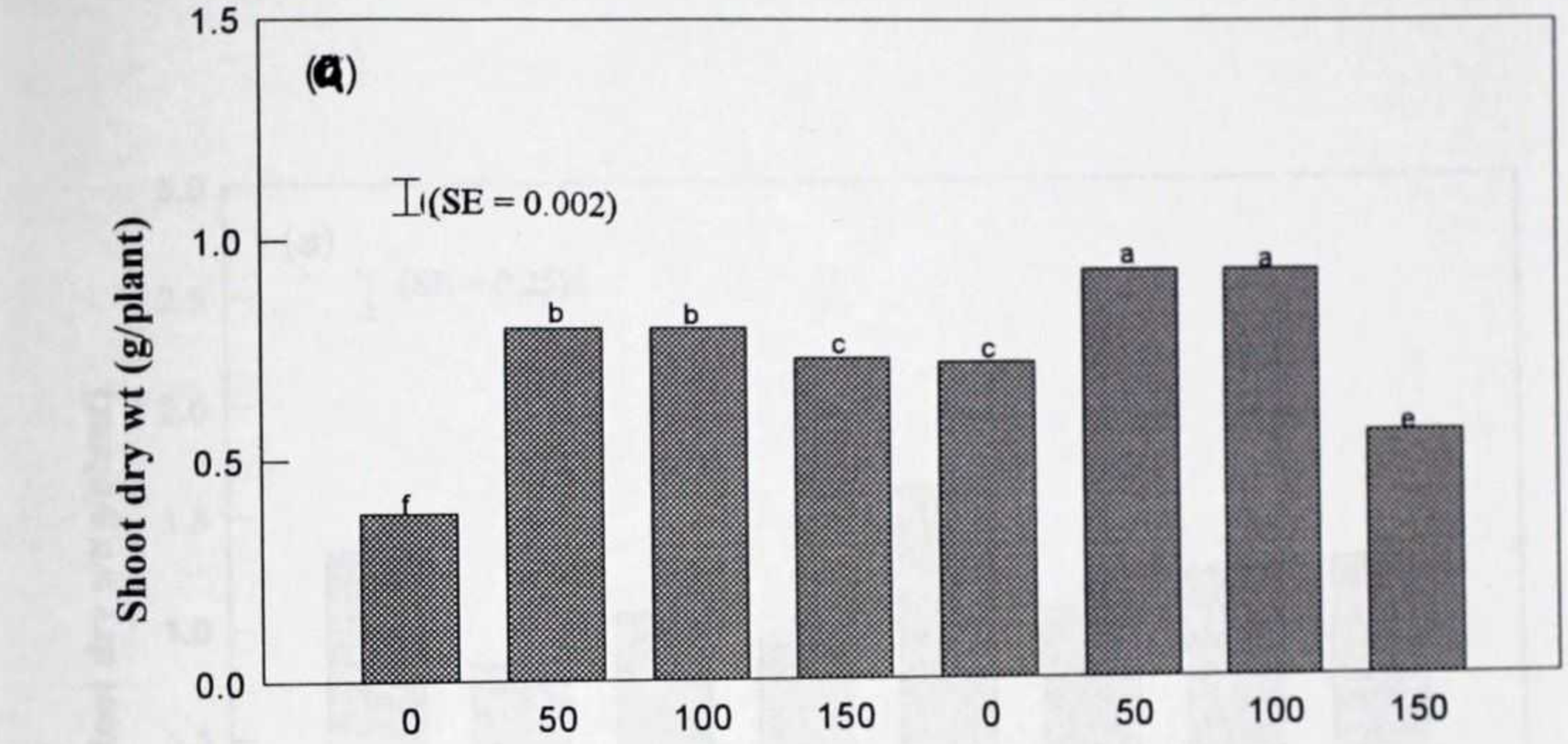


Figure 22. Effect of phosphorus and *Glomus clarum* on Plant dry weight of *Albizia ferruginea*.
(a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments.
Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.



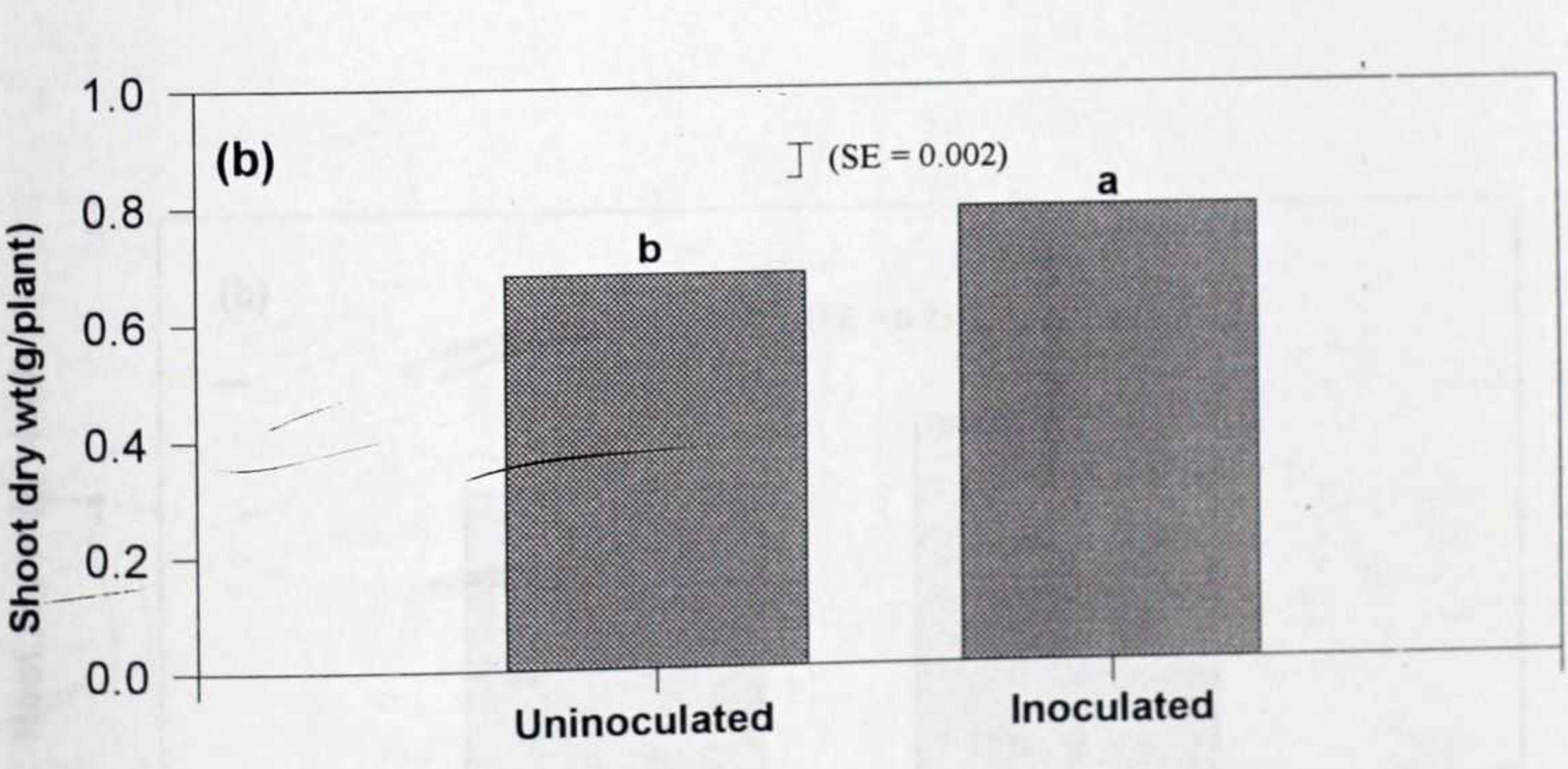
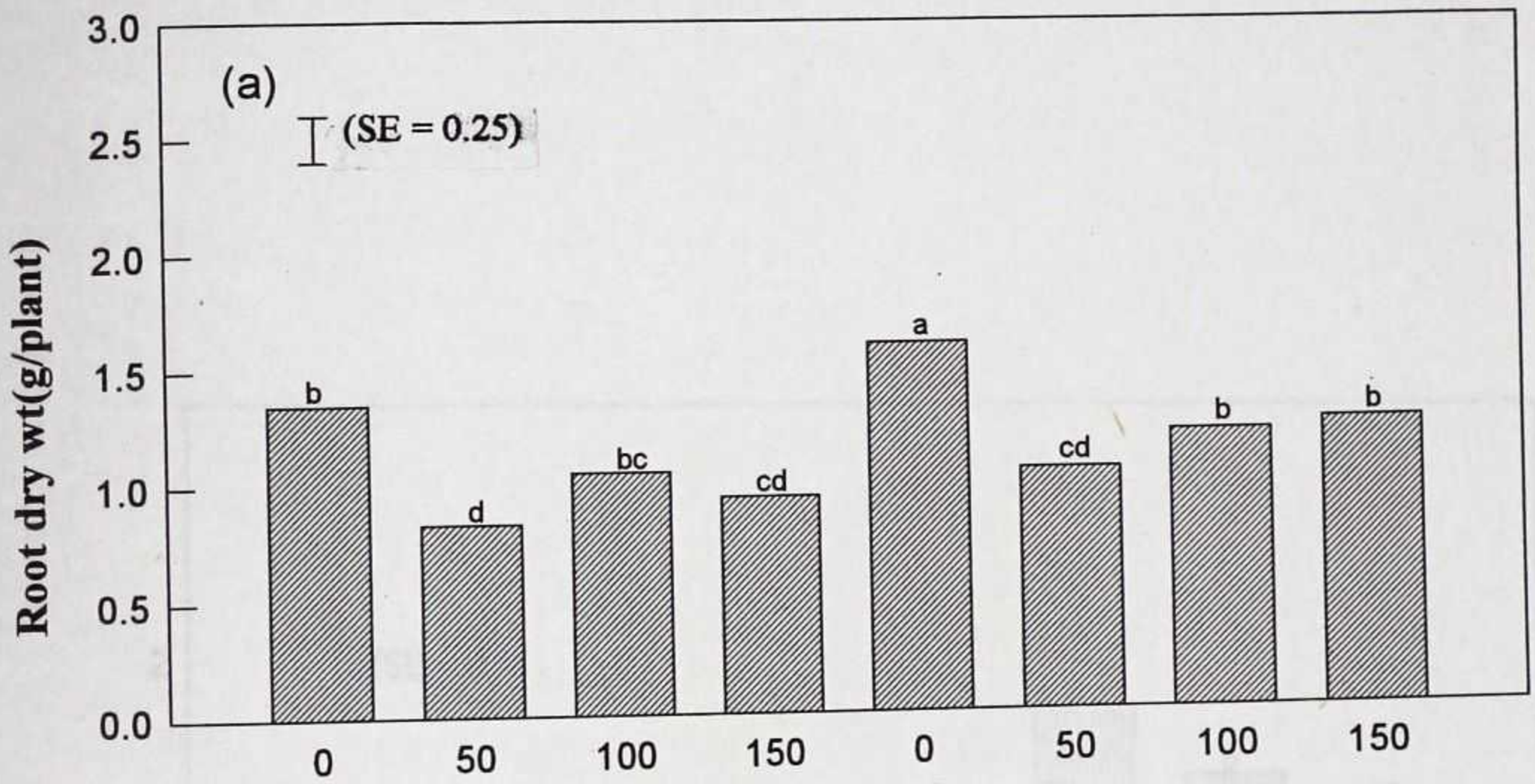


Figure 23. Effect of phosphorus and *Glomus clarum* on Shoot dry weight of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.



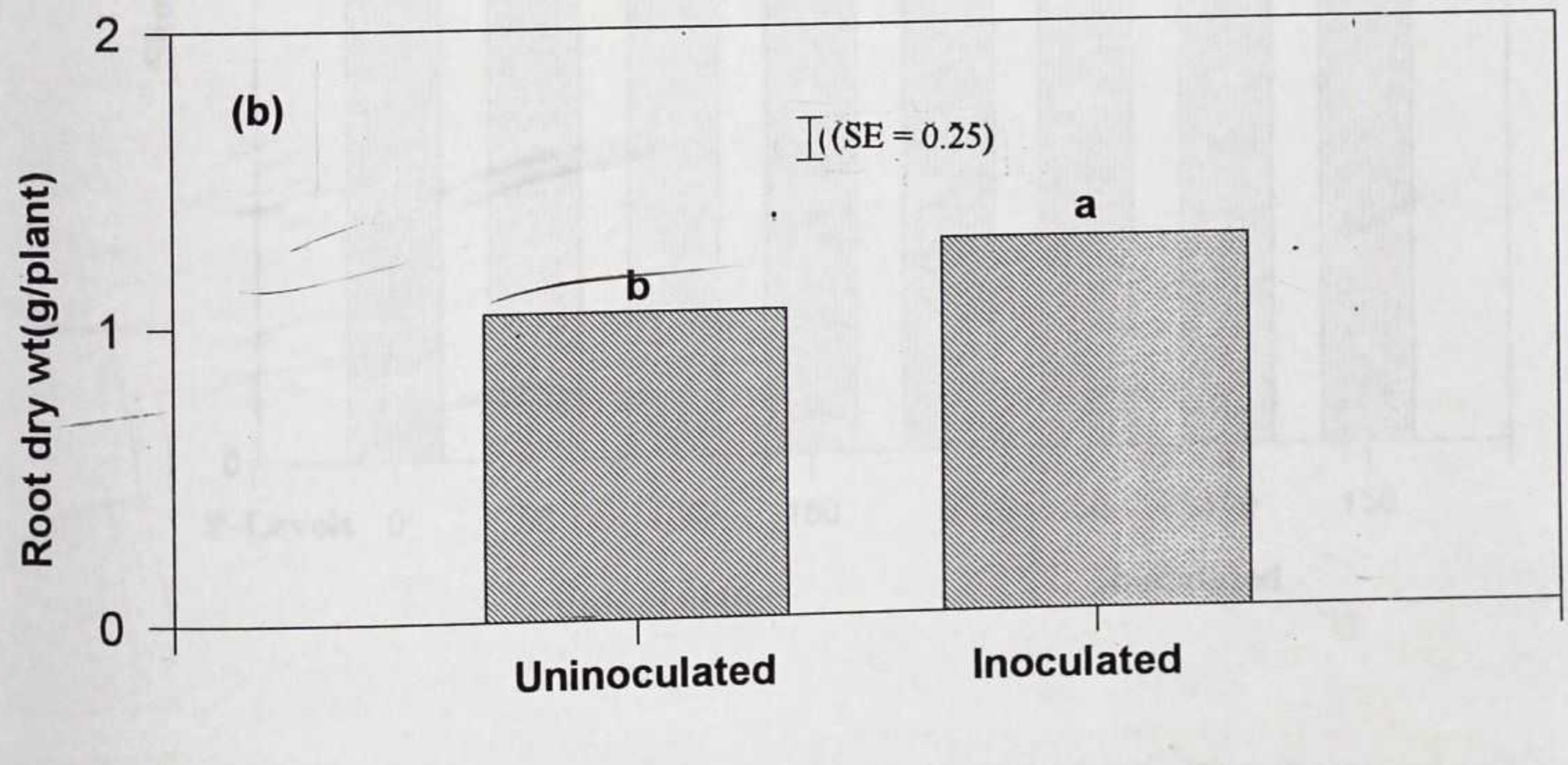


Figure 24. Effect of phosphorus and *Glomus clarum* on Root dry weight of *Albizia ferruginea*.
(a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

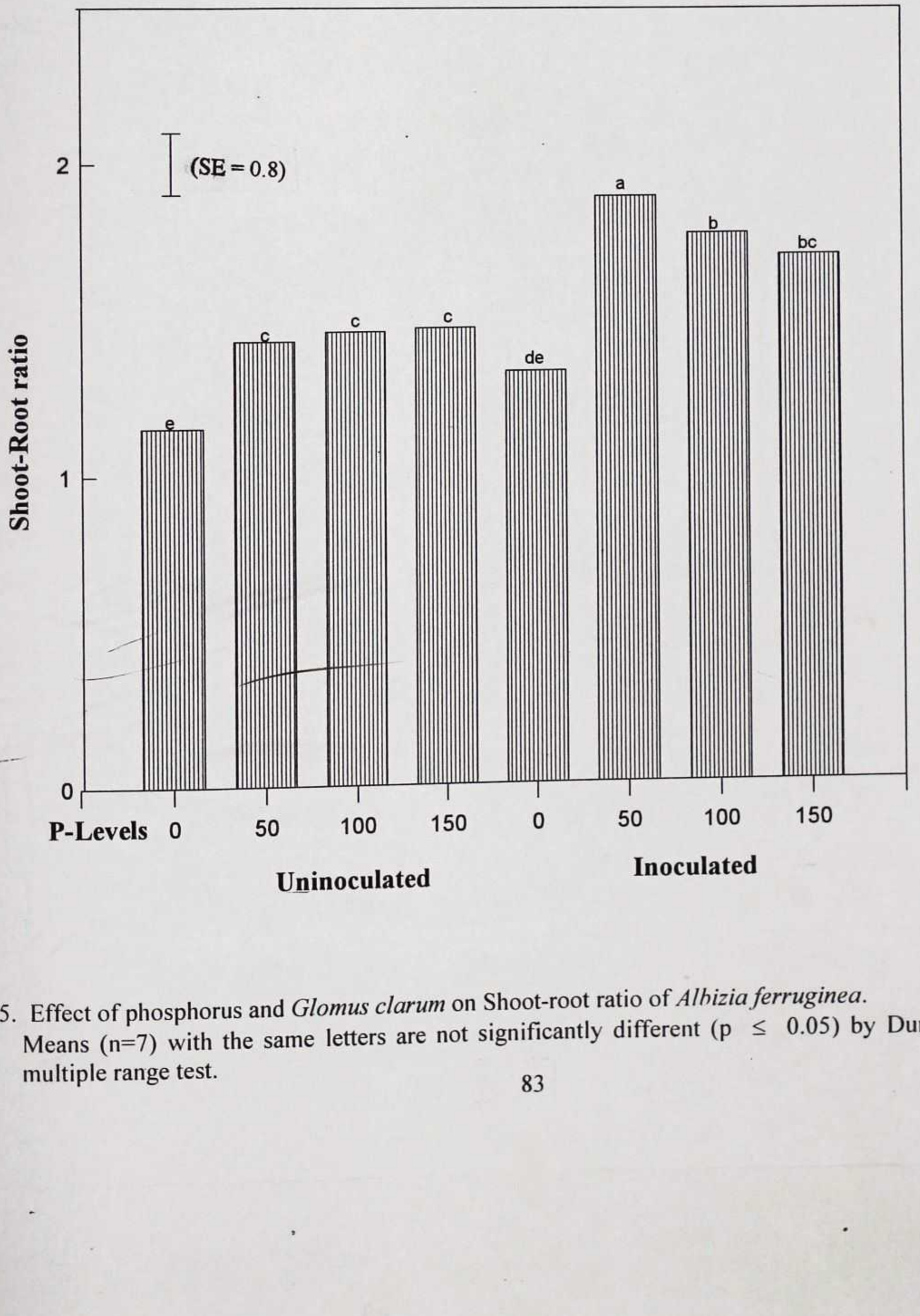


Figure 25. Effect of phosphorus and *Glomus clarum* on Shoot-root ratio of *Albizia ferruginea*. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

However, considering the partitioning of the main effects, inoculated seedlings recorded the highest ratios compared to the uninoculated treatments (figure 25). Main effect and interactions were not significant.

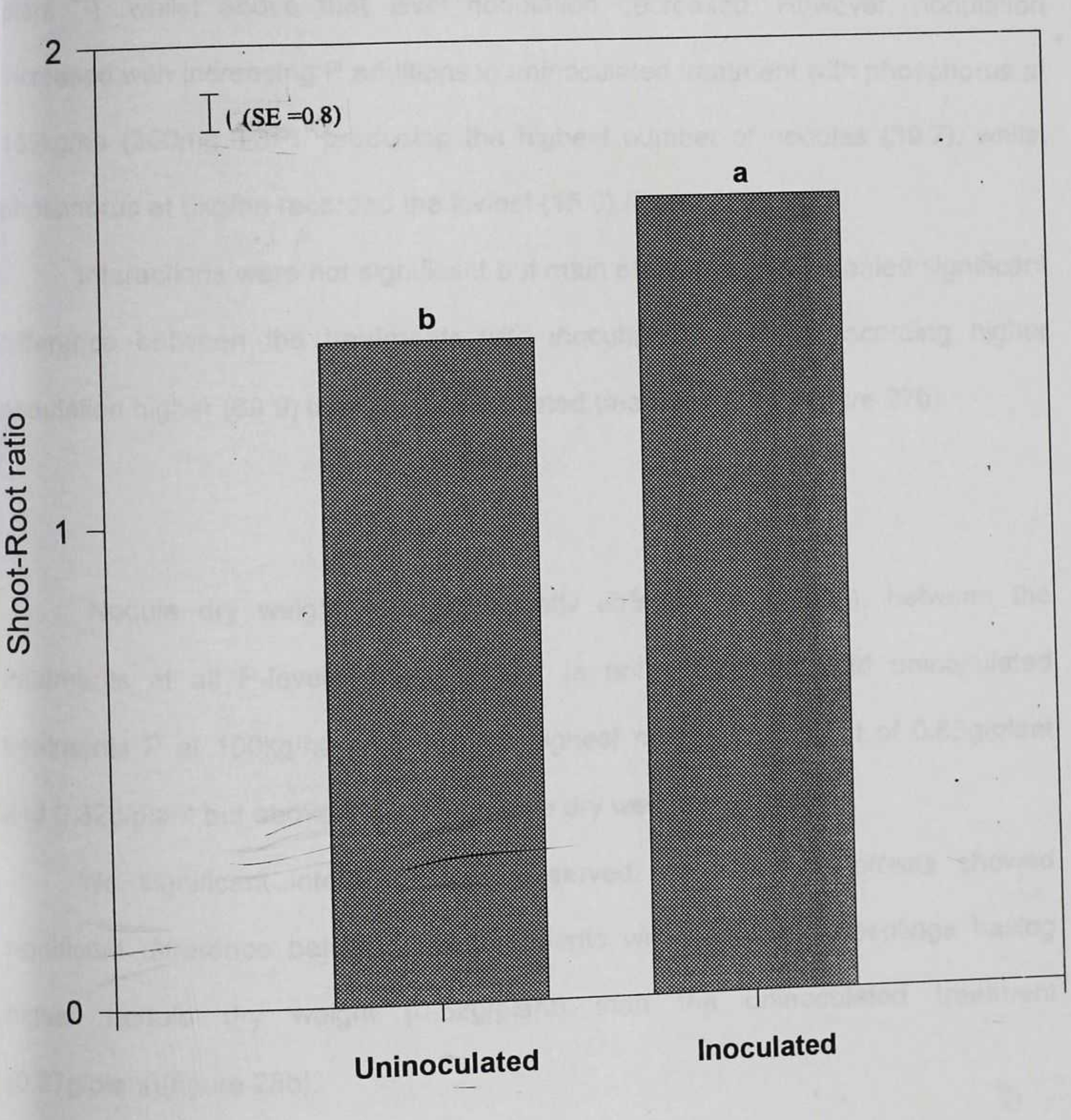


Figure 26. Effect of phosphorus and *Glomus clarum* on Shoot-root ratio of *Albizia ferruginea*. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

4.2.4 Nodulation

Phosphorus stimulated nodulation at all P-levels and was significantly different ($p \leq 0.01$), among the treatments (figure 27a). In the inoculated seedlings, P at 100kg/ha increases nodulation and recorded the highest nodule number (88.4 plant $^{-1}$), whilst above that level nodulation decreased. However, nodulation increased with increasing P additions in uninoculated treatment with phosphorus at 150kg/ha (300mg TSP), producing the highest number of nodules (39.7), whilst phosphorus at 0kg/ha recorded the lowest (15.0) (figure 27a).

Interactions were not significant but main effect analysis revealed significant difference between the treatments with inoculated seedlings recording higher nodulation higher (69.9) than the uninoculated treatment (29.2)(figure 27b).

Nodule dry weight was significantly different ($p \leq 0.01$), between the treatments at all P-levels (figure 28a). In both inoculated and uninoculated treatments P at 100kg/ha recorded the highest nodule dry weight of 0.63g/plant and 0.32g/plant but above 100kg/ha nodule dry weight decreases.

No significant interaction was observed but the main effects showed significant difference between the treatments with inoculated seedlings having higher nodule dry weight (0.52g/plant) than the uninoculated treatment (0.27g/plant)(figure 28b).

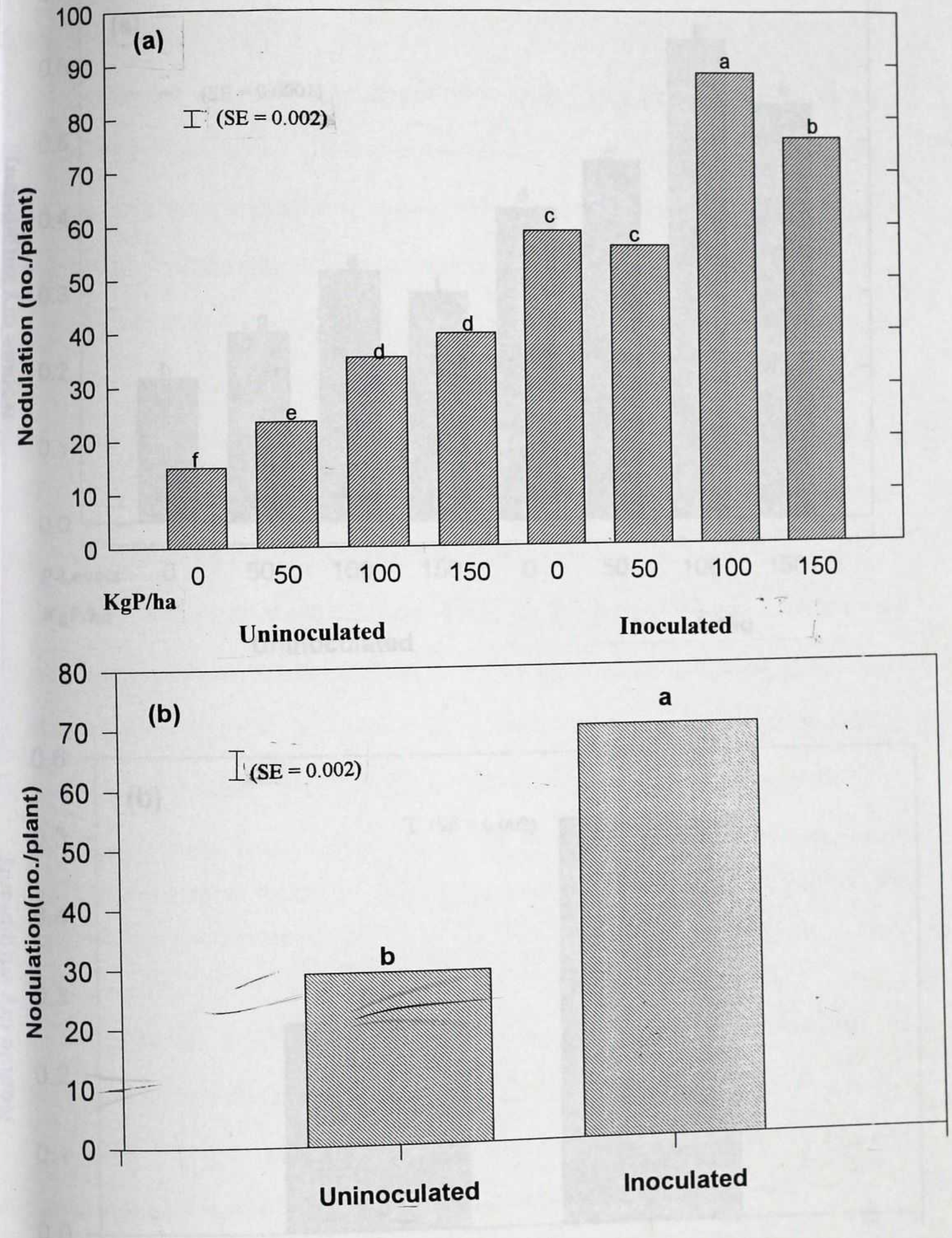


Figure 27. Effect of phosphorus and *Glomus clarum* on Nodulation of *Albizia ferruginea*.
(a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments.
Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

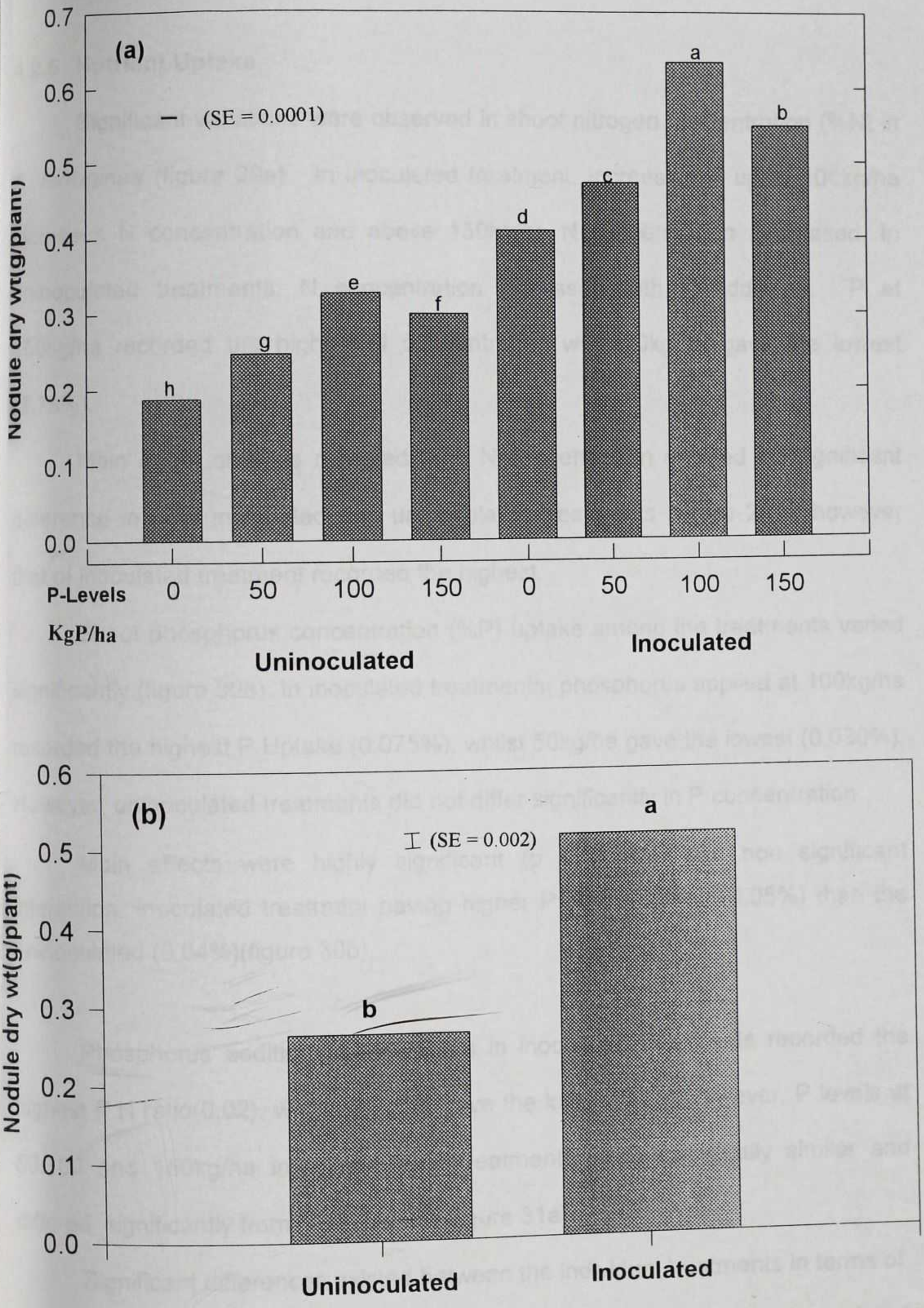


Figure 28. Effect of phosphorus and *Glomus clarum* on Nodule dry weight of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

4.2.5 Nutrient Uptake

Significant variations were observed in shoot nitrogen concentration (%N) in *A. ferruginea* (figure 29a). In inoculated treatment, increasing P up to 100kg/ha increases N concentration and above 150kg/ha N concentration decreased. In uninoculated treatments, N concentration increased with P additions. P at 150kg/ha recorded the highest N concentration whilst 0kg/ha gave the lowest (2.78%).

Main effect analysis revealed that, N concentration showed no significant difference in both inoculated and uninoculated treatments (figure 29b), however that of inoculated treatment recorded the highest.

Shoot phosphorus concentration (%P) uptake among the treatments varied significantly (figure 30a). In inoculated treatments, phosphorus applied at 100kg/ha recorded the highest P Uptake (0.075%), whilst 50kg/ha gave the lowest (0.030%). However, uninoculated treatments did not differ significantly in P concentration.

Main effects were highly significant ($p \leq 0.001$), with non significant interaction. Inoculated treatment having higher P concentration (0.05%) than the uninoculated (0.04%)(figure 30b).

Phosphorus additions at 100Kg/ha in inoculated treatments recorded the highest P:N ratio(0.02), whilst 50kg/ha gave the lowest(0.01) However, P levels at 50,100 and 150kg/ha in uninoculated treatments were statistically similar and differed significantly from P at 0kg/ha (figure 31a).

Significant differences existed between the individual treatments in terms of shoot P:N ratio's with the inoculated treatments being higher than the uninoculated (figure 31b). Main effects and interaction were not significant.

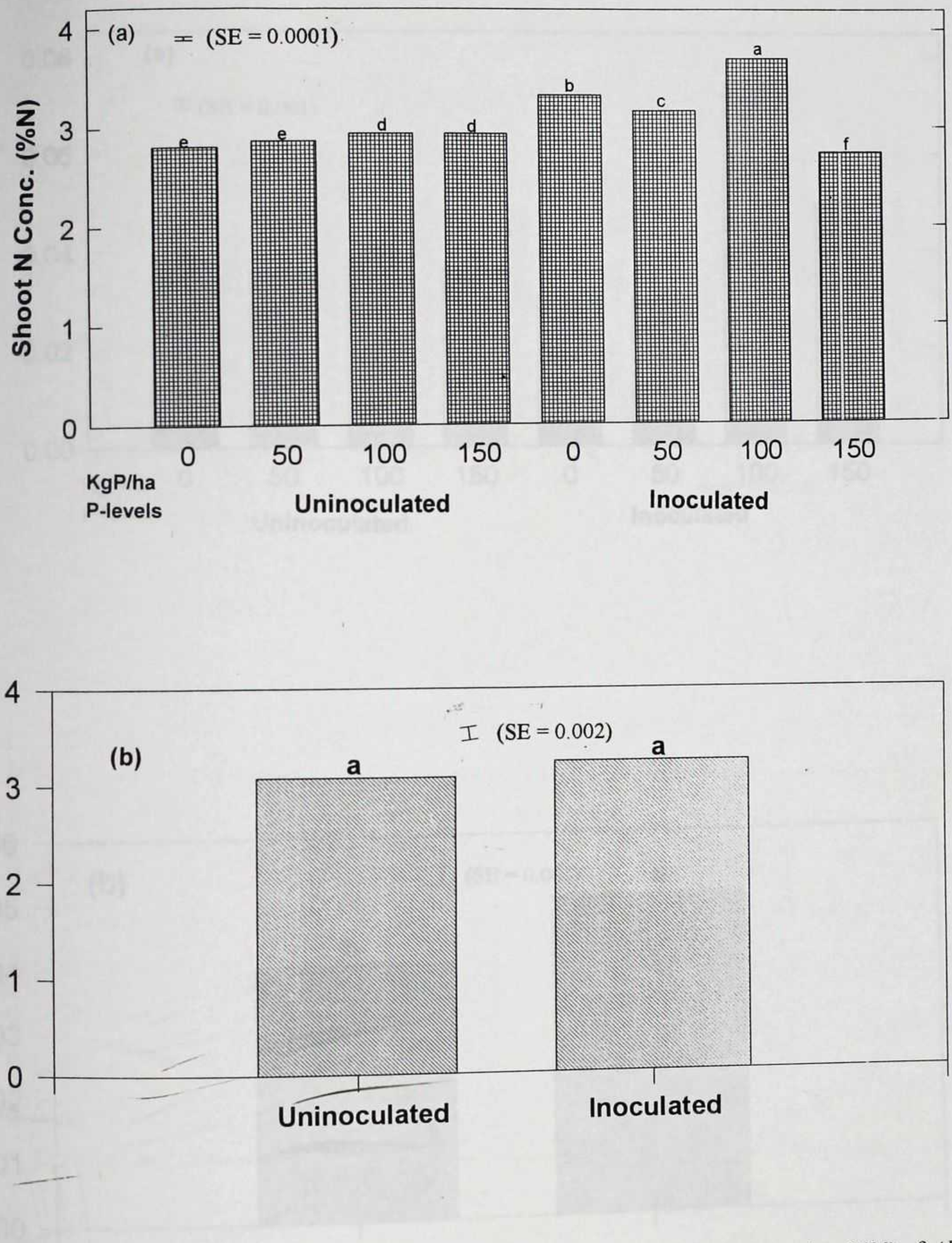
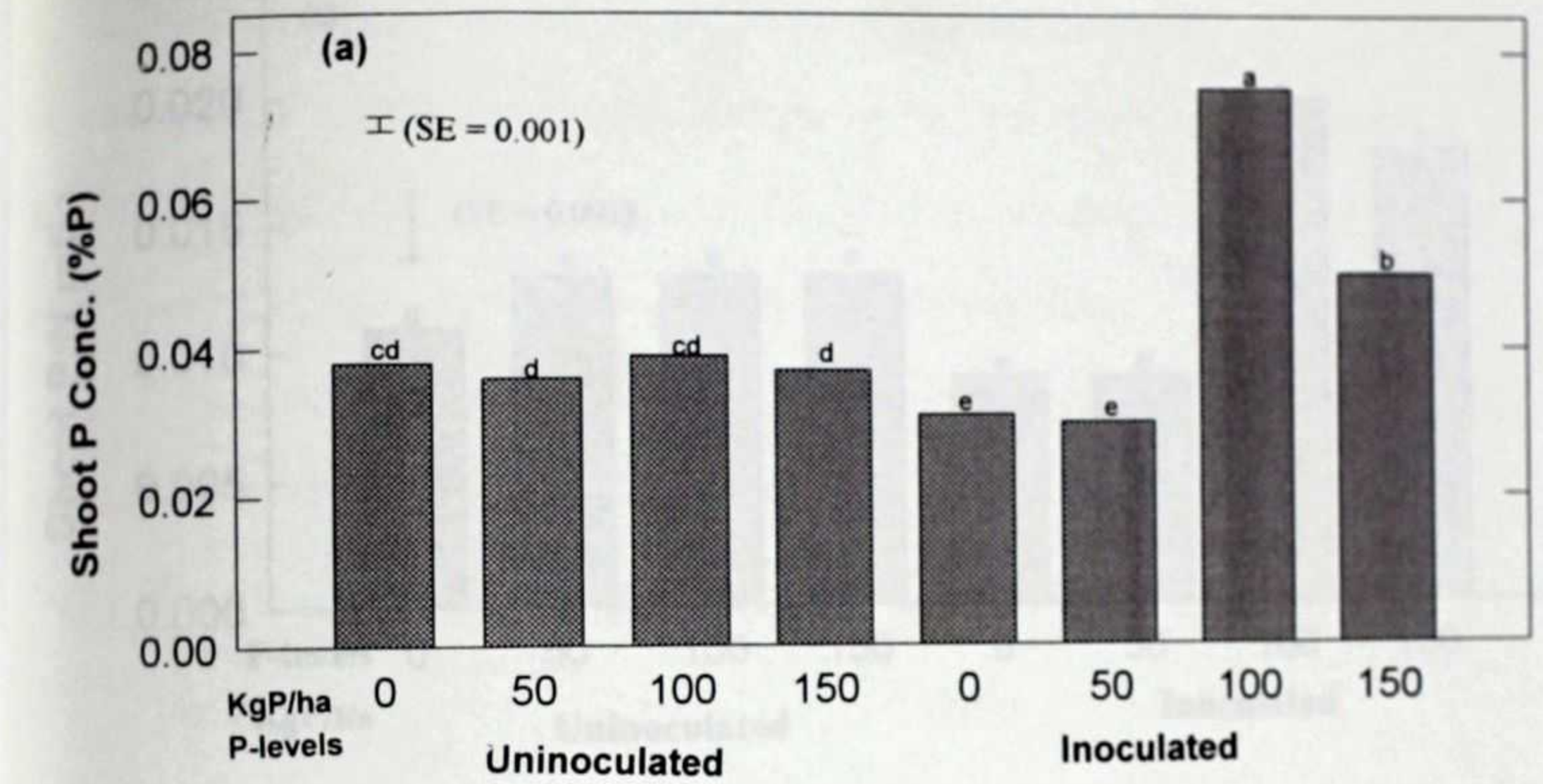


Figure 29. Effect of phosphorus and *Glomus clarum* on Shoot Nitrogen concentration (%N) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.



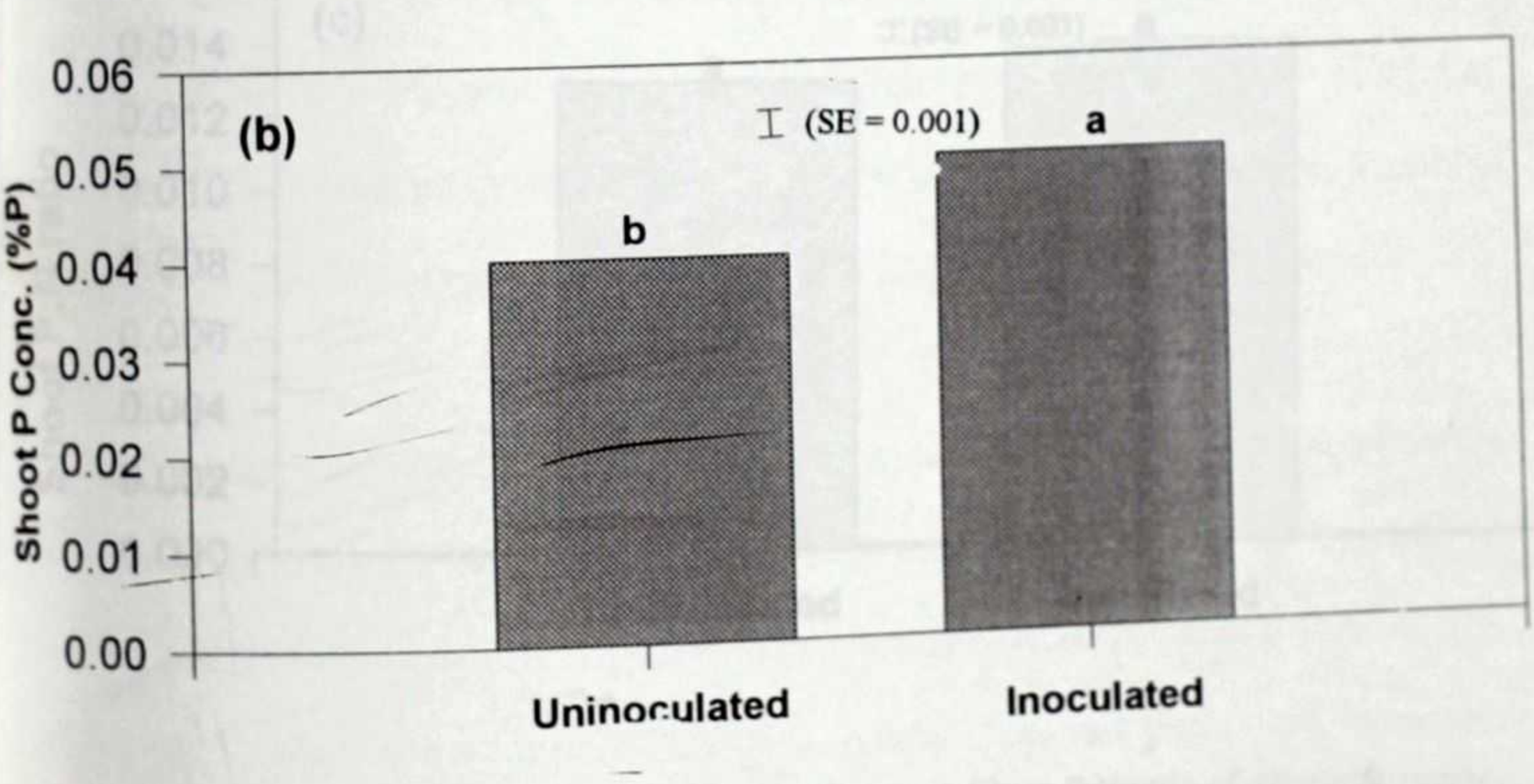


Figure 30. Effect of phosphorus and *Glomus clarum* on Shoot Phosphorus concentration (%P) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

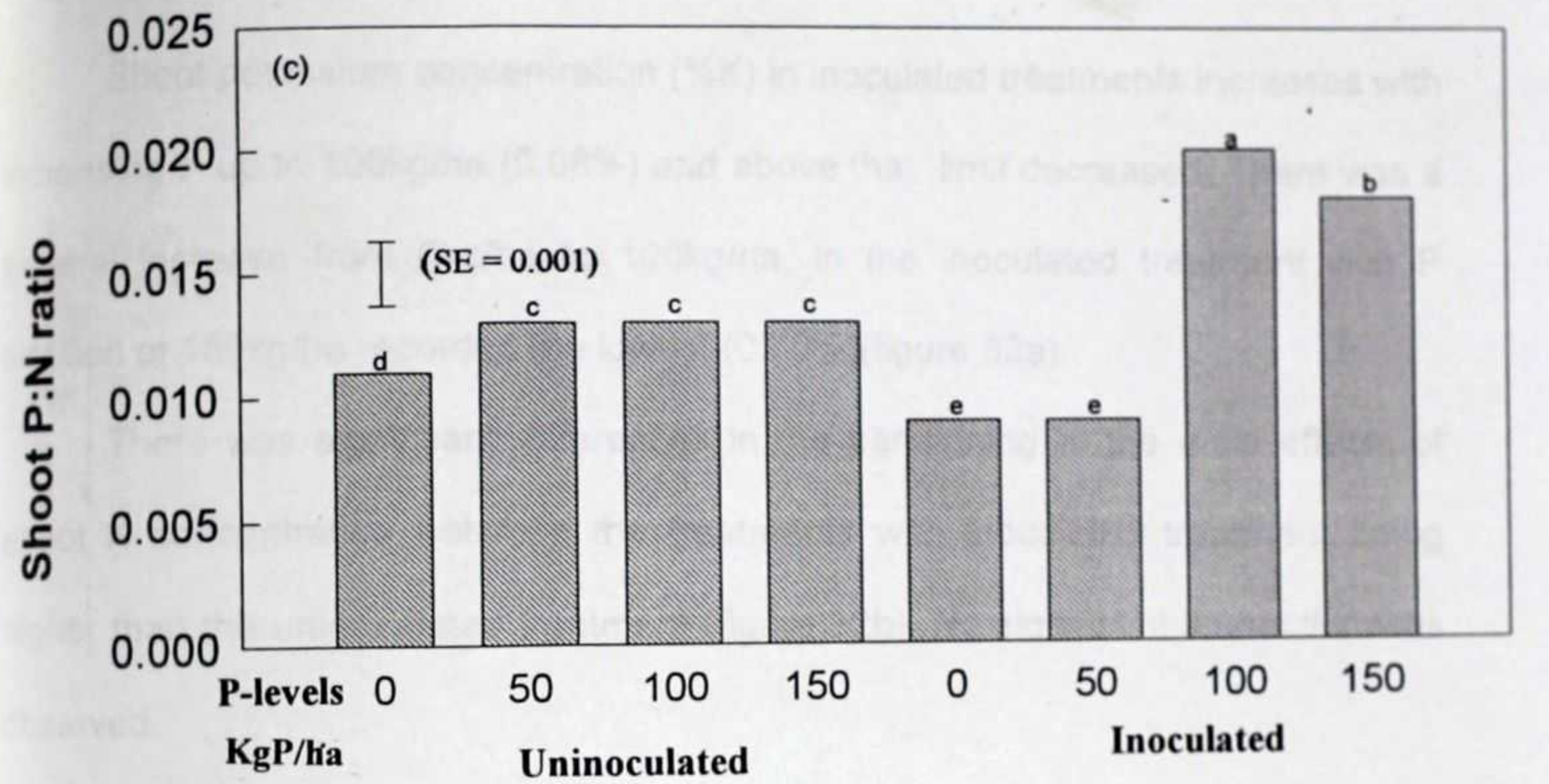


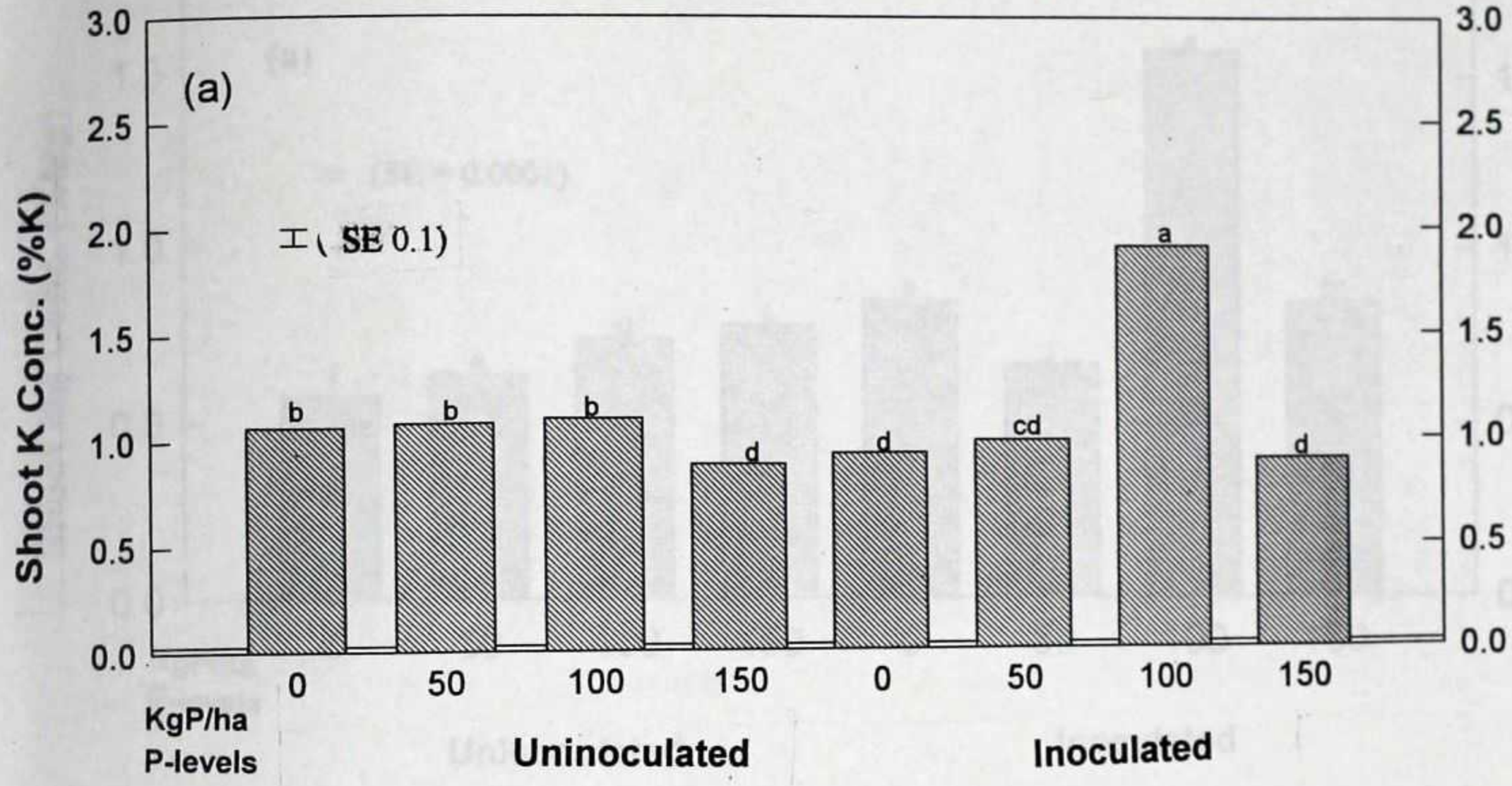
Figure 31. Effect of phosphorus and *Glomus clarum* on Shoot P:N ratio of *Albizia ferruginea*.
(a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments
Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multipl
range test.

Shoot potassium concentration (%K) in inoculated treatments increases with increasing P up to 100kg/ha (0.08%) and above that limit decreased. There was a general increase from 0kg/ha to 100kg/ha, in the inoculated treatment with P addition at 150kg/ha recorded the lowest (0.70%)(figure 32a).

There was significant differences in the partitioning of the main effects of shoot K concentration between the treatments with inoculated treatment being higher than the uninoculated treatment (figure 32b). No significant interaction was observed.

Significant differences in terms of shoot magnesium concentration (%Mg) between the treatments were observed ($P \leq 0.01$)(figure 33a). In inoculated treatments, P at 100kg/ha increased Mg concentration and recorded the highest (1.57%), above that limit Mg concentration decreased. However, in uninoculated treatments Mg concentration increases with increasing P additions. Main effects of Mg concentration were significantly higher in inoculated treatment(0.99%) than the uninoculated treatment with non significant interaction (0.69%)(figure 33b).

Phosphorus additions at 50kg/ha in inoculated treatments significantly increased calcium concentration (%Ca) and then decreased it at 0kg/ha. In uninoculated treatments, Ca concentration increases with increasing P additions (figure 34a). Main effect for shoot Ca concentration was not significant, however, inoculated treatment recorded the highest (0.69%)(figure 34b). The interactions were also not significant.



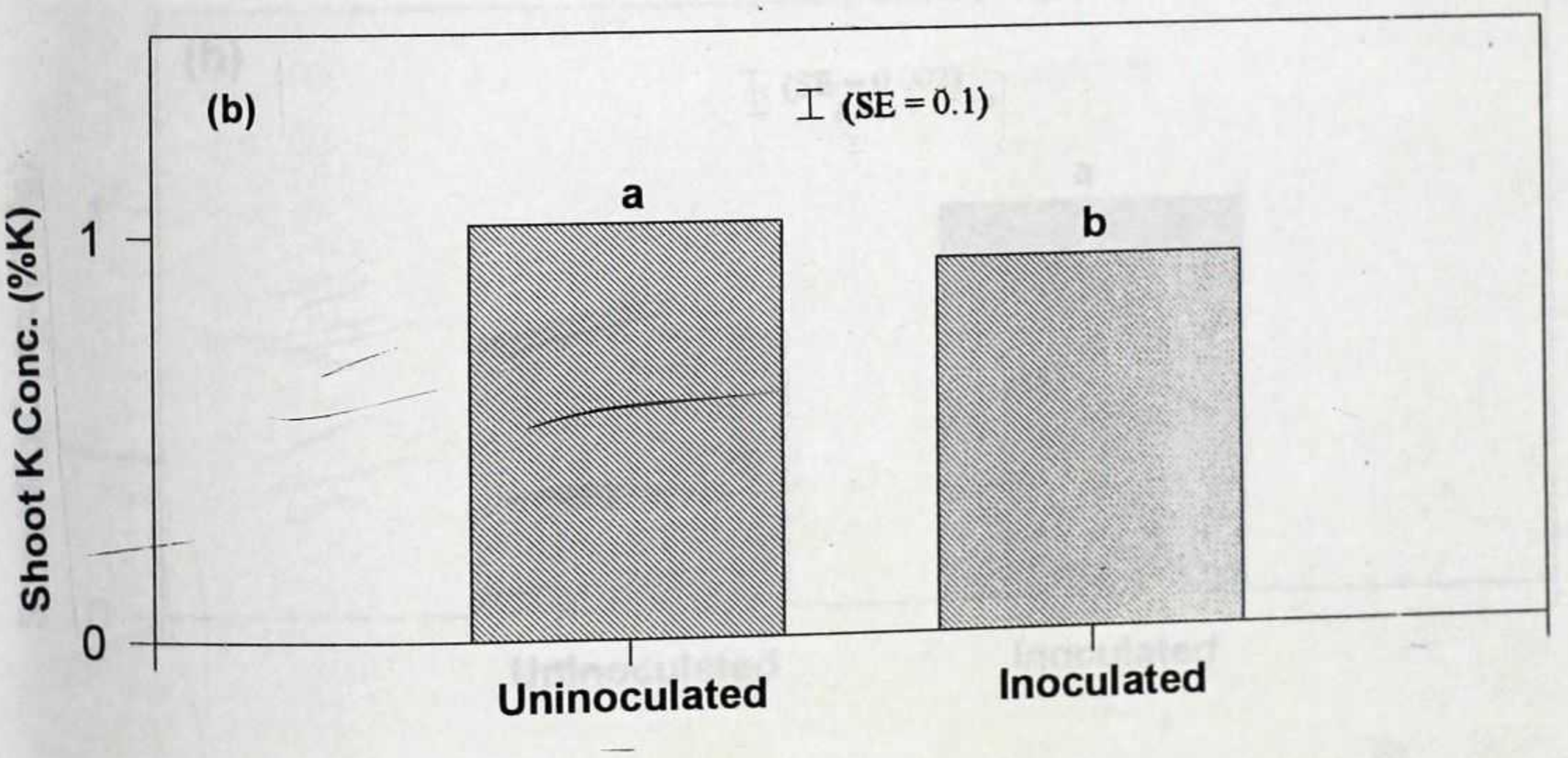


Figure 32. Effect of phosphorus and *Glomus clarum* on Shoot Potassium concentration (%K) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

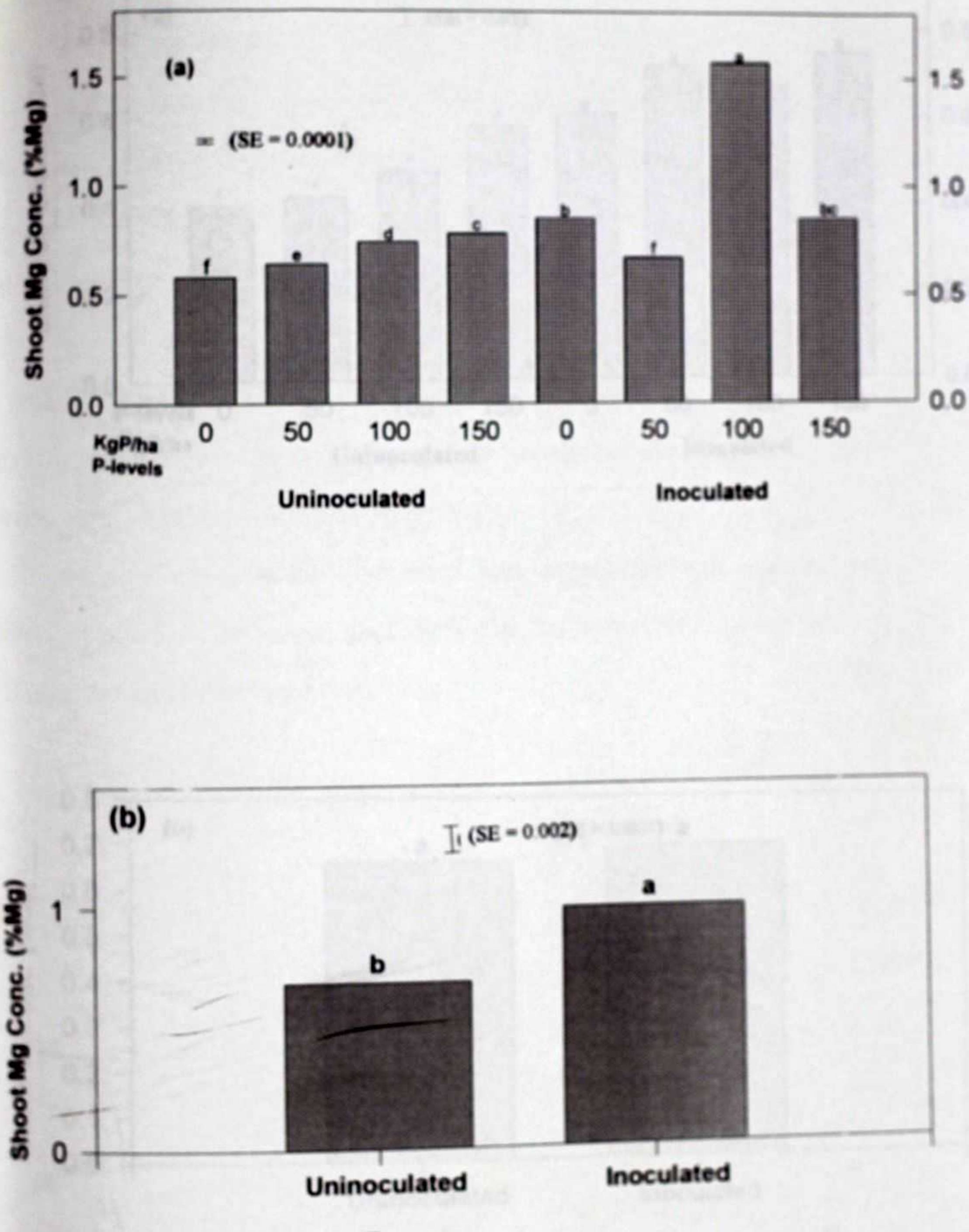
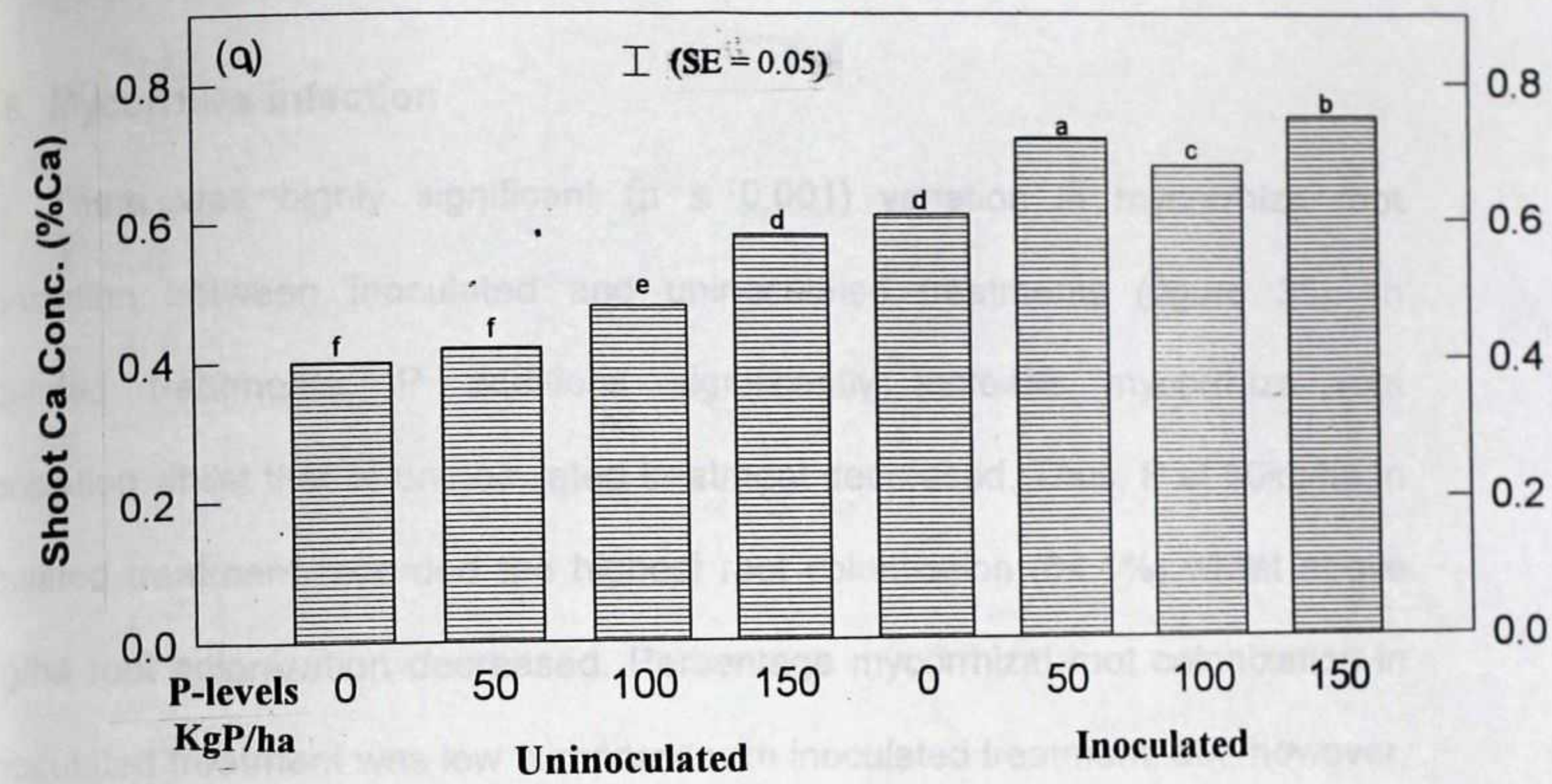


Figure 33. Effect of phosphorus and *Glomus clarum* on Shoot Magnesium concentration (%Mg) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.



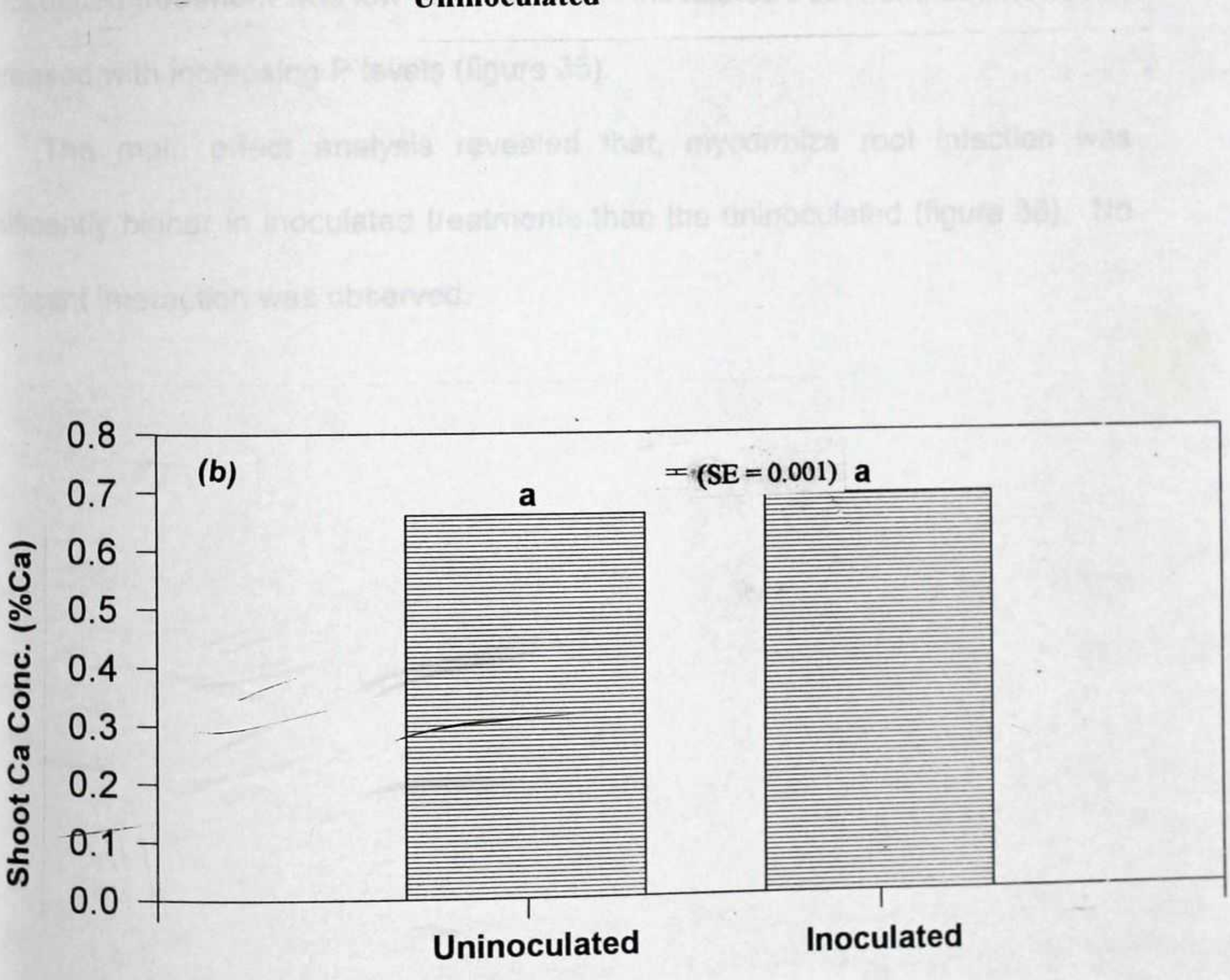


Figure 34. Effect of phosphorus and *Glomus clarum* on Shoot Calcium concentration (%Ca) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* (b) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test

4.2.6 Mycorrhiza Infection

There was highly significant ($p \leq 0.001$) variation in mycorrhiza root colonization between inoculated and uninoculated treatments (figure 35). In inoculated treatments, P additions significantly increase mycorrhiza root colonization whilst that of uninoculated treatment decreased. Thus, P at 50kg/ha in inoculated treatment recorded the highest root colonization (64.1%) whilst above 50kg/ha root colonization decreased. Percentage mycorrhizal root colonization in uninoculated treatment was low compared with inoculated treatment, but, however, decreased with increasing P levels (figure 35).

The main effect analysis revealed that, mycorrhiza root infection was significantly higher in inoculated treatments than the uninoculated (figure 36). No significant interaction was observed.

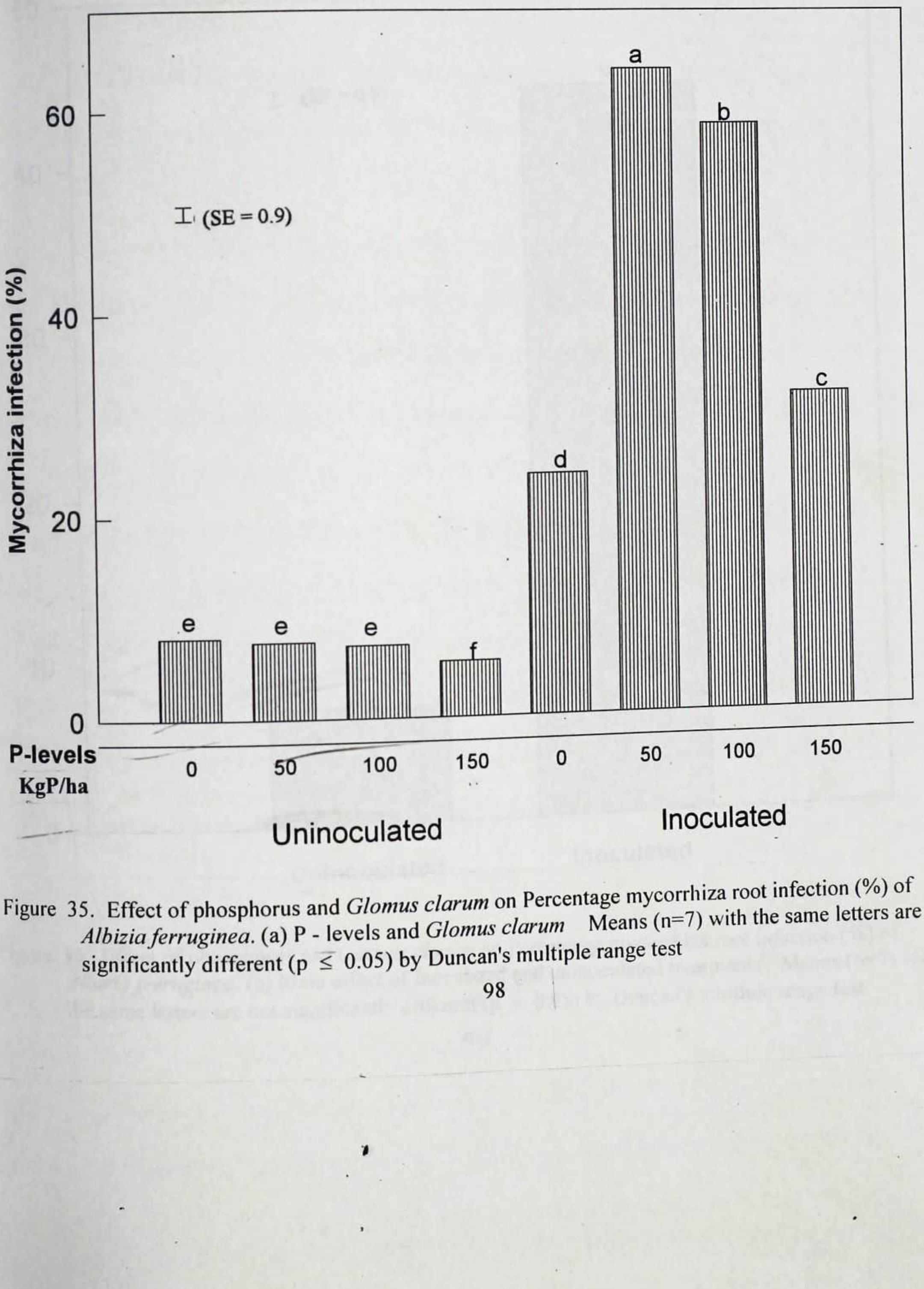


Figure 35. Effect of phosphorus and *Glomus clarum* on Percentage mycorrhiza root infection (%) of *Albizia ferruginea*. (a) P - levels and *Glomus clarum* Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test

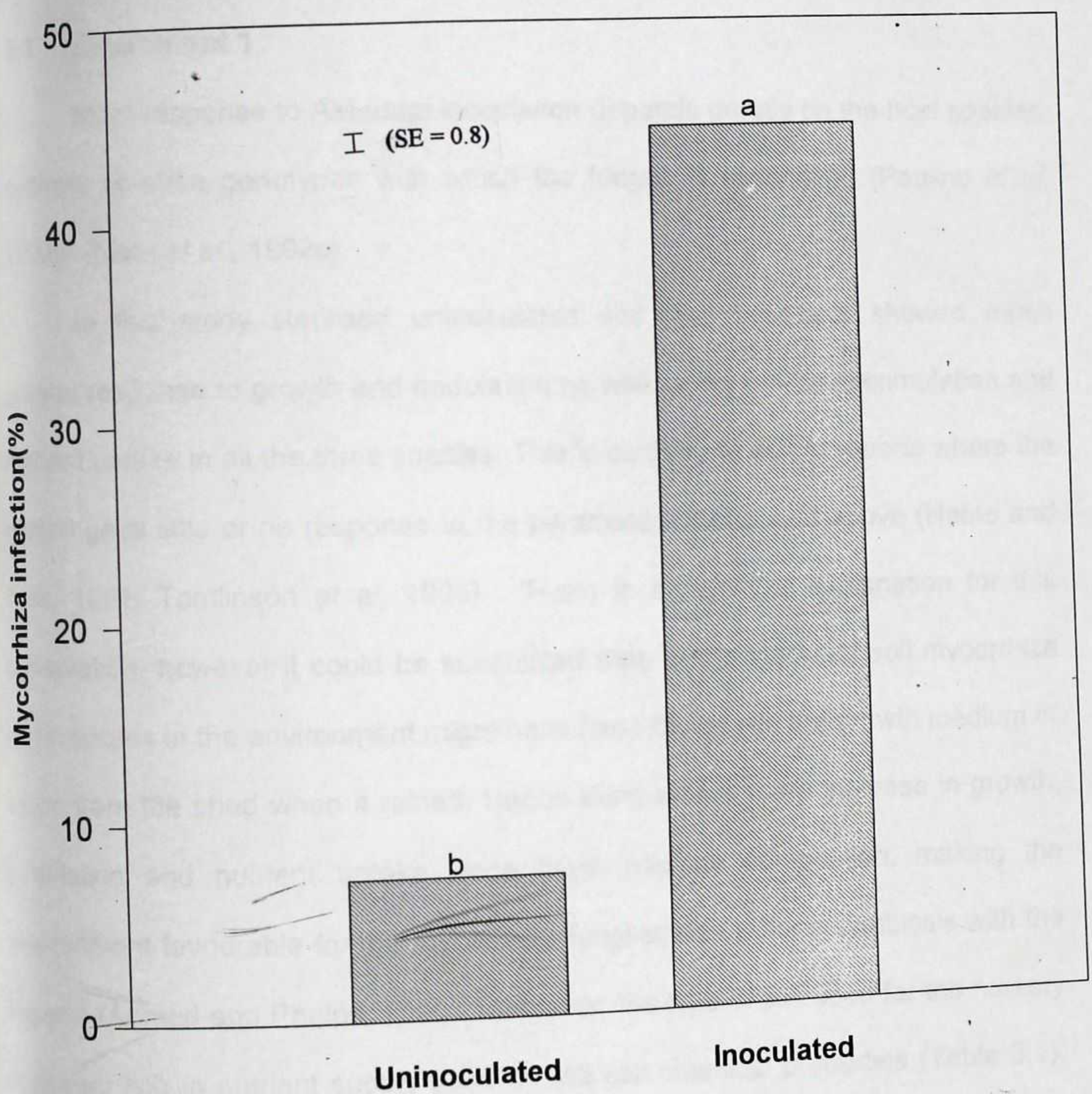


Figure 36. Effect of phosphorus and *Glomus clarum* on Percentage mycorrhiza root infection (%) of *Albizia ferruginea*. (a) Main effect of inoculated and uninoculated treatments. Means (n=7) with the same letters are not significantly different ($p \leq 0.05$) by Duncan's multiple range test

CHAPTER FIVE

5.0 DISCUSSION

5.1 Experiment 1

Plant response to AM fungi inoculation depends greatly on the host species, cultivars or even genotypes with which the fungus is associated (Paulino *et al*, 1992b; Costa *et al.*, 1992c).

In this study sterilised uninoculated soil (Su) treatment showed much greater response to growth and nodulation as well as dry matter accumulation and nutrient uptake in all the three species. This is contrary to earlier reports where the control gave little or no response to the parameters mentioned above (Habte and Turk, 1991; Tomlinson *et al*, 1995). There is no obvious explanation for this observation, however it could be speculated that, in the sterilised soil mycorrhiza strain spores in the environment might have been blown into the growth medium or came from the shed when it rained. Hence there was a sharp increase in growth, nodulation and nutrient uptake since there was no competition, making the environment favourable for the mycorrhiza fungi strains to form symbiosis with the legume (Ahmed and Phelps, 1990). Moreover, the type of soil used for the nursery was very rich in nutrient supply base on the soil chemical properties (Table 3.1), hence might have also contributed to the positive response shown by the sterilised uninoculated treatment. Although sterilised uninoculated treatment gave a lot of positive results in the parameters measured, it produced very low mycorrhiza infection compared to the inoculated treatment, suggesting lack of effectiveness of the native mycorrhizal fungi strains blown into the growth medium (Sanginga *et al*, 1990 and Ndoye *et al*, 1995). There was a positive correlation between AM

infection and P-content in both *A. ferruginea* and *A. adianthifolia* respectively (r^2 = 0.61 and r^2 = 0.69 ; p ≤ 0.05).

However, the AM fungi strains used enhanced some plant growth and nutrient uptake in some of the *Albizia* species but have little or no effect on others. Liyanage *et al*, (1994), also observed significant trend of variation in dry matter, total nitrogen, and nodulation among *Gliricidia sepium* genotypes. Similar differences in nitrogen fixation and growth have been reported in provenances of *L. leucocephala, Casuarinas, Faidherbia albida* and *Gliricidia sepium* (Rai, 1992; Gitonga, 1994; Twum-Ampofo, 1995). Species response to AM fungi inoculation indicate that, *Albizia ferruginea* responded very well to growth in height and diameter followed by *Albizia adianthifolia*, and *Albizia zygia*. Although some reports in the literature indicated that AM fungi strains are non-host specific, they seem to show some preferences to their host plant making them more efficient than others (Dela Cruz *et al*., 1988). Thus in *A. adianthifolia* the most probable preferred strain was *Gigaspora rosea* and *Glomus intraradices* whilst *Glomus etunicatum* could be selected for A. *ferruginea* and *A. zygia* to promote plant initial growth.

Albizia species, which has recently gained popularity as a promising nitrogen fixing trees (NFTs), exhibited significant variation in nodulation, dry matter yield, nutrient uptake as well as percentage root infection. In this study, each *Albizia* species significantly differed in nodulation when inoculated with different AM fungi strains. Thus, in *A. adianthifolia, Glomus intraradices* might be recommended for its growth and development, whilst *Glomus clarum* in *Albizia ferruginea* and *Glomus clarum* or *Glomus etunicatum* may be recommended for *A zygia*. These variations may be attributed to the differences in the genotypes of the various *Albizia* spp (Paulino *et al*, 1992a; Costa *et al*., 1992c). Variability in

nodulation has been reported in other NFTs such as *L. leucocephala*, *Gliricidia sepium* (Liyanage *et al*, 1994, Goi, 1993).

The symbiotic success of a strain with a host may be measured directly by a plant dry matter yield and nitrogen production (Hardarson and Danso, 1993). The differences in the effectiveness of the strains were reflected in the significant variation in the total plant dry matter accumulation. Each of the three *Albizia* species responded differently to the various AM fungal strains, thus *Glomus intraradices*, *Glomus clarum* and *Glomus etunicatum* respectively responded well to *Albizia adianthifolia*, *Albizia ferruginea, and Albizia zygia* in terms of root dry weight. *Gigaspora rosea* showed the highest shoot dry weight in *Albizia adianthifolia*, while *Glomus clarum*, recorded the highest in both *Albizia ferruginea and Albizia zygia*. *Glomus etunicatum* strain proved to be superior to the other strains with the exception of the sterilised uninoculated treatment and thereby recorded the highest dry matter yield in *Albizia ferruginea*. However, *Glomus clarum* and *Gigaspora rosea* proved superior in *Albizia zygia* and *Albizia adianthifolia* respectively.

Shoot to root ratio was very high in *A. zygia*, followed by *A. adianthifolia* and *A ferruginea* in that order. This means that, most of the assimilates in the seedlings might have been invested in the shoot production rather than the root. *Glomus clarum* was significantly different from other treatments in *A zygia*, *Gigaspora rosea* in *A. adianthifolia* and *Glomus clarum* in *A. ferruginea*, whilst that of sterilised uninoculated soil was very low in almost all them. This has also been reported by Smith (1982), that shoot-root ratio is high in plants inoculated with mycorrhiza strains than non mycorrhizal plants which is due to improved mineral nutrition particularly nitrogen. This is of importance in Agroforestry systems in terms of efficient nutrient cycling through proper management by regulating the

quality, quantity, timing, and method of application. Thus, one of the factors considered when selecting Agroforestry tree is high biomass production above ground. Nutrient release through decomposition of tree biomass can be synchronised with the peak period of the crop's nutrient demand, especially in alley cropping, trees on crop land such as *Albizia* species in cocoa farm, coffee and shade tree combination such as *Erythrina poeppigiana* and *Cordia alliodiora*, etc. Moreover, other management practices will lead to the improvement of organic matter status of the soil making it more productive as well as soil conservation (Nair, 1995).

Significant variation in species treatment interaction show that *Albizia ferruginea* responded well to mycorrhiza colonization followed by *Albizia adianthifolia* and *Albizia zygia* respectively.

Large differences were shown between the treatments in each species with *Glomus clarum and Glomus etunicatum* proving to be superior in all the three species in terms of mycorrhiza root infection (figure 23b).

The mycorrhiza root infection of all the three species were also observed to be generally enhanced by *Glomus clarum*. The higher mycorrhiza root infection of plants inoculated with *Glomus clarum* may be attributed to the improved plant N uptake and P-uptake that might be linked to good nodulation and high mycorrhizal infection (Aziz and Habte, Sylvia, 1993).

Increased uptake of nutrients especially phosphorus has been reported as the most important mechanism in which AM fungi improves legume tree growth and nutrition through the P-mediated effect of the mycorrhiza in nitrogen fixation (Barea *et al*, 1990; Azcon *et al*, 1991).

Generally, there was an improvement in the nutrient uptake in almost all the three *Albizia* species under study in terms of Magnesium, Calcium and Potassium

uptake as a result of AM fungal inoculations, attesting to the role AM fungi play in nutrient mobilisation for plant growth (Li *et al.*, 1991).

The consistent superiority of *Glomus clarum* in plant growth, dry matter accumulation as well as nutrient uptake was manifested in its high rate of root infection, hence the most preferred AM fungus strain throughout the experiment.

5.2 Experiment 2

Arbuscular mycorrhizal inoculation (*Glomus clarum*), significantly improved growth of plants, dry matter accumulation, nutrient uptake and percentage root infection over uninoculated seedlings irrespective of phosphorus in the form of tripple superphosphate (TSP) nutrition. This result agrees with the observation that AM inoculation of nitrogen fixing trees has a marked influence on growth as reported earlier by these authors (Jasper *et al.*, 1989; Dela Cruz *et al*, 1990).

Generally, all the plant parameters examined indicated significant differences ($p \leq 0.05$) between the tree species and AM fungi inoculation. The same effect of positive response to phosphorus nutrition on growth of legume trees has been documented in *Acacia* spp (Sun *et al*, 1992) and *Leucaena leucocephala* (Sanginga *et al.*, 1992).

Tripple superphosphate application at the highest level of 200mg (100kg/ha of P), significantly stimulated growth in both inoculated and uninoculated plant seedlings and thereafter decreases. However, *Glomus clarum* improved growth of inoculated seedlings over that of uninoculated plants at the same TSP levels. This is in line with earlier reports made by some authors that, AM fungi inoculation of NFTs has a marked influence on growth (Habte and Turk, 1991; Dala Cruz *et al.*, 1990; Read,1991).

Increasing TSP - concentration significantly increased both nodule numbers and a nodule dry weight in both inoculated and uninoculated seedlings but that of inoculated seedlings was comparably higher. This agrees with the reports that, phosphorus has an active role in the process of nodulation and that alleviating P deficiency causes highly significant increases in nodule numbers and dry weight (Jakobsen, 1985; Sanginga *et al*, 1988; Adu-Gyamfi *et al*., 1989; Barea *et al*., 1989; Twum-Ampofo, 1995).

Inoculation with *Glomus clarum* significantly increased shoot P–uptake in inoculated seedlings than uninoculated ones indicating more efficient absorption of phosphorus from soil by AM inoculated plants. This is attributed to better exploitation of phosphorus from soil by the extension net work of external hyphae increasing the absorption area of roots. Improved plant P uptake from soil by AM fungus inoculation was documented (Costa *et al*, 1992b; Osunde *et al*, 1992; Paulino *et al*, 1992b). Higher P uptake in inoculated seedlings resulted in significant higher accumulation of nitrogen uptake. This confirms observations made by the authors (Adu-Gyamfi *et al*, 1989; Jakobsen, 1985) that phosphorus significantly influence nodulation and nitrogen fixation in legumes.

There are various reports indicating that, low rates of P application increase AM fungal infection (Manjunath and Bagyarai,1994; Habte and Manjunath,1987;Paulino *et al*,1986; Miranda and Harris,1994), whilst high P levels decrease root infection (Abbott *et al*.,1984; Amijee *et al*.,1993; Pearson *et al*.,1994; Auge *et al*.,1995). The same observation was made in this study when tripple superphosphate in addition to *Glomus clarum* inoculation significantly increased

root infection far above that of uninoculated seedlings. However, phosphorus level above 100kg/ha (200mgTSP), root infection rate decreased which suggests that TSP might have become toxic to plant growth. But the degree of inhibition is known to depend on the host species involved (Habte and Turk, 1991; Habte and Manjunath, 1991). The fact that uninoculated seedlings with P applications (TSP) gave some mycorrhizal infection, indicates that phosphorus increased native mycorrhizal infection in uninoculated plants. This pre-supposes that native mycorrhizal fungi can support phosphorus uptake. Interestingly, a similar observation was made by Adu Gyamfi *et al*, 1989 and Jesper *et al*, 1990 where the effect of *Bradyrhizobia* spp or mycorrhizal strains infections was compared with unfertilised plants. P fertilizer and mycorrhiza promote the same degree of root colonization in uninoculated plants at the level of 40kgha^{-1}. However, contrary ideas have been reported to show the existence of differences between legumes inoculated with mycorrhiza species; and results in general support the suggestion that different plants exert different controls on the degree of root infection (Arias *et al*., 1991).

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CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

The data suggest several important conclusions from this study. Species and strains of AM fungi have been reported showing variations in the extent to which they enhance plant development and nutrient uptake as well as mycorrhiza colonization cited in the literature. Hetrick *et al.*, (1985) reported that, although AM fungi are not host specific, they exhibit certain host preferences, whereas some species of AM fungi have the ability to colonise certain plant host than others (Jensen, 1982). Based on the findings from this studies (experiment 1), some of the AM fungi inoculation did not succeed with the biological materials utilised, thus showed preference for their host plant in terms of plant development and root infection. *Glomus intraradices* was the most preferred fungus in *Albizia adianthifolia*, *Glomus clarum* in *A. ferruginea* and *Glomus etunicatum* in *Albizia zygia*. However, *Glomus clarum* proved to be the most efficient fungus in almost all the parameters considered in experiment 1. This superiority of *Glomus clarum* has also been exhibited in other strains in maize, sorghum and Pigeonpea (Simpson & Daft, 1990), and even in tree legumes like *Gliricidia sepium* (Habte and Turk(1991), and Twum-Ampofo(1995). Therefore, it may be a good competitor with other *Rhizobium* strains, hence its suitability as a potential inoculation of *Albizia* species should be further investigated thoroughly. *Gigaspora rosea* was the least effective, probably the species is not effective with the tree legume or not competitive with the native mycorrhiza in the soil.

The study again clearly demonstrated observations made by other authors on the existence of considerable amount of variability in nodulation and plant development in Nitrogen-fixing trees under nursery conditions. Findings from the studies indicated that *A ferruginea* gave the most promising initial growth characteristics as well as nutrient uptake and root colonization. This may offer a promising approach for species selection, and for evaluation of nursery practices, thereby helping the development of Agroforestry.

Phosphorus supply has also been reported to influence nodulation, dry matter accumulation, nutrient uptake, mycorrhiza infection, among others, as indicated in the literature. Experiment 2 demonstrated that, P deficiency affected nodulation and growth of *Albizia ferruginea* and that alleviating phosphorus deficiency resulted in significant increase in the parameters mentioned above. However, at the lowest P levels of 50kglha (100TSP), plant P required for growth was satisfied and further P supply above 100kg/ha (200TSP), may not give any significant returns in the plant growth hence extra cost or becoming toxic and hinder plant development.

The findings from this study indicate that, microbial interactions concerning AM fungal and nitrogen fixing - bacteria are of relevance because they can improve plant establishment, development and nutrient acquisition. Hence, there is a great potential for improving nitrogen fixation and growth of *Albizia ferruginea* by selecting effective AM fungus strain in conjunction with judicious application of mineral fertilizer (TSP). This is because P has a specific function to play on nodule initiation, growth and metabolism as well as stimulating host plant growth. Therefore in most tropical soil where P is limiting, judicious application of TSP may promote nodulation, mycorrhiza infection and growth of *Albizia ferruginea* and

other nitrogen fixing trees. However, high levels of TSP was found to suppressed *Glomus clarum* infection.

6.2 Recommendations:

The outcome of this study indicates significant symbiotic variation in the efficiency of different AM fungi strains to *Albizia* species.

The use of *Glomus clarum* and *Albizia ferruginea* in combination with other crops in the farmers' fields should be widely promoted since they showed very high symbiotic functional compatibility and gave better initial growth characteristics This will help in species selection in Agroforestry development. Moreover, AM fungi inoculations resulted in high biomass production by the *Albizia* species which will lead to efficient nutrient cycling for soil improvement.

Application of P fertilizer (TSP) or combination of P fertilizer (TSP) and AM fungus strain by farmers to their fields should not exceed 50kgha^{-1} (100mg TSP). This is because at the lowest P concentration, plant P required for growth was satisfied and that further P gave no significant returns in plant growth hence extra cost. However, the effect of TSP plus *Glomus clarum* was far above that of the uninoculated seedlings, therefore the use of AM fungi inoculations by farmers should also be promoted.

A lot of on-farm researches into the potential use of different AM fungus on *Albizia* species to specific soil types in Ghana should also be considered. This will help in improving fertilizer use efficiency so that in the absence of adequate mineral nutrition it could be explore to improve the growth of *Albizia* species and other NFT's in nutrient deficient soils.

There is also the need for researchers to look into the economic analysis of these organisms in fertilizer recovery and then throw more light on the profitability of AM fungus application so as to enhance its adoption by farmers.

Researchers should also come out with various Agroforestry technologies where *Albizia* species are part of the tree components taking into account the several potential attributes shown in this study.

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