Effect of mycorrhizal inoculation and phosphorus levels on growth and yield of wheat and maize crops grown on a phosphorus deficient sandy soil

By

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Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

An understanding of the mycorrhiza of agronomic crops is essential because of their potential involvement in systems of agricultural sustainability. Mycorrhiza is an important component of soil life and soil chemistry as they channel carbon to the soil, thus improving soil aggregation and also excrete organic acid which aids in ion displacement. Despite numerous reports on the positive effects of arbuscular mycorrhizal (AM) fungi on plant growth and yield in phosphorus (P) deficient soils, surprisingly little data exist on the importance of AM fungi for crop growth and yield on P deficient soils of semi-arid regions of Southern Africa.

The general objective of the study was to evaluate superphosphate as a P source for wheat and maize in pot and in field trials with P deficient sandy soil and investigate the possibility of improving the crop responses to P fertilization by seed inoculation with AM fungi, afterwards referred to as mycorrhiza. To achieve this, temperature controlled glasshouse experiments were conducted with wheat (*Triticum aestivum* L: SST 027) and maize (*Zea mays* L: CRN 3505) at the Department of Agronomy of the University of Stellenbosch, in the Western Cape Province of South Africa in 2009 winter and summer cropping seasons respectively while a field experiment was conducted with maize (*Zea mays* L: KEP) in the 2009/2010 summer growing season in the North-West District of Botswana.

Pot trials in the temperature controlled glasshouse with P deficient sandy soil (18 mg P kg^{-1} soil ; citric acid method), showed that both crops responded positively to P application levels of between 0 and 40 kg P ha^{-1}. At early growth stages both wheat and maize showed little or even negative responses to seed inoculation with AM fungi mainly due to the fact that at these early growth stages of the plant the mycorrhiza is still in the process of colonizing the plant roots. However, at later growth stages root moisture content, shoot mass, number of grains per plant and 1000 kernel weight responses of wheat to P application level were significantly enhanced by mycorrhizal seed inoculation, while mycorrhizal seed inoculation significantly increased leaf area, root mass, root moisture

content, shoot mass, grain yield and 1000 kernel weight. A positive interaction between P application level and mycorrhizal seed inoculation was also observed with regard to plant height and shoot mass of maize plants while mycorrhizal seed inoculation significantly increased leaf area, plant height, root mass, as well as shoot mass of maize plants. The results of this experiment also showed that nutrient contents were higher in mycorrhizal plants compared to non-mycorrhizal plants.

The field experiment done in Botswana in a sandy soil with P content of 7 mg P kg^{-1} soil (citric acid method) comprised of 40 plots half of which (20 plots) were sown with inoculated seeds while the other half was sown with uninoculated seeds designated mycorrhiza (AM fungi) inoculated (M_1) and uninoculated (M_0) respectively. Mycorrhizal seed inoculation was done at sowing by applying 7.35 g of inoculum obtained from Biocult (Pty) Ltd Somerset West, RSA to 1225 g of maize seed. Phosphorus was applied at planting as single superphosphate (10.5%) at five different levels (control P_1 = 0 kg P ha^{-1}, P_2 = 10 kg P ha^{-1}, P_3 = 20 kg P ha^{-1}, P_4 = 30 kg P ha^{-1} and P_5 = 40 kg P ha^{-1}). In this experiment, increasing P application levels significantly increased shoot mass, percent mycorrhizal colonization, grain yield per cob and number of grains per cob. Mycorrhizal seed inoculation had a significant positive effect on grain yield per cob as well as on 100 grain weight. However, no significant interaction between P fertilization and mycorrhizal seed inoculation was observed in this experiment and this may be due to the fact that even at the highest application level of 40 kg P ha^{-1}, the growth and yield response of maize to P were still positive.

This study confirmed that seed inoculation with AM fungi may improve crop growth and responses to P fertilizer in P deficient soils.

UITTREKSEL

Kennis van interaksies tussen gewasse en mikorrisa is belangrik omdat dit die volhoubaarheid van produksiesisteme mag beïnvloed. Mikorrisa is ‘n belangrike komponent van grondlewe en grond chemiese aktiwiteite omdat dit koolstof na die grond kan kanaliseer om grondstruktuur te verbeter en ook organiese sure kan afskei wat help met die vrystelling van ione in die grond. Ondanks verskeie publikasies oor die positiewe uitwerking van arbuskulêre mikorrisa (AM) op groei en opbrengs van plante in fosfor (P) gebrekkige grond, is verbasend min inligting beskikbaar oor die belang daarvan op groei en opbrengs van gewasse in semi-ariede omgewings van Suider-Afrika.

Die algemene doel van die studie was om superfosfaat as bron van fosfaat bemesting vir koring en mielies in pot en veldproewe te evalueer en om vas te stel of die effektiwiteit daarvan in P gebrekkige grond verhoog kan word deur die saad met endomikorrisa te ent. Om dit te bereik is potproewe met koring (*Triticum aestivum* L; cv SST027) gedurende die winter van 2009 en mielies (*Zea mays* L; cv CRN 3505) gedurende die somer van 2009 in ‘n temperatuurbeheerde glashuis van die Departement Agronomie van die Universiteit van Stelllenbosch in die Weskaap Provinsie van Suid Afrika uitgevoer, terwyl veldproewe met mielies in Botswana gedoen is.

Resultate van die pot proewe wat in P gebrekkige grond (18 mg P kg^{-1}, sitroensuurmetode) uitgevoer is, toon dat koring en mielies positief gereageer het teenoor toenemende P vlakke tussen 0 en 40 kg P ha^{-1}. Tydens vroeë groeistadiums het beide gewasse egter min of selfs negatiewe reaksies teenoor saadbehandeling met mikorrisa getoon omdat mikorrisa tydens hierdie vroeë groeistadiums nog besig was om die plantwortels te koloniseer. Op latere groeistadiums is die invloed van P op die wortelvoginhoud, stammassa, aantal korrels $plant^{-1}$ en duisendkorrelmassa van koring egter verbeter deur saadbehandeling met mikorrisa. Saadbehandeling met mikorrisa het assulks die blaaroppervlakte, wortelmassa, wortelvoginhoud, stammassa, graanopbrengs en duisendkorrelmassa van koring verhoog. ‘n Positiewe interaksie tussen die P toedieningsvlak en saadbehandeling met mikorrisa ten

opsigte van plant hoogte en stammassa van mielie plante is waargeneem, terwyl saadbehandeling met mikorrisa assulks die blaaroppervlakte, planthoogte asook wortel- en stammassa van mielieplante verhoog het. Resultate toon ook dat die minerale inhoud van plante wat met mikorrisa geënt is, hoër was in vergelyking met ongeënte plante.

Die veld eksperiment wat in Botswana uitgevoer is op ‘n sanderige grond met ‘n P inhoud van 7 mg P kg^{-1} grond (sitroensuurmetode), het uit 40 persele bestaan waarvan die helfte geplant is met mikorrisa behandelde mieliesaad (7.35 g entstof bevattende *Glomus intraradices* per 1225 g saad) en die ander helfde met onbehandelde saad. Fosfor is toegedien as enkel superfosfaat teen vlakke van 0 (P_1), 10 (P_2), 20 (P_3), 30 (P_4) en 40 (P_5) kg P ha^{-1}). Toenemende P vlakke het in hierdie eksperiment die plante se stammassas, mikorrisa kolonisasie, pitmassa kop^{-1} en pitte kop^{-1} verhoog. Saad behandeling met mikorrisa het die pitmassa kop^{-1} en 100 pitmassa verhoog. Geen interaksie is egter waargeneem tussen die P-vlakke en mikorrisa behandelings, wat waarskynlik daaraan toe te skryf is, dat plante se P behoefte selfs by die hoogste P vlakke steeds nie bevredig is nie.

Hierdie studie bevestig dus die resultate van vorige navorsing wat toon dat saadbehandeling met mikorrisa die groei van gewasse en reaksie teenoor P bemesting op P gebrekkige grond kan verbeter.

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DEDICATION

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CONTENTS

CHAPTER 1

INTRODUCTION

Crop production is an important agricultural activity in semi-arid regions and crops are widely grown in many countries in the world. Production of hybrid crops has been given wide publicity among crop farmers in the world in recent times. However, hybrid crops are known for their high demand for plant nutrients and other production inputs. Soil degradation and nutrient depletion have been increased by continuous crop production with low inputs and have become a serious threat to agricultural production in semi-arid regions. A primary limitation of crop production in semi-arid regions is the deficiency of available nutrients especially phosphorus (P) and water (Nagarathna *et al.,* 2007). Phosphorus is a vital plant nutrient and a major yield-determining factor required for crop production (Holford, 1997). It is an essential nutrient both as a part of several key plant structure compounds and as a catalyst in the conversion of numerous key biochemical reactions in plants (Griffith, 2004).

Phosphorus has to be added to most soils to make sure that adequate levels are available for optimum crop growth and yield. The use of inorganic fertilizers by the resource poor small scale farmers and large scale farmers in some poor developing countries is made difficult by their scarcity or high cost. In Botswana, traditional farmers who grow crops do not apply fertilizer or manure to the crop. However, soil moisture status seems to be the first limiting factor for crop production in Botswana with the mean annual rainfall varying from a maximum of about 650 mm in the extreme northeast area of the Chobe District to a minimum of less than 250 mm in the extreme southwest part of the Kgalagadi District. Limited soil moisture availability can inhibit plant growth both directly due to water stress, and indirectly as a result of limited nutrient availability, the latter specifically with P (Ramolemana, *et al.,* 2002). Most of the soils under cultivation in Botswana are also reported to have low soil P availability which limits crop production (Ramolemana, *et al*., 2002). The FAO Soil Mapping Project found that the majority (69%) of soils tested in Botswana, excluding the Western Region have soil P less than 5 mg kg^{-1} (Beynon, 1991).

Research on several crops in Botswana therefore showed large responses to P applications (Beynon, 1991; Tacheba & Moyo, 1994; Ramolemana, 1999; Ramolemana *et al.,* 2000).

It will be advisable to develop cheaper solutions such as mycorrhizal inoculation to enhance nutrient and water use efficiency of annual crops in semi-arid developing countries such as Botswana. Inoculation of plant roots with arbuscular mycorrhizal (AM) fungi may be effective in improving crop production under nutrient and drought stress conditions, because AM fungi associated with plant roots were found to enhance productivity under drought conditions by improving the mineral nutritional status, mainly P (Nagarathna *et al.,* 2007).

Objective

The general objective of the study was to evaluate superphosphate as a P source for wheat and maize in pot and in field trials and investigate the possibility of improving its value by seed inoculation with AM fungi which will be referred to as mycorrhiza.

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CHAPTER 2

LITERATURE REVIEW

Introduction

Soil degradation and nutrient depletion have increased and have become a serious threat to agricultural production in semi-arid regions. A primary limitation of crop production in semi-arid regions is the deficiency of available nutrients especially P and water (Nagarathna *et al.,* 2007). The use of inorganic fertilizers by the poor small scale farmers and large scale farmers in some poor developing countries is made difficult by their scarcity or high cost. The incorporation of factors enabling plants to withstand nutrient deficiency and toxicity as well as drought stress would therefore be helpful to improve crop production. Cheaper solutions such as mycorrhizal inoculation to enhance nutrient and water use efficiency of annual crops should thus be investigated.

Inoculation of plant roots with AM fungi may be effective in improving crop production under nutrient and drought stress conditions (Nagarathna *et al.,* 2007), because improved productivity of AM plants was attributed to enhanced uptake of immobile nutrients such as P, Zn and Cu (Al-Karaki *et al.,* 2004).

Mycorrhizal fungi live in a symbiotic relationship with plants. These fungi live symbiotically with the roots of the plant and they take up water and nutrients from the soil, making them available to the plant and in exchange, the plant supplies the fungus with carbohydrates (Joubert & Archer, 2000; Farahani *et al.,* 2008). Mycorrhizal roots due to their extrametrical hyphae that are capable of absorbing and translocating nutrients can explore more soil volume than the non-mycorrhizal roots and increase the supply of the slowly diffusing ions such as phosphate to the plant (Khaliq & Sanders, 2000) and by so doing the nutritional requirements of the plant as well as water are more efficiently provided and the performance of the plant is considerably enhanced (Joubert & Archer, 2000). In return, the plant meets the carbon requirement of the fungus (Khaliq & Sanders, 2000). AM fungi are associated with improved growth of many plant species (Turk *et al.,* 2006), but crops differ with regard to their dependency on these organisms (Seymour, 2009).

The term mycorrhiza was coined by A. B. Frank, a scientist in Germany, more than 100 years ago (Habte, 2000). It literally means fungus-root, and describes the mutualistic association existing between a group of soil fungi and higher plants (Habte, 2000). AM fungi are considered to be obligate biotrophs as they are unable to grow and reproduce in regions of the soil where the host plant roots are absent (Meyer, 2007). Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the cortical tissue of roots during periods of active growth (Sylvia *et al.,* 2004).

Types of mycorrhiza

Seven types of mycorrhizal associations are known namely: ectomycorrhiza, endomycorrhiza, ect-endomycorrhiza, arbutoid mycorrhiza, ericoid and orchidoid mycorrhiza (Mukerji *et al.,* 2002). They are distinguished by their morphology and to a certain extent in their physiology (Turk *et al.,* 2006). Of the many types of mycorrhizal association, two are of major economic and ecological importance: ectomycorrhizal associations and the endomycorrhizal association of the vesicular arbuscular type. The ectomycorrhiza is characterized by an external sheath of fungal cells surrounding the root, typical of forest trees, i.e. pine. While endomycorrhiza like vesicular arbuscular mycorrhizal (AM) fungi forms no sheath. The fungus colonizes root systems of most cultivated crops and it usually invades several layers of the outer root cortex forming arbuscules and in most cases vesicles (Turk *et al.,* 2006). However, only endomycorrhizal fungi will be discussed in this study.

Arbuscular mycorrhiza (AM)

The diagnostic feature of AM fungi is the development of a highly branched arbusculus within root cortical cells (Sylvia *et al.,* 2004). It is an intracelular finely branched hyphae (haustoria) where the symbiotic metabolic exchanges between the fungus and the host plant takes place (Davies, 2010). The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is endomycorrhiza (Sylvia *et al.,* 2004). In this association neither the fungal cell wall nor the host cell membrane are breached. The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type (Sylvia *et al.,* 2004).

Effect of mycorrhiza on plant growth

Enhanced nutrient uptake

Mycorrhizal fungi can play an important role in plant nutrient and water uptake, particularly on soils with low P availability (Bagayoko *et al.,* 2000; Meyer, 2007). Results of experiments suggest that AM fungi absorb N, P, K, Ca, S, Cu, Zn and other micro-elements from the soil and translocate them to associated plants ((Joubert & Archer, 2000; Tinker & Gildon, 1983). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu and Zn (Pacovsky, 1986; Manjunath & Habte, 1988).

The supply of immobile nutrients to roots is largely determined by the rate of diffusion. Mycorrhiza may increase nutrient uptake by reducing the distance that nutrients must diffuse to plant roots (Abbott & Robson, 1984). In soils not adequately supplied with nutrients, uptake of nutrients by plants far exceeds the rate at which the nutrients diffuse into the root zone, resulting in a zone around the roots depleted of the nutrients. Mycorrhizal fungi help overcome this problem by extending their external hyphae to areas of soil beyond the depletion zone, thereby exploring a greater volume of the soil than is accessible to the unaided root. The distribution of hyphae in soil zones where the roots are absent (the micro-sized cross-section of the hyphae facilitates the penetration of much smaller soil pore where roots cannot penetrate), as well as the bigger contact of the hyphae with the soil contributes largely to the increased nutrient and water uptake (Joubert & Archer, 2000). Enhanced nutrient uptake by AM fungi is often associated with dramatic increase in dry matter yield, typically amounting to several-fold increases for plant species having high dependency on mycorrhiza.

Phosphorus uptake

Inorganic phosphate (Pi) is required in relatively large amounts by plants and is often a limiting nutrient for plant growth. Even if P was added to soil in soluble form, soon it becomes immobilized as organic P, calcium phosphates or other fixed forms (Turk *et al*., 2006). An important impact of the AM fungi on plants is the alleviation of phosphate deficiency (Soil Health, 2010). AM fungi may have biochemical capabilities for increasing the supply of available P and other immobile nutrients. These capabilities may involve increases in root phosphatase activity, excretion of chelating agents, and rhizosphere acidification (Habte & Fox, 1993). AM fungi can help increase the effectiveness of P fertilizer added to soils that are P deficient or having high P-fixing capacity. For example, in an acidic soil, addition of AM fungi and rock phosphate fertilizer together were more effective in enhancing the growth of corn than when rock phosphate was added alone (Inoue *et al.,* 2009).

The establishment of mycorrhiza can prove beneficial to plants in soils that are low in available P. In fact mycorrhizal effect decreases with increased supply of soluble phosphate (Mukerji *et al.,* 2002). In the absence of applied P the mycorrhizal plants made better growth than the non-mycorrhizal plants in the P deficient soil (Paraskevopoulou-Paroussi *et al.,* 1997). Also Al-Karaki *et al*. (2004) indicated that shoot dry matter, shoot P and root dry matter were higher for mycorrhizal infected wheat (*Triticum aestivum* L.) plants than for non infected plants. On the other hand, mycorrhizal infection has been shown to depress plant growth in soils with optimum P availability, these effects were attributed to competition for carbon between the host plant and the AM fungi (Turk *et al.,* 2006).

Nitrogen uptake

The most important sources of inorganic N for plants are nitrate (NO_3^-) and ammonium (NH_4^+) ions. Nitrates predominate in agricultural soils where they are readily mobile and can be easily taken up by plants. However, some ecophysiological situations, such as drought stress may interfere with the movement of the nutrients to the root surface (Coetzee, 2001). Mycorrhizal associations are therefore, important in nitrate uptake in water deficient soils (Coetzee, 2001). Plant growth is often enhanced by this association, mainly due to an increased ability to take up nutrients, principally P (Mullen & Schmidt, 1993; Smith & Read, 1997) and N (Tobar *et al*., 1994). AM fungal hyphae take up and transport N to the host from inorganic N sources (Ames *et al.,* 1983; Mader *et al.,* 2000) and organic N sources (Ames *et al.,* 1983; Hawkins *et al.,* 2000; Hodge *et al.,* 2001). The extraradical hyphae of AM fungi are able to take up and assimilate ammonium (NH_4^+) (Johansen *et al.,* 1992, 1993, 1996), nitrate (NO_3^-) (Bago *et al.,* 1996; Johansen *et al.,* 1996) and amino acids (Hawkins *et al.,* 2000; Hodge *et al.,* 2001) from their surroundings and translocate N from diverse sources to the plant (Hawkins *et al.,* 2000; Azcón *et al.,* 2001; Vazquez *et al.,* 2001).

Other nutrients

In addition to P and N, mycorrhizal infection has been shown to increase the assimilation of Cu, Zn and other heavy metals (Pacovsky, 1986). The hyphal mycelium increases the total absorption surface of infected plants and thus improve its access of immobile elements such as P, Cu, Zn (Lambert *et al.,* 1979; George *et al.,* 1994; George *et al.,* 1996; Ortas *et al.,* 1996) and cadmium (Cd) (Guo *et al.,* 1996) in areas beyond the root's depletion zone. A more novel effect is the decreased Fe and Mn uptake by AM plants. Phosphorus fertilized soybeans accumulated Mn to toxic levels, but AM plants moderated Mn uptake to less than critical concentration for Mn toxicity (Pacovsky, 1986).

Water uptake

In many arid and semiarid regions of the world, drought limits crop production. Inoculation of plant roots with AM fungi may be effective in improving crop production under drought conditions (Al-Karaki *et al.,* 2004). AM symbiosis can affect water relations of host plants. AM fungi infection has been reported to increase nutrient uptake in water stressed plants, enable plants to use water more efficiently and to increase root hydraulic conductivity (Turk *et al.,* 2006). The improved water uptake of plants caused by AM fungi can be ascribed to phosphate uptake which improves stoma conduction as well as improved hormone balances in the plant which regulate stoma closure (Joubert & Archer, 2000). In general, AM plants show higher stomatal conductance, transpiration rates, hydraulic conductivity and leaf water potential under water stress conditions, inducing greater water relations of host plants (Wu *et al.,* 2006).

Mycorrhizal fungi have been suggested as having a role in mediating the uptake of water at times of drought stress and of heavy metals on contaminated ground (Farahani *et al.,* 2008). Plants colonized by AM fungi have been shown to deplete soil water more thoroughly than nonmycorrhizal plants (Auge', 2001). One reason for this is the fact that the shoots of plants with AM fungi usually have a larger biomass (more evaporative leaf surface area) than non-AM fungi plants (Fitter, 1985; Nelsen, 1987). Also the root systems of plants with AM fungi are often more finely divided and thus have more absorptive surface area for water and nutrient absorption.

Factors affecting mycorrhizal associations

Nutrient availability

The establishment of mycorrhiza can prove beneficial to plants in soils that are low in available P. However, it has been also recognized that high soil P levels severely limit arbuscular mycorrhizal infection (De Miranda & Harris, 1994). The direct effects of soil P on spore germination and hyphal growth of the AM fungi were investigated by De Miranda & Harris, (1994). Spore germination was stimulated when 12.5 μg P g^{-1} was applied to the soil and decreased with further soil P increments. Hyphal growth decreased significantly with soil P amendments above 37.5 μg P g^{-1}. It has been postulated that this could be possibly due to P inhibition of the mycorrhizal fungus activity in the soil or during host fungus interaction (Coetzee, 2001).

pH

Plant growth is generally depressed by severe conditions that include excessive H^+ ions, the toxicity of aluminium (Al) and/or manganese (Mn) and the deficiency of some essential mineral nutrients, primarily P, Ca, Mg and Mo (Marschner, 1991). Growth depression in acid soils might also result from lack of activity of micro-organisms that form mutualistic associations with the plants, such as bacteria that form root nodules, e.g. *Rhizobium* spp. (Robert, 1995), and soil fungi that form arbuscular mycorrhizas (Abbott & Robson, 1985; Yost & Fox, 1979).

Kapoor *et al.* (2002) stated that mycorrhizal inoculation potential decrease with increase in initial soil pH and AM fungal spore germination occurs within a range of pH that is acceptable to plant growth. Optimum pH for colonization of *Glomus fasciculatum* in maize roots was found to be between pH 5.6 and 6.2 and in wheat roots between pH 6.7 and 6.9 (Kapoor *et al.,* 2002). The relationship between soil pH and mycorrhization is complex and depends on the plant species and soil type, forms of P and fungal species involved. Soil pH could affect the ability of AM fungi to colonize and persist by influencing spore germination and survival, growth of hyphae in soil, penetration and colonization of root propagule formation (Kapoor *et al.,* 2002).

Flooding

Mycorrhizal fungi have been suggested as having a role in mediating the uptake of water at times of drought stress. However, very little work has been done on presence of mycorrhiza on plants growing under flooded conditions and the information available is often contradictory. Flooding is usually expected to decrease mycorrhizal fungal colonization of wetland and aquatic plants because the fungi require aerated soil and are thought to be poorly suited to be living in wet environments (Miller, 2000). Hence, excessive soil moisture and the resulting reduction-oxidation levels are expected to have adverse effects on mycorrhizal formation (Coetzee, 2001).

However, literature reports either the presence or absence of mycorrhiza on flooded plants. Some wetland species have been reported to be mycorrhizal in well-drained soils and non-mycorrhizal in flooded soils (Jackson & Drew, 1984). Rutto *et al.* (2002) examined the effect of root-zone flooding on mycorrhizal and non-mycorrhizal peach seedlings growing in a low P medium. It was clear that AM fungi development confers limited tolerance to flooding on peach seedlings. This could be due to improved plant nutrition, the suppression of ethanol accumulation in roots and the extension of the duration of root activity in a flooded environment.

Organic wastes

The materials we refer to as organic wastes are merely those which are not put to use in our existing technological systems. Animal and plant wastes differ in their chemical and biological composition depending on the source of the material. Kale *et al.* (1992) found that AM fungi in roots of a summer crop was 2.85% in soil that previously received chemical fertilizers compared to 10% in the soil with half the recommended dosage of chemical fertilizers and organic matter amendment. Inoculation with AM fungi did not significantly affect seed yield of pea (*Pisum sativum* L.) plants in soil which is rich in organic matter but seed yield was significantly enhanced with AM fungal inoculation in soil which is poor in organic matter and P (Turk *et al.,* 2006).

Temperature

The development of AM fungi and the formation of mycorrhiza are affected by soil environmental conditions, plant nutrient level, light intensity, cropping systems (Furlan & Fortin, 1977; Jasper *et al.,* 1989a, 1989b, 1991; Evans & Miller, 1990; Reinharts *et al.,* 1994) as well as temperature which is a very important factor (Jakobsen & Andersen, 1982; Fabig *et al.,* 1989; Bowen 1991). In plants of cool temperate climates, AM fungal development is usually optimal at 20–25^{o}C (Zhang *et al*., 1995; Matsubara *et al.,* 2000) and maximal spore germination occurs between 20^{o}C and 28^{0}C, depending on the species (Wang *et al*., 1997).

The effect of temperature on the rate and extent of colonization however is complex and the responses vary according to the host plant and the fungus. Many plants, both wild and cultivated, grow and develop mycorrhizas at lower temperatures in temperate regions (Smith & Read, 1997). Baon (1994) found that barley failed to become colonized by *Glomus etunicatum* when root temperatures were held at 10^{o}C, although it became colonized at 15^{o}C. However, Allen *et al.* (1989) found high (40 – 60%) colonization of *Agropyron* by both field (*Glomus* spp) and pot-culture (*Gigaspora margarita*) inoculums at 12^{o}C, and Daft *et al.*

(1980) commented that in English bluebells colonization increased rapidly in the winter months when soil temperatures were near to 5^{o}C.

Liu *et al.* (2004) evaluated the effects of root zone temperature (10, 15 and 23°C) on the formation and development of AM fungi in greenhouse-grown sorghum [*Sorghum bicolor* L. Moench]. Colonization was markedly reduced at 15°C compared with 23°C, and almost completely inhibited at 10°C. It is clear that generalizations are dangerous and that there is need for more work which is specifically directed at understanding the biology of propagule survival, germination and colonization of roots in particular habitats (Smith & Read, 1997).

From this literature review it became clear that the effect of AM fungi on crop growth and yield may differ between crops and may be affected by growing conditions. Research to test the effect under local conditions is for this reason needed.

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CHAPTER 3

THE EFFECT OF MYCORRHIZAL INOCULATION ON EARLY GROWTH OF WHEAT AND MAIZE CROPS GROWN ON A PHOSPHORUS DEFICIENT SANDY SOIL OF SOUTH AFRICA

Abstract

Pot experiments were conducted during the winter and summer of 2009 in a temperature controlled glasshouse at the Department of Agronomy of the University of Stellenbosch, in the Western Cape Province of South Africa to determine the effect of mycorrhizal inoculation on early growth of wheat and maize crops in a P deficient sandy soil. Plants of wheat (10, 20 and 30 days after planting) and maize (14, 21 and 28 days after planting) were harvested to determine leaf area, root fresh and dry mass, shoot fresh and dry mass, moisture content of both roots and shoots as well as maize plant height and stem diameter. The results showed that mycorrhiza (AM fungi) varied in their ability to stimulate plant growth at different growth stages. In general mycorrhizal inoculation negatively affected both wheat and maize during the early stages of growth (14 – 21 and 10 – 30 days after planting respectively), but root mass of maize were increased as a result of mycorrhizal inoculation at 28 days after planting (DAP). Although wheat performance was adversely affected by addition of mycorrhiza during the early growth stages (10 – 30 DAP), the opposite may be true at later growth stages, because mycorrhiza improves nutrient and water use efficiency of crops. This study however indicated that beneficial responses due to mycorrhizal inoculation may need some time to develop even in highly dependent crops such as maize.

Introduction

Most plant species form a symbiotic association with some beneficial soil fungi (Babana & Antoun, 2006). The roots are colonized by the AM fungus, which ramifies through the soil. Typically, fungal spores germinate, infect fine roots of host plants, and form characteristic structures, vesicles and arbuscules, inside the roots. Outside the roots, mycelia spread profusely in the soil (Dowdle, 1980). The AM fungus enhances the function of the plant's root hairs and acts as an extension of the root system allowing the mycorrhizal plants to explore and capture nutrients and water (Meyer, 2007) from a larger volume of soil compared to non-mycorrhizal plants (Muchovej, 2001; Joubert & Archer, 2004). With mycorrhizal colonization in the plant roots, there is an increase in absorption surface area, greater soil area exposed to plant roots, greater longevity of absorbing roots, better utilization of low-available nutrients and better retention of soluble nutrients, thus reducing reaction with soil colloids or leaching losses (Muchovej, 2004; Selvaraj & Chellappan, 2006). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky, 1986; Manjunath & Habte, 1988).

The combination of root and fungus is called mycorrhiza (St John, 2000). It is neither the fungus nor the root, but rather the structure formed between these two partners that is called a mycorrhiza (Yost & Fox, 1979; Abbott & Robson, 1984; Muchovej, 2001). Since the association is mutualistic, both organisms benefit from the association as the fungus receives carbohydrates from the plant, which in turn receives water and nutrients from the fungus (Joubert & Archer, 2000).

This association is particularly important to agricultural plants because they require large amount of nutrients to achieve optimum yields. The association also offers the host plant protection against soil pathogens (Pacovsky, 1986). Mycorrhizal fungi may have biochemical capabilities that increases root phosphatase activity, excretion of chelating agents and rhizosphere acidification (Habte & Fox, 1993). The AM fungi excrete chemicals that dissolve mineral nutrients, absorb water, retard soil pathogens, and glue (glomalin) soil particles together into a porous structure (Amaranthus, 2004).

The establishment of mycorrhiza can prove beneficial to plants in soils that are low in available P. However, it has been recognized that high soil P levels severely limit AM colonization (De Miranda & Harris, 1994).

Soil degradation and nutrient depletion have increased and have become a serious threat to agricultural production in semi-arid regions. A primary limitation of crop production in semi-arid regions is the deficiency of available nutrients especially P and water (Nagarathna *et al.,* 2007). Therefore, the incorporation of factors enabling plants to withstand nutrient deficiency and toxicity as well as drought stress would be helpful to improve crop production. The use of inorganic fertilizers by poor small scale farmers and large scale farmers in some developing countries is made difficult by their scarcity or high cost. It is thus advisable to develop cheaper solutions such as mycorrhizal inoculation to enhance nutrient and water uptake and efficiency in annual crops. Mycorrhizal fungi do not only enhance P uptake, but also enhances Zn, S, K and Se uptake (Dowdle, 1980). Without AM fungi, much higher amounts of P and/or Zn fertilizer is required to attain the same level of productivity as when plants are mycorrhizal (Seymour, 2009). The improved nutrient and water uptake of mycorrhizal plants has huge potential benefit to farmers in that it may reduce the fertilization requirements and therefore bring about savings (Joubert & Archer, 2000).

The objective of this study was

- To determine the effect of mycorrhizal seed inoculation on early growth of wheat and maize in a sandy soil with low phosphorus level.

Materials and Methods

Experiments were conducted during the 2009 winter (30th July – 31st August 2009) and summer (28th September – 26th October 2009) cropping seasons under temperature controlled glasshouse conditions at the Department of Agronomy of the University of Stellenbosch, in the Western Cape Province of South Africa. Wheat (*Triticum aestivum* L. SST 027) was planted in winter while maize (*Zea mays* L. CRN 3505) was planted in summer. The temperatures of the glasshouse were 20/12^{o}C day/night in winter and 25/17^{o}C day/night in summer. The soil used for the study was coarse sand. The soil was analyzed for chemical properties before the start of the experiment and showed low nutrient content levels as compared to the amount required by wheat and maize for optimum growth (Table 3.1). The plants were fertilized (all nutrients except P) according to soil analysis recommendations for optimum wheat and maize production by means of the irrigation system (Table 3.2). Irrigation frequency was determined by solar radiation and the volume per irrigation was adjusted for different growth stages to ensure 10% drainage with each irrigation.

Table 3.1 Soil characteristics at planting and optimum soil nutrient levels for cereal crops (sampled before planting 2009)

Soil characteristics	Measured values	0ptimum levels	Reference
Texture	Sand		
pH (KCL)	5.4	>6.0	DAR, 2010
Calcium	0.40 cmol+/kg	>1.00 cmol+/kg	DAR, 2010
Magnesium	0.08 cmol+/kg	>0.30 cmol+/kg	DAR, 2010
Potassium	0.028 cmol+/kg	>0.10 cmol+/kg	DAR, 2010
Sodium	0.021 cmol+/kg	<1.00 cmol+/kg	DAR, 2010
Phosphorus (citric acid)	18 mg/kg	>30 mg/kg	FSSA, 2007
Total cations	0.68 cmol+/kg	>2.50 cmol+/kg	DAR, 2010
Copper	0.07 mg/kg	0.5 – 3 mg/kg	Aubert & Pinta, 1977
Zinc	0.26 mg/kg	0.6 mg/kg	Aubert & Pinta, 1977
Manganese	5.04 mg/kg	1.0 mg/kg	Aubert & Pinta, 1977
Boron	0.01 mg/kg	0.1 – 2.0 mg/kg	Aubert & Pinta, 1977
Carbon	0.02%	>0.2%	DAR, 2010
Sulphur	1.50 mg/kg	5 – 10 mg/kg	Jez, 2008
Total Nitrogen	0.01%	-	

Plastic pots (12.5 cm high x 10.5 cm diameter) filled with sterilized coarse sand were used. The soil was fumigated with methyl bromide to kill all fungi. There-after it was aerated for 2-3 days before it was used. In the case of wheat, six seeds of wheat (*Triticum aestivum* L. SST 027) were planted and thinned to three plants per pot during the seedling stage. In the case of maize (*Zea mays* L. CRN 3505) four seeds were planted and thinned to two plants per pot during the seedling stage. Half of the pots (12 pots for each crop) were sown with AM fungi inoculated seed (M_1) while the other half was sown with uninoculated seed (M_0). The experimental treatments were arranged according to a complete randomized block design having four replications and making provision for three sampling times.

Table 3.2 Nutrient applied by fertigation to wheat and maize in a pot trial

Nutrient Solution lacking P	**EC = 2.5 mS/cm**
Macro nutrients (g/900L)	
KNO_3	327.6
K_2SO_4	266.4
$Ca(NO_3)_2.2H_2O$	810
$MgSO_4.7H_2O$	442.8
Micro nutrients (g/900L)	
Fe:Libfer (Fe-EDTA)	7.36
Mn: Manganese Sulphate	2.51
Zn: Zinc Sulphate	1.50
B: Soluber	1.65
Cu: Copper Sulphate	0.22
Mo: Sodium molibdate	0.14

Mycorrhizal inoculation was done at sowing for both crops by applying 3 g of mycorrhizal inoculum (*Glomus intraradices*) obtained from Biocult (Pty) Ltd Somerset West, RSA to 400 g of wheat seeds and 1.50 g of inoculum to 250 g of maize seed. Wheat sampling was done at 10 day intervals after emergence and maize sampling was done at 7 day intervals after emergence.

Growth measurements

At sampling days, the following measurements were taken on wheat plants: leaf area, root mass, shoot mass and percent moisture. Maize sampling was done as on wheat plants with the inclusion of stem diameter and plant height. Plant height of maize was measured from the ground level to the tip of the tallest leaf (Fageria, *et al*., 2006) using a meter ruler. The stem diameter of maize plants was measured after the maize plants were cut at the ground level. After watering thoroughly to loosen the soil plant shoots and roots of both crops were gathered. Roots were collected by soaking off the soil around the entire root system in water. Several washings were done to remove all the sand granules and thin layers of soil still coating the roots. Each plant was then separated into roots and shoots (leaves and stems). Leaf area was measured using the Li-Cor Model LI3100 Area Meter. After that, components were weighed to determine the fresh mass before being oven dried at 70°C for 72 hours to determine dry weight (Fageria, *et al.,* 2006).

The percent moisture was calculated using the following formula:

- % moisture = [(Fresh Weight – Dry Weight) ÷ Fresh Weight)] * 100

Data analysis

The data recorded at the different sampling stages was subjected to analysis of variance by Statistica Version 9.0 System (StatSoft Inc., 1993) procedure to determine the least significant differences between the treatment means. Tukey HSD test was used to separate the means of the measured parameters at $P \leq 0.05$.

Results

Wheat

Increased plant growth is usually attributed to mycorrhizal colonization through increased capacity of plant roots to take up water and nutrients. In this study, mycorrhizal inoculation had a highly significant effect ($P \leq 0.05$) on wheat root dry mass at 10 days after planting (9.38 mg for mycorrhizal inoculated plants compared to 6.13 mg of plants not inoculated with mycorrhiza), but did not have any significant effect at this sampling date on the other growth parameters (leaf area, root fresh mass, shoot fresh and dry mass, percent moisture) (Table 3.3).

At 20 days after planting, the non-mycorrhizal plants had a significantly higher leaf area, percent root moisture, shoot fresh mass as well as shoot dry mass compared to plants from mycorrhizal inoculated seeds. However, no significant differences were found between the root mass of the non-mycorrhizal compared to the mycorrhizal inoculated plants (Table 3.3). At 30 days after planting, the only differences were due to a significant higher fresh and dry shoot mass of non-mycorrhizal plants compared to plants from mycorrhizal inoculated seeds (Table 3.3).

Table 3.3 Response of wheat during the seedling and early growth stages to mycorrhizal inoculation

Parameters	10 days after planting		20 days after planting		30 days after planting	
	M_0	**M_1**	**M_0**	**M_1**	**M_0**	**M_1**
Leaf area (cm^2)	-	-	12.359a	7.7372b	18.26a	8.7825a
Root fresh mass (g)	0.0351a	0.05353a	0.31625a	0.255a	1.5125a	1.0578a
Root dry mass (g)	0.00613b	0.00938a	0.04025a	0.06175a	0.141a	0.09425a
Root moisture (%)	81.875a	81.975a	87.75a	75.075b	90.7a	90.875a
Shoot fresh mass (g)	0.1453a	0.14883a	0.48275a	0.291b	1.3938a	0.85575b
Shoot dry mass (g)	0.02212a	0.0244a	0.06075a	0.04025b	0.23225a	0.14425b
Shoot moisture (%)	84.75a	83.6a	87.35a	86.125a	83.325a	82.9a

* Means with different letters within the same row are significantly different (P≤0.05).

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

Maize

In this experiment, mycorrhizal inoculation did not have a positive effect on maize growth at 14 days after planting and plants from seed that were not inoculated even resulted in a significantly higher shoot fresh and dry mass (Table 3.4). The absence of any positive response in mycorrhizal inoculated plants could be due to the fact that at this early stage the mycorrhiza was still forming an association with plant roots and did not yet improve the nutrient uptake of inoculated plants (Khan, 1974).

Most probably for the same reason as at 14 days after planting, no significant differences between inoculated and non-inoculated plants were shown at 21 days after planting (Table 3.4).

At 28 days after planting, plants from mycorrhizal inoculated seed had a significantly higher root fresh and dry mass (Table 3.4). These plants also tend to have higher leaf area, plant height, shoot fresh mass and shoot dry mass, but differences were not significant. This response indicates that at 28 days after planting, the mycorrhiza has formed an association with plant roots and was now beginning to increase nutrient uptake by plants in exchange of the carbohydrates from the plants.

Table 3.4 Response of maize during the seedling and early growth stages to mycorrhizal inoculation.

Parameters	14 days after planting		21 days after planting		28 days after planting	
	M_0	M_1	M_0	M_1	M_0	M_1
Stem diameter (mm)	3.75a	3.25a	5.2075a	5.00a	6.00a	6.00a
Leaf area (cm^2)	-	-	44.443a	38.578a	64.813a	67.358a
Plant height (cm)	109.75a	96.75a	270.88a	245.21a	296.25a	301.00a
Root fresh mass (g)	1.4908a	1.332a	3.1335a	2.993a	4.9648b	6.528a
Root dry mass (g)	0.23325a	0.24375a	0.3865a	0.34475a	0.39475b	0.5255a
Root moisture (%)	84.125a	81.775a	87.45a	88.425a	92.075a	91.95a
Shoot fresh mass (g)	0.72a	0.536b	2.1828a	1.899a	3.162a	3.5758a
Shoot dry mass (g)	0.6625a	0.485b	0.2125a	0.18475a	0.338a	0.39325 a
Shoot moisture (%)	90.825a	90.9a	90.2a	90.2a	89.55a	89.00a

* Means with different letters within the same row are significantly different (P≤0.05).

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

Discussion

Wheat

The main function of mycorrhiza is to increase the soil volume explored for nutrients, especially P uptake and to enhance the efficiency of nutrient absorption from the soil solution and in return the fungus obtains carbohydrates from the plant (Sylvia *et al.,* 2004). With the exception of root dry mass at 10 days after planting, mycorrhizal inoculation did not have any positive effect on wheat seedling growth in this experiment. Several growth parameters were however negatively affected by mycorrhizal inoculation.

Mycorrhiza therefore seemed to have a suppressing effect on wheat growth during the seedling and early growth stages. This response could be due to the fact that the coarse sandy soil had a low nutrient content and although other nutrients were applied, no P was added. This meant that the mycorrhiza could not enhance the efficiency of P uptake but still obtained carbohydrates from the plants. Wheat also has a low mycorrhizal dependency (Seymour, 2009), and this enabled the crop to perform well without mycorrhizal inoculation with the result that the effect of mycorrhizal inoculation on wheat growth could be very variable. Another possible reason may be that because AM fungi are known to be obligate fungal symbionts, they have to form an association with plant roots to survive (Grantham, 2009) and it may take some time for mycorrhiza to form this association. Therefore, crops might not show any response to mycorrhiza during the early growth stages.

Maize

The effects of AM fungi are a result of their ability to increase the uptake of P, but their advantages differ between plant species depending on the species dependency on mycorrhiza. Maize is considered to be highly dependent on mycorrhiza (Seymour, 2009). In this experiment, mycorrhiza seemed to have a suppressing effect on maize growth at the first week or two after planting. At these early growth stages of the plant when the mycorrhiza is still in the process of colonizing the plant roots, it may not be able to enhance the efficiency of nutrient uptake but still obtained carbohydrates from the plants to ensure its survival and thereby suppresses crop growth.

Bougher *et al.* (1990) stated that at low levels of soil P, height of seedlings from mycorrhizal inoculated seed was similar to that of uninoculated seedlings till about 35 days after planting, but started to accelerate after this initial period of growth. This observation supports earlier results (Khan, 1975; Owusu-Bennoah & Mosse, 1979) that suggested that the positive effect of mycorrhizal inoculation becomes evident only after about one month.

Conclusion

Low soil fertility and non-efficient use of nutrients and water lead to low crop yields, particularly under traditional farming systems. During the early stages of growth (10 – 30 and 14 – 21 DAP respectively), both wheat and maize were negatively affected by mycorrhiza. Contrarily, maize growth was improved by mycorrhiza in later growth stages (28 DAP) which indicate that mycorrhiza have formed an association with plant roots to survive and therefore enhanced water and nutrient uptake by plant roots. Compared with wheat, maize benefited from mycorrhiza because it is considered to be highly dependent on mycorrhiza as compared to wheat which has a lower mycorrhizal dependency. Although wheat performance was adversely affected by addition of mycorrhiza during the early growth stages (10 – 30 DAP), its performance can be improved as the crop matures. Because mycorrhiza improves nutrient and water use efficiency, it can improve crop performance and yield particularly under semi-arid conditions.

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CHAPTER 4

THE EFFECT OF MYCORRHIZAL INOCULATION AND PHOSPHORUS LEVELS ON GROWTH AND YIELD OF WHEAT AND MAIZE CROPS GROWN ON A PHOSPHORUS DEFICIENT SANDY SOIL OF SOUTH AFRICA

Abstract

Phosphorus is one of the three (nitrogen, potassium and phosphate) most important nutrients required for plant growth and one of the most important constraints in crop production in developing countries. Pot experiments with P deficient sandy soil (18 mg P kg^{-1} soil; citric acid method) were conducted in a temperature controlled glasshouse at the Department of Agronomy of the University of Stellenbosch, in the Western Cape Province of South Africa to study the response of wheat (*Triticum aestivum* L. SST 027) and maize (*Zea mays* L. CRN 3505) to different P levels (0, 10, 20, 30 or 40 kg P ha^{-1}) and seed inoculation with AM fungi (*Glomus intraradices*).

Results indicated that wheat growth and yield and maize growth were significantly enhanced by the application of different P levels and mycorrhizal seed inoculation. Mycorrhizal inoculation did not have a significant effect on wheat and maize growth during the early growth stages, but the effect became more pronounced as the crops matured. Phosphorus application levels significantly increased all the measured parameters of wheat plants except for root fresh mass, root moisture and thousand kernel weight while the effect of seed inoculation with AM fungi varied between different wheat growth stages.

Phosphorus application levels had a significant increasing effect on all the measured parameters of maize plants except for percentage plant moisture while the effect of seed inoculation with AM fungi varied between different growth stages. The results of this experiment showed that nutrient contents were significantly higher in mycorrhizal plants than in non-mycorrhizal plants and this may be due to increased above ground parts growth of mycorrhizal plants. Compared with wheat, maize benefited more from mycorrhiza

because it is considered to be highly dependent on mycorrhiza as compared to wheat which has a lower mycorrhizal dependency.

Introduction

Crop production is an important agricultural activity in semi-arid regions and crops are widely grown in many countries in the world. Production of hybrid crops has been given wide publicity among crop farmers in developing countries in recent times. However, hybrid crops are known for their high demand for nutrients and soils are being depleted of essential nutrient elements by continuous crop production with low inputs. Mineral nutrition is one of the most important factors affecting plant processes (Skudra & Skudra, 2004) and proper nutrition is essential for satisfactory crop growth and production (McKenzie, 1998).

Most soils in semi-arid regions are deficient in N and P, and this constitutes a serious constraint to adequate food production (Iniobong *et al.,* 2008). However, P is the nutrient most frequently limiting yield on newly farmed and formerly unfertilized soils and its deficiency is one of the major constraints to crop production in semi-arid regions (Mullen & Gammie, 2003; Skudra & Skudra, 2004). Phosphate is the most temperamental of the major nutrients, with low mobility and low efficiency of use (Tivy, 1990). Although P fertilizer applications can improve crop production in semi-arid areas, small scale or resource poor farmers often cannot afford such applications due to the scarcity and high cost of P fertilizers.

Phosphorus is a nutrient required in relatively small amounts by plants compared with N, but is together with N and K, the most important nutrients. Phosphate contributes to many vital functions in the plant, such as early root and seedling growth, improved winter hardiness, promotion of early heading and uniform maturity, seed formation and quality and increased water use efficiency (Johnston, 2001). While these effects are more visible, P also plays a number of unseen roles such as in photosynthesis, energy storage and transfer, respiration and cell division, fat and albumen formation (McKenzie & Middleton, 1997; El-Ghamry *et al.,* 2009). Crops that experience P deficiency tend to develop slower, exhibit limited growth potential, evidenced by weak vegetation and finally leads to poor yields (Johnston, 2001). This P deficiency is attributed to the low availability of soil P, especially in arid and semi-arid regions, caused by intense calcium P fixation (Diez *et al.,* 1992). In such conditions, P application will show a spectacular increase in crop yield.

Several factors can enhance the uptake of P by crops. Soil organic matter plays an important role in soil fertility as it increases the soils nutrient holding capacity and contributes to soil fertility upon its breakdown (Relf, 1997; FSSA, 2007). Generally, higher soil organic matter levels are related to greater P availability and studies have emphasized the importance of organic P in plant nutrition (Armstrong, 1999). Gradual release of organic P provides a steady supply of P under conditions which would otherwise result in P fixation.

Soil pH also affects the availability of nutrients such as P to crops. A low pH (pH $CaCl_2$ <5) has a negative effect on plant growth, mostly because of imbalances in nutrient levels. Macronutrients tend to be less available in soils with low pH, while micronutrients are less available in soils with high pH (Relf, 1997). Phosphorus availability is often restricted in both alkaline and acid soils because of extremes in pH (Goos & Johnson, 2000).

Mycorrhizal fungi live in a symbiotic relationship with plants. They grow in close association with the roots and play an important role in the uptake and transfer of nutrients from the soil to the plant in exchange for sugars from the plant (Farahani *et al.,* 2008). Mycorrhizal roots due to their extrametrical hyphae that are capable of absorbing and translocating nutrients can explore more soil volume than the non-mycorrhizal roots and thus increase the supply of the slowly diffusing ions such as P to the plant (Khaliq & Sanders, 2000). Most crop plants are colonized by AM fungi (Babana & Antoun, 2006). Besides improving the potential for uptake of poorly mobile nutrients, AM symbioses benefit plant growth by other mechanisms of action such as improving drought tolerance, protecting plants against pathogens and improving soil aggregation (Joubert & Archer, 2000; Babana & Antoun, 2006).

Plants colonized by the AM fungi have an improved water consumption than uncolonized plants which is attributed to the improved phosphate uptake which improves stoma conduction as well as improved hormone balances in the plant which regulate stoma closure (Joubert & Archer, 2000). The AM plants also have increased lignifications of the endodermal root cells which offer more resistance to infection by other plant pathogens (Joubert & Archer, 2000). Garg & Chandel (2010) concluded that the AM fungal symbiosis mainly affected the host-pathogen relationship by improving P nutrition leading to greater resistance to root-infecting fungi. However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky, 1986; Manjunath & Habte, 1988).

The beneficial effects of mycorrhiza on P uptake and crop growth may however differ between crops and may be affected by growth conditions. Some crops rely strongly on AM fungi for P uptake (Baylis, 1975; Janos, 1980) and AM formation and activity may differ between seasons of the year (Fitter, 1989). Many summer crops such as maize (*Zea mays* L.) have a very high AM dependency while the major winter crops such as wheat (*Triticum aestivum* L.) are lower down on the dependency scale (Seymour, 2009). Some crops like canola and lupis are even regarded as AM independent (Seymour, 2009).

Wheat and maize are important crops in semi-arid areas. Maize requires about 4.5 kg of P to produce a grain yield of 1000 kg ha^{-1}, while wheat requires about 4.8 kg of P to produce the same yield. Both crops require soil P content (Bray 1) of 21-27 mg P kg^{-1} of soil for optimum growth (FSSA, 2007). Little is however known on the effect of AM fungi on P uptake and P responses of these crops grown in P deficient soils of semi-arid areas in Southern Africa.

The objective of this study was

- To evaluate the growth response of wheat and maize to seed inoculation with AM fungi in combination with different P fertilizer application levels in a pot trial with P deficient sandy soil at the University of Stellenbosch, in the Western Cape Province of South Africa.

Materials and Methods

Study area

The experiments were conducted under temperature controlled glasshouse conditions at the Department of Agronomy of the University of Stellenbosch, in the Western Cape Province of South Africa. The wheat (*Triticum aestivum* L. SST 027) experiment was conducted during the 2009 winter (30th July – 27th November 2009) cropping season while the maize (*Zea mays* L. CRN 3505) experiment was conducted during the 2009 summer (28th September – 27th November 2009) cropping season. The winter temperatures of the glasshouse were 20/12^{o}C day/night while the summer temperatures were 25/17^{o}C day/night.

Coarse sand collected from a local crop field was analyzed for fertility status before the start of the experiments and showed low nutrient content levels as compared to the amount required by both wheat and maize for optimum growth (Table 4.1). The soil was sterilized with methyl bromide to kill all fungi and there-after aerated for 2-3 days before use. The plants were fertilized (all nutrients except P) according to soil analysis recommendations for optimum wheat and maize production by means of the irrigation system (Table 4.2). Irrigation frequency was determined by solar radiation method and the volume per irrigation was adjusted for different growth stages to ensure 10% drainage with each irrigation.

The crops were planted in plastic pots (12.5 cm high x 10.5 cm diameter) filled with sterilized coarse sand. The soil was fumigated with methyl bromide to kill all fungi. There-after it was aerated for 2-3 days before being used. In the case of wheat, six seeds of wheat (*Triticum aestivum* L: SST 027) were planted and thinned to three plants per pot during the seedling stage. In the case of maize (*Zea mays* L: CRN 3505) four seeds were planted and thinned to two plants per pot during the seedling stage. Half of the pots (60 pots for each crop) were sown with AM fungi inoculated seeds while the other half was sown with uninoculated seeds designated mycorrhiza inoculated (M_1) and uninoculated (M_0)

respectively. Phosphorus was applied as single superphosphate (10.5%) at five different levels at planting. The experimental treatments comprising of control P_1 = 0 kg P ha^{-1}, P_2 = 10 kg P ha^{-1}, P_3 = 20 kg P ha^{-1}, P_4 = 30 kg P ha^{-1} and P_5 = 40 kg P ha^{-1}, were arranged as a 2 x 5 factorial design with each treatment combination replicated four times and making provision for three sampling times.

Table 4.1 Soil characteristics at planting and optimum soil nutrient levels for cereal crops (sampled before planting 2009)

Soil characteristics	Measured values	0ptimum levels	Reference
Texture	Sand		
pH (KCL)	5.4	>6.0	DAR, 2010
Calcium	0.40 cmol+/kg	>1.00 cmol+/kg	DAR, 2010
Magnesium	0.08 cmol+/kg	>0.30 cmol+/kg	DAR, 2010
Potassium	0.028 cmol+/kg	>0.10 cmol+/kg	DAR, 2010
Sodium	0.021 cmol+/kg	<1.00 cmol+/kg	DAR, 2010
Phosphorus (citric acid)	18 mg/kg	>30 mg/kg	FSSA, 2007
Total cations	0.68 cmol+/kg	>2.50 cmol+/kg	DAR, 2010
Copper	0.07 mg/kg	0.5 – 3 mg/kg	Aubert & Pinta, 1977
Zinc	0.26 mg/kg	1.5 - 2 mg/kg	FSSA, 2007
Manganese	5.04 mg/kg	1.0 mg/kg	Aubert & Pinta, 1977
Boron	0.01 mg/kg	0.1 – 2.0 mg/kg	Aubert & Pinta, 1977
Carbon	0.02%	>0.2%	DAR, 2010
Sulphur	1.50 mg/kg	5 – 10 mg/kg	Jez, 2008
Total Nitrogen	0.01%	-	

Table 4.2 Nutrient applied by fertigation to wheat and maize in a pot trial

Nutrient Solution lacking P	
EC = 2.5 mS/cm	
Macro nutrients (g/900L)	
KNO_3	327.6
K_2SO_4	266.4
$Ca(NO_3)_2.2H_2O$	810
$MgSO_4.7H_2O$	442.8
Micro nutrients (g/900L)	
Fe:Libfer (Fe-EDTA)	5.88
Mn: Manganese Sulphate	2.00
Zn: Zinc Sulphate	1.20
B: Soluber	1.32
Cu: Copper Sulphate	0.18
Mo: Sodium molibdate	0.11

Seed inoculation

Mycorrhizal inoculation was done at planting for both crops by applying 3 g mycorrhizal inoculum (*Glomus intraradices*) obtained from Biocult (Pty) Ltd Somerset West, RSA to 400 g of wheat seeds and 1.50 g of mycorrhizal inoculum to 250 g of maize seed. Wheat sampling was done at 50 (tillering), 90 (flowering) and at 120 (maturity) days after planting, while maize plants were sampled at 30, 45 and 60 days after planting (DAP) respectively.

Growth measurements

Wheat

The first sampling was done at tillering stage (50 DAP) and the following measurements were done: leaf area, number of tillers per plant, root and shoot mass as well as percent moisture of both roots and shoots. The second sampling which was at anthesis (90 DAP) was done as at tillering stage with the inclusion of the number of ears per plant. The final sampling was done at maturity (120 DAP) and the following parameters were measured: number of ears per plant, grains per ear, grain yield per plant, thousand kernel weight, protein content of grains and nutrient content of above ground plant parts. After watering thoroughly to loosen the soil plants and roots were gathered. Roots were collected by soaking off the soil around the entire root system in water and then by several washings to remove all the sand granules and thin layers of soil still coating the roots.

Each plant was then separated into roots and shoots (leaves and stems). Leaf area of wheat plants was measured using the Li-Cor Model LI3100 Area Meter. After that, components were weighed to determine the fresh mass before being oven dried at 70^{o}C for 72 hours to determine dry weights (Fageria, *et al.*, 2006). The number of grains per plant of wheat were determined using the Numigral Seed Counter while the grain protein content was determined using the Technicon™ InfraAnalyzer™ 400.

Maize

At sampling days 30 and 45, the following measurements were made: leaf area, stem diameter, plant height, root and shoot mass as well as the percent moisture of both roots and shoots. In addition to that, nutrient content of above ground plant parts was measured at 60 days after planting. Plant height was measured from the ground level to the tip of the tallest leaf (Fageria *et al.*, 2006) using a meter ruler while the stem diameter of maize plants was measured after plants were cut at the ground level. Due to the size of the pots which might restrict root development and plant growth, it was decided to terminate the maize trial at 60 days after planting.

After watering thoroughly to loosen the soil, plants and roots were gathered. Roots were collected by soaking off the soil around the entire root system in water and then by several washings to remove all the sand granules and thin layers of soil still coating the roots. Each plant was then separated into roots and shoots (leaves and stems). Leaf area was measured using the Li-Cor Model LI3100 Area Meter. After that, components were weighed to determine the fresh mass before being oven dried at 70°C for 72 hours to determine dry weights (Fageria, *et al*., 2006).

The percent moisture content of dry matter was calculated using the following formula:

- % moisture = [(Fresh Weight – Dry Weight) ÷ Fresh Weight)] * 100

Chemical Analysis

Samples of wheat straw (120 DAP) and maize stover (60 DAP) of each treatment were taken. The samples were analyzed chemically for N, P, K, Mg, Na, Fe, Cu, Zn, Mn, B, Al, S and OC concentrations. The mass per plant of elements was then calculated as follows:

- Mass plant^{-1} (g) = [(% Nutrient concentration ÷ 100) x Total dry mass plant^{-1}]

or

- Mass plant^{-1} (g) = [(Nutrient concentration (mg/g) ÷ 1000) x Total dry mass plant^{-1}]

Data analysis

Analysis of variance was performed using the Statistica Version 9.0 System (StatSoft Inc., 1993) procedure to determine the least significant differences between the treatment means. Post hoc analyses were performed using the Turkey HSD Test to separate the means of the measured parameters at P≤0.05.

Results and discussion

Wheat

The effect of P application levels, seed inoculation with AM fungi and their interaction on the growth of wheat were measured at tillering (50 DAP) and anthesis (90 DAP), while the effect on yield components were measured at maturity (120 DAP). Results from the analysis of variance are summarized in Table 4.3.

At tillering stage, P application levels had a significant increasing effect on all the measured parameters except for root fresh mass and root moisture (Table 4.3). At anthesis P application levels had a significant increasing effect on all parameters except for root moisture, while at maturity P application levels had a significant increasing effect on all parameters except thousand kernel weight.

Table 4.3 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on growth and yield of wheat plants at different growth stages

Sampling stages	Treatment	Leaf area (cm^2 $plant^{-1}$)	No. of tillers $plant^{-1}$	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
Tillering (50days after planting)	P	*	*	NS	*	NS	*	*	*
	M	*	NS	NS	NS	*	*	NS	*
	M*P	NS	NS	NS	NS	NS	NS	NS	NS
Anthesis (90days after planting)	Treatment	Leaf area (cm^2 $plant^{-1}$)	No. of ears $plant^{-1}$	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
	P	*	*	*	*	NS	*	*	*
	M	NS	NS	NS	NS	*	NS	NS	*
	M*P	NS	NS	NS	NS	*	NS	NS	NS
Maturity (120days after planting)	Treatment	Grain yield $plant^{-1}$ (g)	No. of grains $plant^{-1}$	No. of grains ear^{-1}	No. of ears $plant^{-1}$	1000 kernel weight (g)	Plant fresh mass (g)	Plant dry mass (g)	Protein content (%)
	P	*	*	*	*	NS	*	*	*
	M	*	NS	NS	NS	*	*	*	NS
	M*P	NS	*	NS	NS	*	*	*	NS

- NS: not significant at P≤0.05
- *: significant at P≤0.05

The effect of seed inoculation with AM fungi varied between different wheat growth stages (Table 4.3). Only percent root moisture was significantly affected during all the wheat growth stages, while percent plant moisture was affected during the tillering stage and at anthesis. At maturity; grain yield, plant fresh mass and plant dry mass were also affected by the seed treatment with AM fungi.

No interaction between the P application levels and mycorrhizal seed inoculation were found at tillering stage, while a significant interaction with regard to percent root moisture was found at anthesis and at maturity stage (Table 4.3). At maturity significant interaction were also found with regard to number of tillers per plant as well as fresh and dry mass per plant.

Only significant ($P \leq 0.05$) results will be discussed as follows.

Tillering stage

Effect of phosphorus

At tillering, increasing P application levels had a significant increasing effect on the leaf area, number of tillers, root dry mass, shoot fresh mass and dry mass per plant as well as percent plant moisture (Figures 4.1-4.6). All measured parameters showed an increase with an increase in P application level, but tend to reach their maximum or at least level off at the P_4 (30 kg P ha^{-1}) or P_5 (40 kg P ha^{-1}) treatments.

Leaf area increased with P application from 33.79 cm^2 per plant where no P was applied (P_1) to 179.63 cm^2 per plant at a P level of 40 kg P ha^{-1} (P_5) (Figure 4.1). No significant differences were however found between P_3 (20 kg P ha^{-1}) and P_5 treatments.

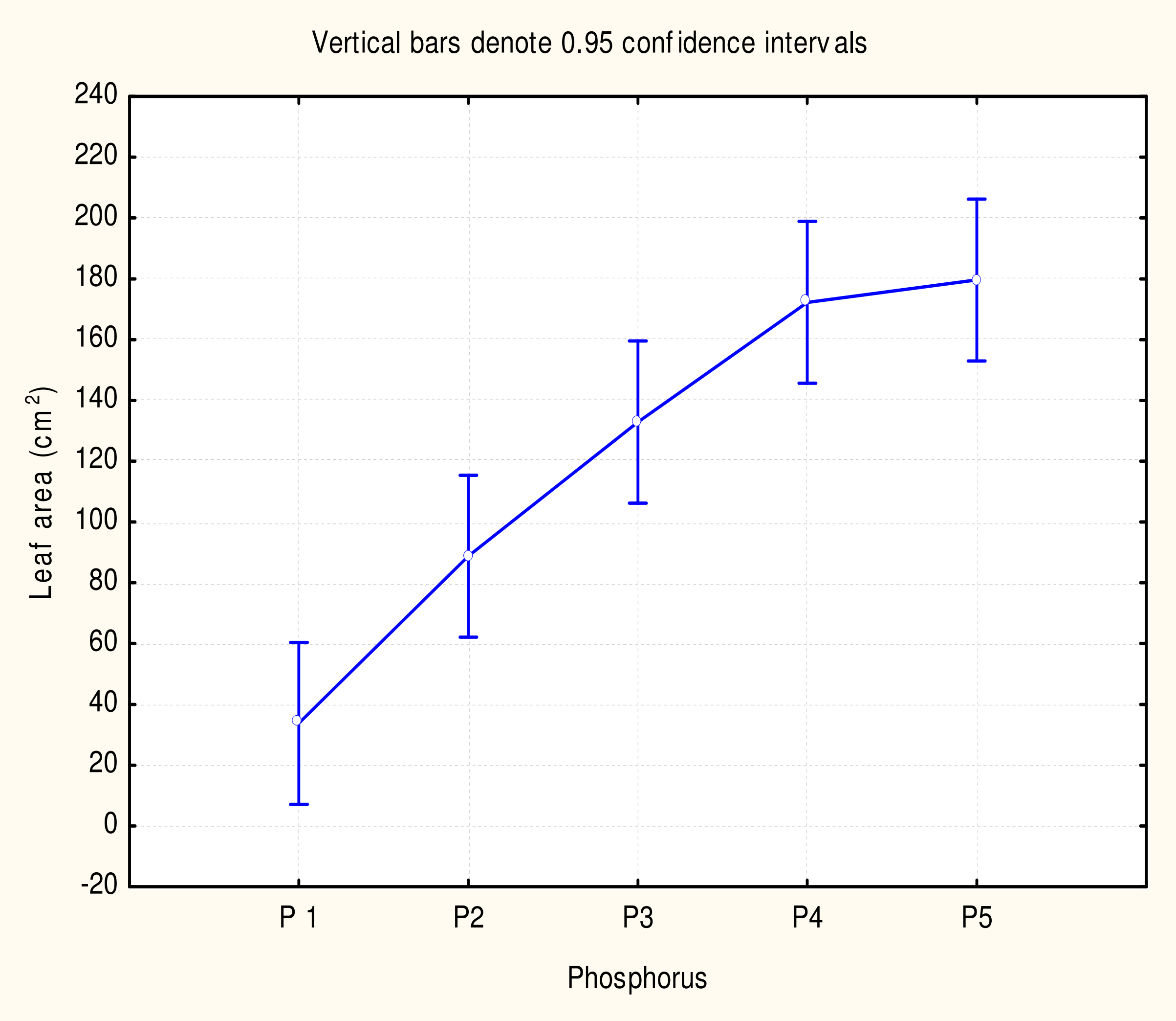

Figure 4.1 Effect of different P levels on leaf area (cm^2 plant^{-1}) of wheat plants at tillering

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The number of tillers increased from 2 tillers plant^{-1} where no P was applied (P_1) to 5 tillers plant^{-1} where 40 kg P ha^{-1} (P_5) was applied (Figure 4.2), but tillers produced per plant with treatment P_5 was not significantly higher than that of P_4 (4 tillers plant^{-1}). Similarly, Abid *et al.* (2002) has also shown that the number of tillers increased significantly with the application of P in pot trials with wheat. The increase in number of tillers could be attributed to better nutrition due to the application of P (Brown *et al.,* 1961; Bhatti *et al.,* 1983; Abid *et al.,* 2002), because Whitney (1997) showed that wheat plants do not tiller well under severe P deficiency. Hergert & Shaver (2009) reported that in winter wheat, P mainly increases tillering in the fall, which eventually increases the number of heads and grain yield.

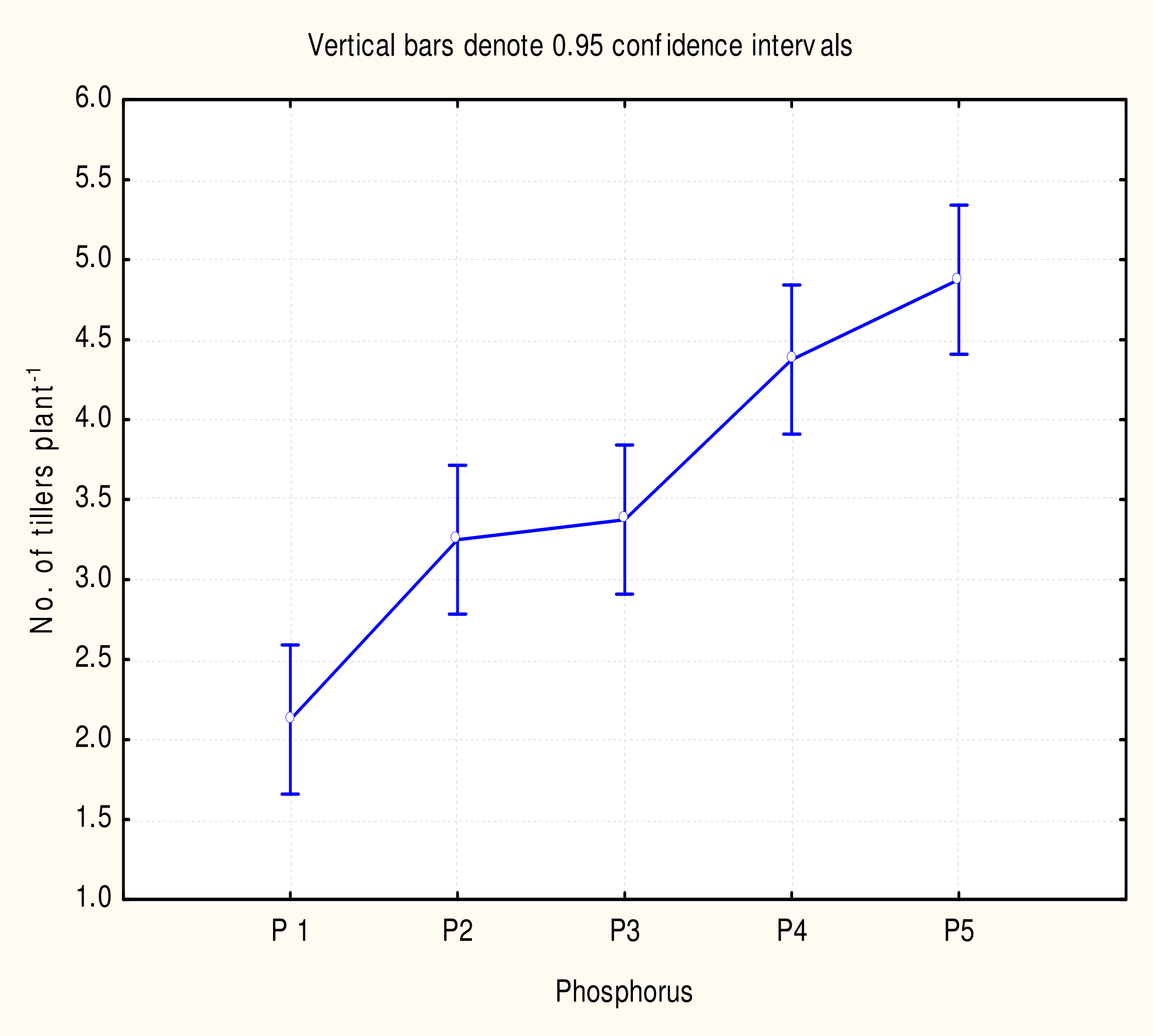

Figure 4.2 Effect of different P levels on number of tillers per plant at tillering stage of wheat

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Root dry mass increased with increasing P application levels (Figure 4.3) from 0.265 g plant^{-1} where no P was applied (P_1), to reach a maximum of 1.116 g plant^{-1} at a P application level of 30 kg P ha^{-1} (P_4). This root dry mass was however not statistically higher than the root dry mass of the P_3 (0.619 g plant^{-1}) and P_5 (1.012 g plant^{-1}) treatments. Higher dry mass due to P application could be due to the direct effect of P on root growth and its development (Purushottam *et al.,* 1995; Srivastava & Ahlawat, 1995), because Singh *et al.* (2005) found that optimum P supply is essential to maintain high root volumes, which enables the plant to explore more soil for nutrients and moisture (McKenzie & Middleton, 1997).

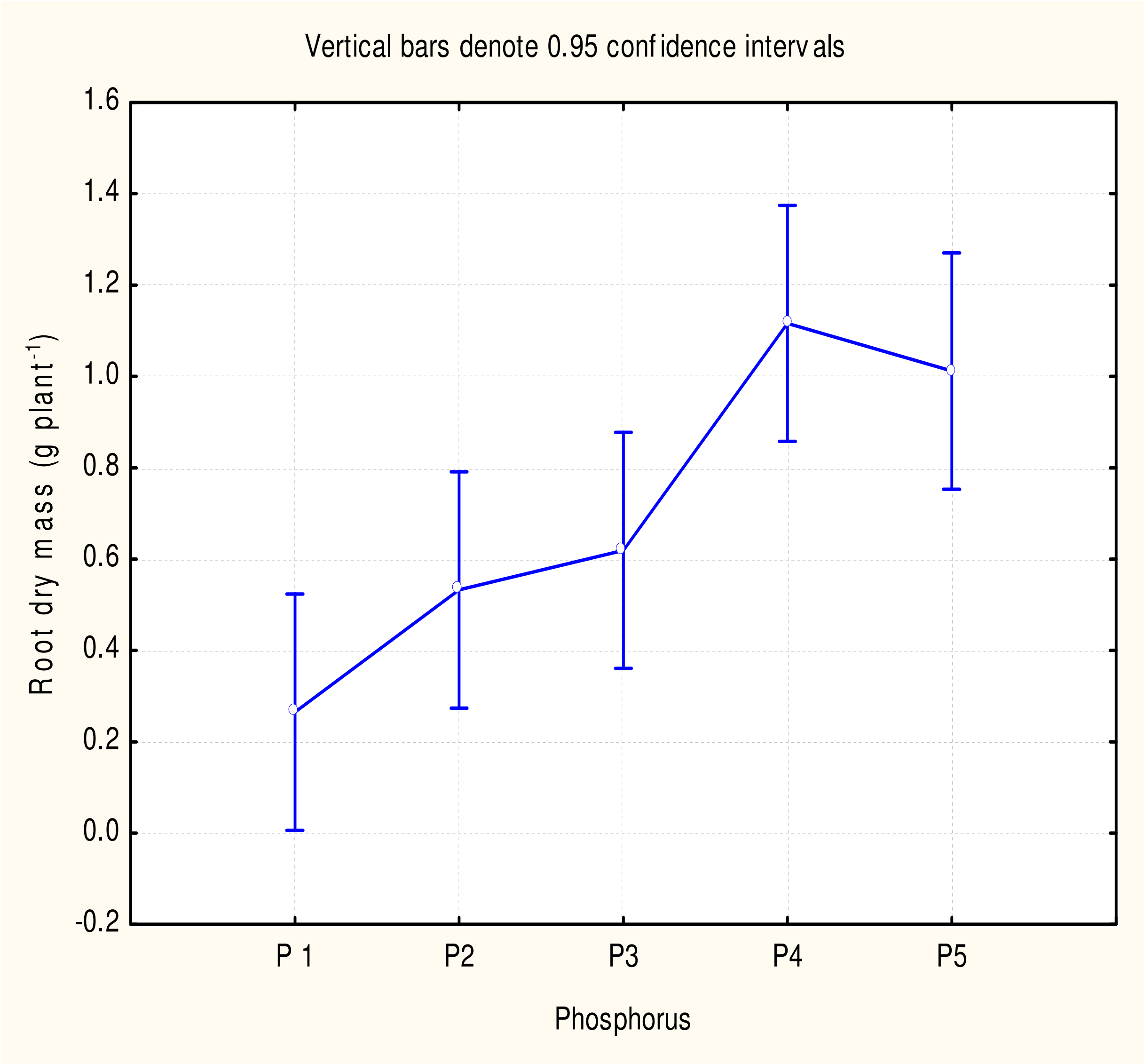

Figure 4.3 Effect of different P levels on root dry mass (g plant^{-1}) of wheat plants at tillering

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Maximum plant fresh mass of 11.85 g plant^{-1} was produced at P_5 (40 kg P ha^{-1}), which was significantly higher than plant fresh mass produced by P_1 (3.13 g plant^{-1}), P_2 (6.25 g plant^{-1}) and P_3 (8.93 g plant^{-1}) but was not statistically different from P_4 (11.49 g plant^{-1}) (Figure 4.4). Plant dry mass showed similar results with a highest value of 2.159 g plant^{-1} at P_5 (Figure 4.5) compared to 0.6036 g plant^{-1} where no P was applied (P_1). The increase in plant mass can be ascribed to the higher leaf area and more tillers recorded in this study as also shown by Muhammad *et al.* (1988) and Zahid & Bhatti (1994). Rehman *et al.* (2006) also reported a significant improvement in biomass of wheat and rice with application of phosphate over nitrogen alone.

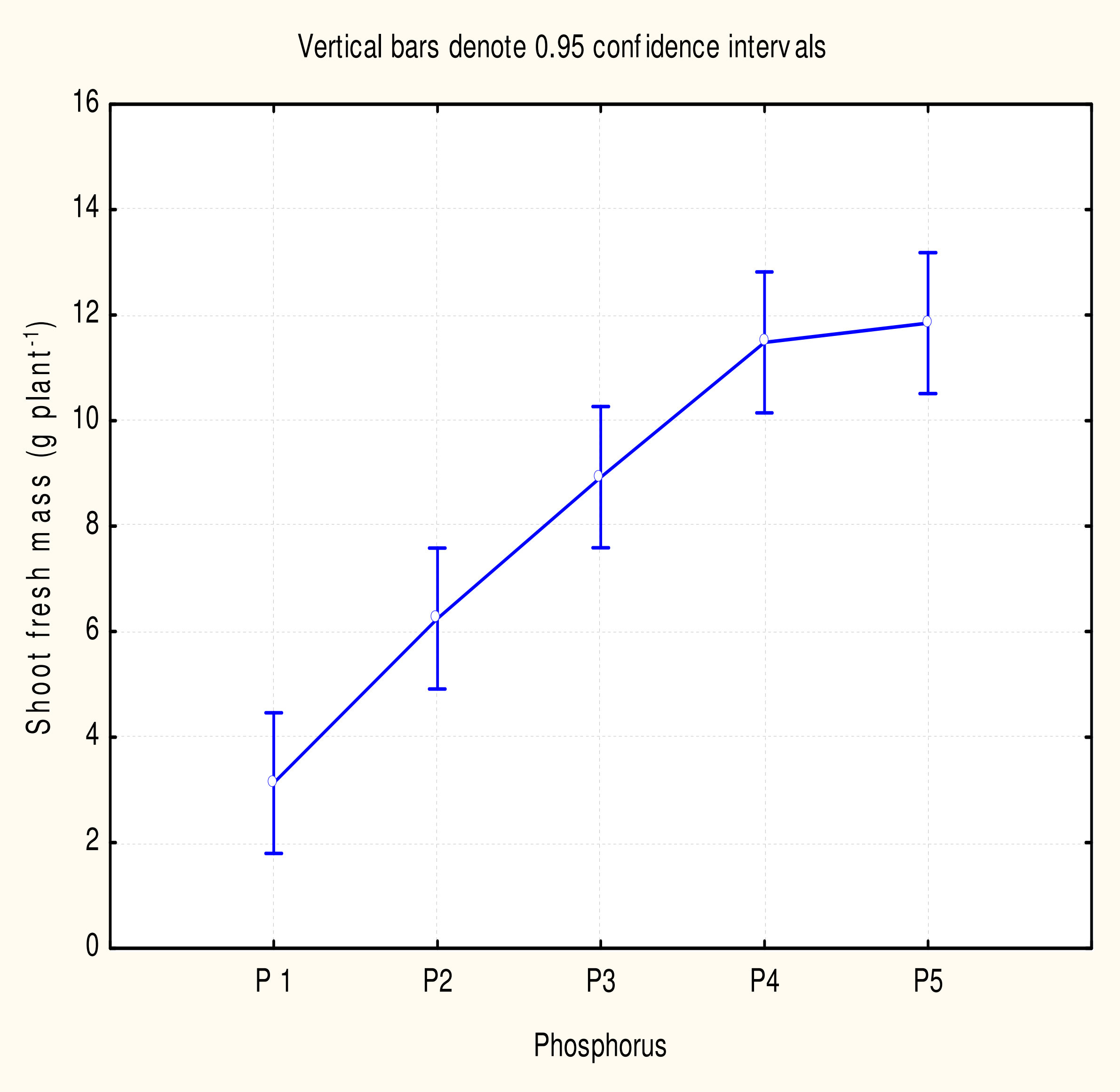

Figure 4.4 Effect of different P levels on plant fresh mass (g plant^{-1}) of wheat plants at tillering

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

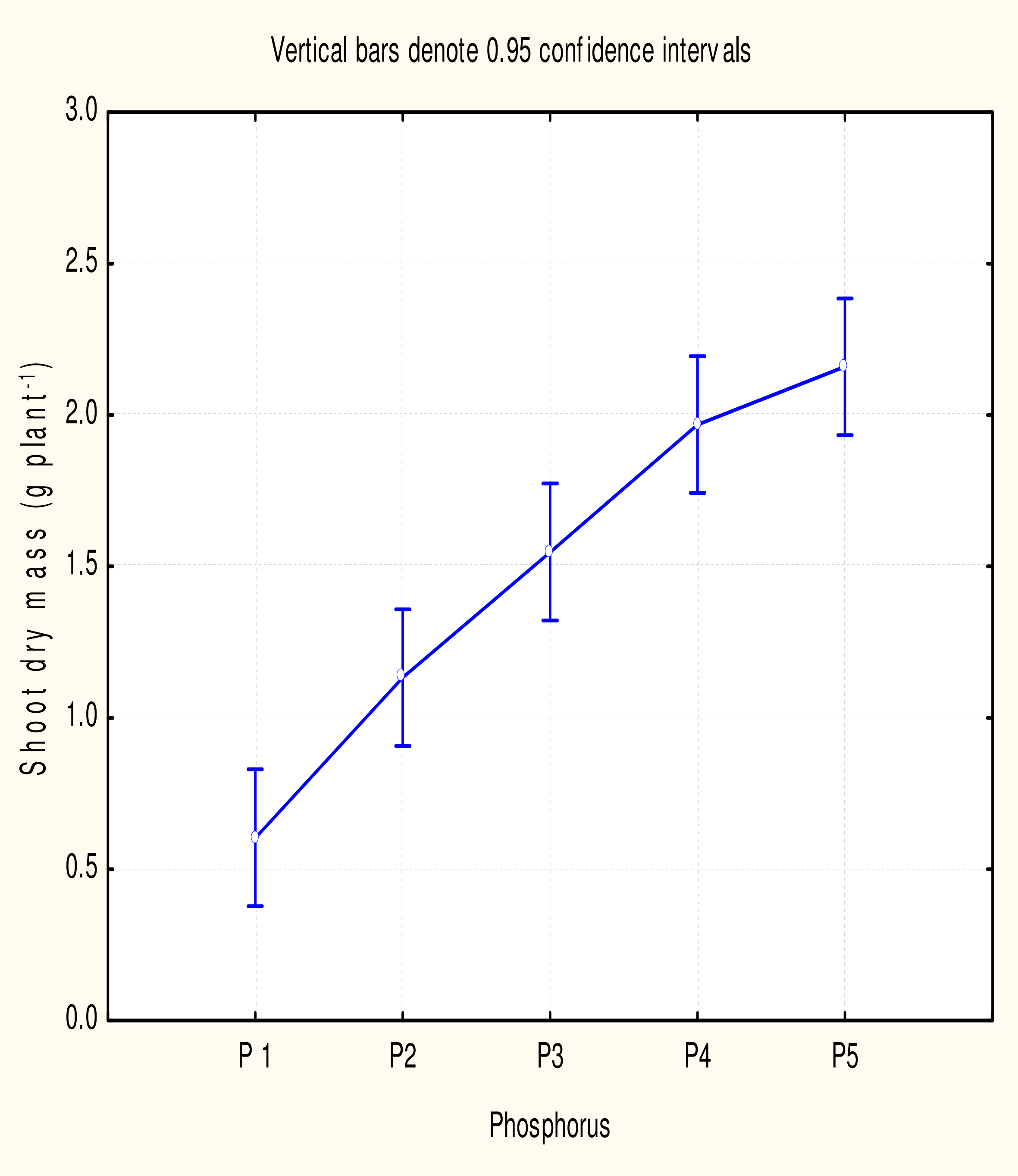

Figure 4.5 Effect of different P levels on plant dry mass (g plant^{-1}) of wheat plants at tillering

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The highest moisture content of 82.74% was measured in plants receiving 30 kg P ha^{-1} (Figure 4.6). This value was however not significantly different from the moisture content of either the P_3 plants (82.46%) or the P_5 plants (81.79%). Plants which received no P (P_1) had the lowest moisture content of 79.99% plant^{-1}.

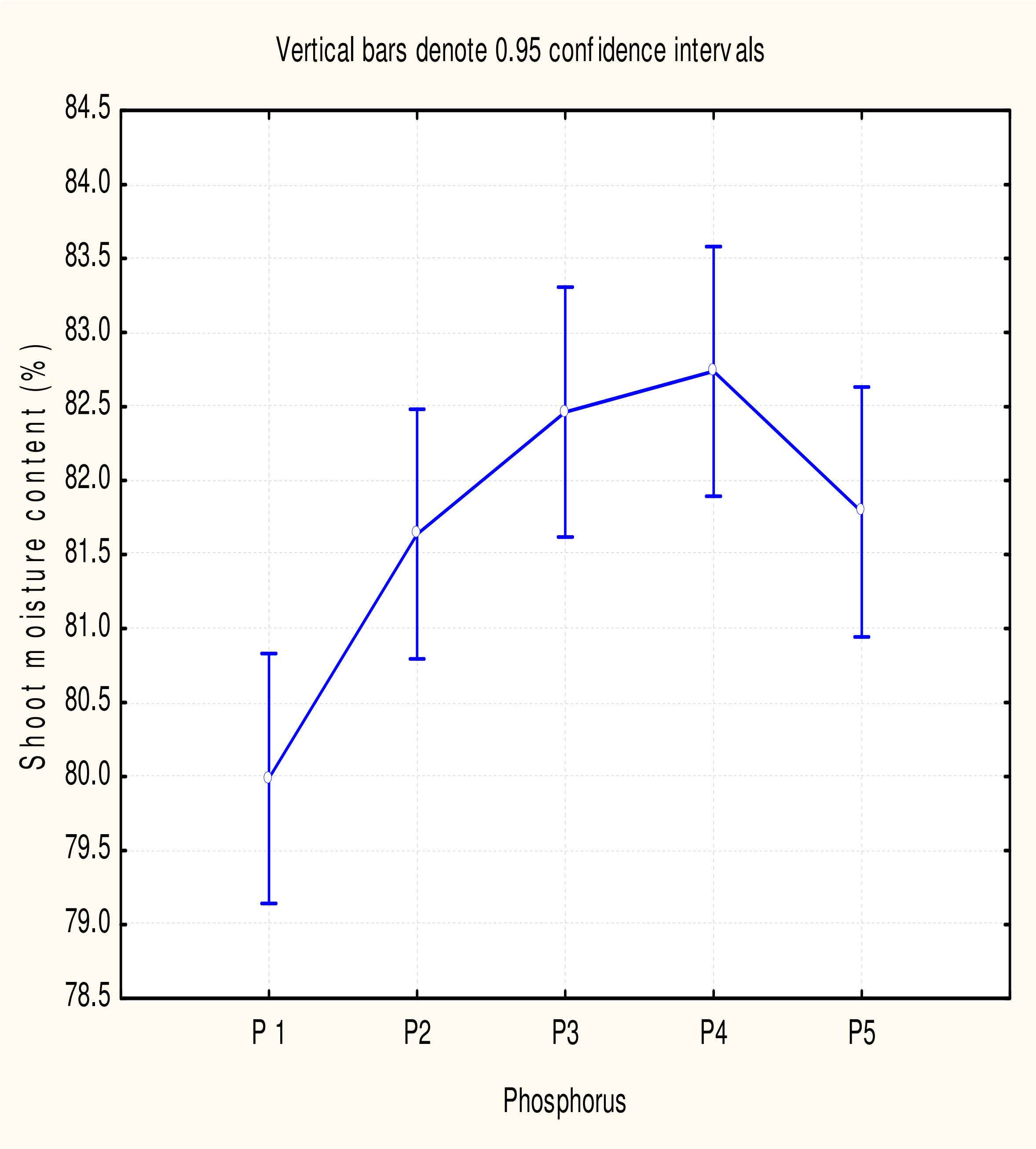

Figure 4.6 Effect of different P levels on the moisture content (%) of wheat plants at tillering

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

These results suggested that the optimum P level for wheat in sandy soil with P content of 18 mg kg^{-1} (citric acid) used in this experiment was between 30 and 40 kg P ha^{-1}, although some parameters tested showed no significant increase when P application rates were higher than 20 kg P ha^{-1}.

Effect of mycorrhizal inoculation

The results showed that seed inoculation with AM fungi had a significant increasing effect on leaf area, percentage root moisture, plant fresh mass and percentage plant moisture (Table 4.4). Seed inoculation with AM fungi increased leaf area per plant at tillering from 103.83 cm^2 to 139.16 cm^2, root moisture from 78.07% to 83.99%, fresh mass $plant^{-1}$ from 7.61 g to 9.05 g and plant moisture content from 80.44 to 81.02% (Table 4.4). Khan (1972) also reported that the increase in leaf area of wheat plants was stimulated by mycorrhizal infection during the early growth phases. Increase in leaf area could improve solar radiation capture by plants and potentially increase yield.

Table 4.4 Effect of mycorrhizal inoculation on growth of wheat plants at tillering (50 days after planting) expressed as mean values per plant

Treatments	Measured parameters							
	Leaf area (cm^2)	No. of tillers	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
M_0	103.83b	3.40a	8.02a	0.66a	78.07b	7.61b	1.45a	80.43b
M_1	139.16a	3.80a	4.43a	0.75a	83.99a	9.05a	1.52a	81.02a

*Means with different letters within the same column are significantly different ($P \leq 0.05$).

M_0 = uninoculated seed M_1= seed inoculated with AM fungi

In this study no significant interaction between P fertilization and mycorrhizal inoculation was found at the tillering stage of the wheat.

Anthesis stage

Effect of phosphorus

At anthesis, increasing P application levels had a significant increasing effect on all the measured parameters except for percentage root moisture (Figures 4.7-4.13), but there was a significant interaction between P application level and seed inoculation with AM fungi on percentage root moisture (Figures 4.13). All measured parameters except for percent root moisture showed a significant increase with an increase in P application levels, but tend to reach their maximum or at least level off at P_3 to P_5 treatments.

Leaf area showed an increasing trend from 40.83 cm^2 $plant^{-1}$ where no P was applied (P_1) and reached a maximum of 135.67 cm^2 $plant^{-1}$ at a P level of 30 kg P ha^{-1} (P_4) (Figure 4.7). No significant differences were however found between P_3 (20 kg P ha^{-1}), P_4 and P_5 (40 kg P ha^{-1}).

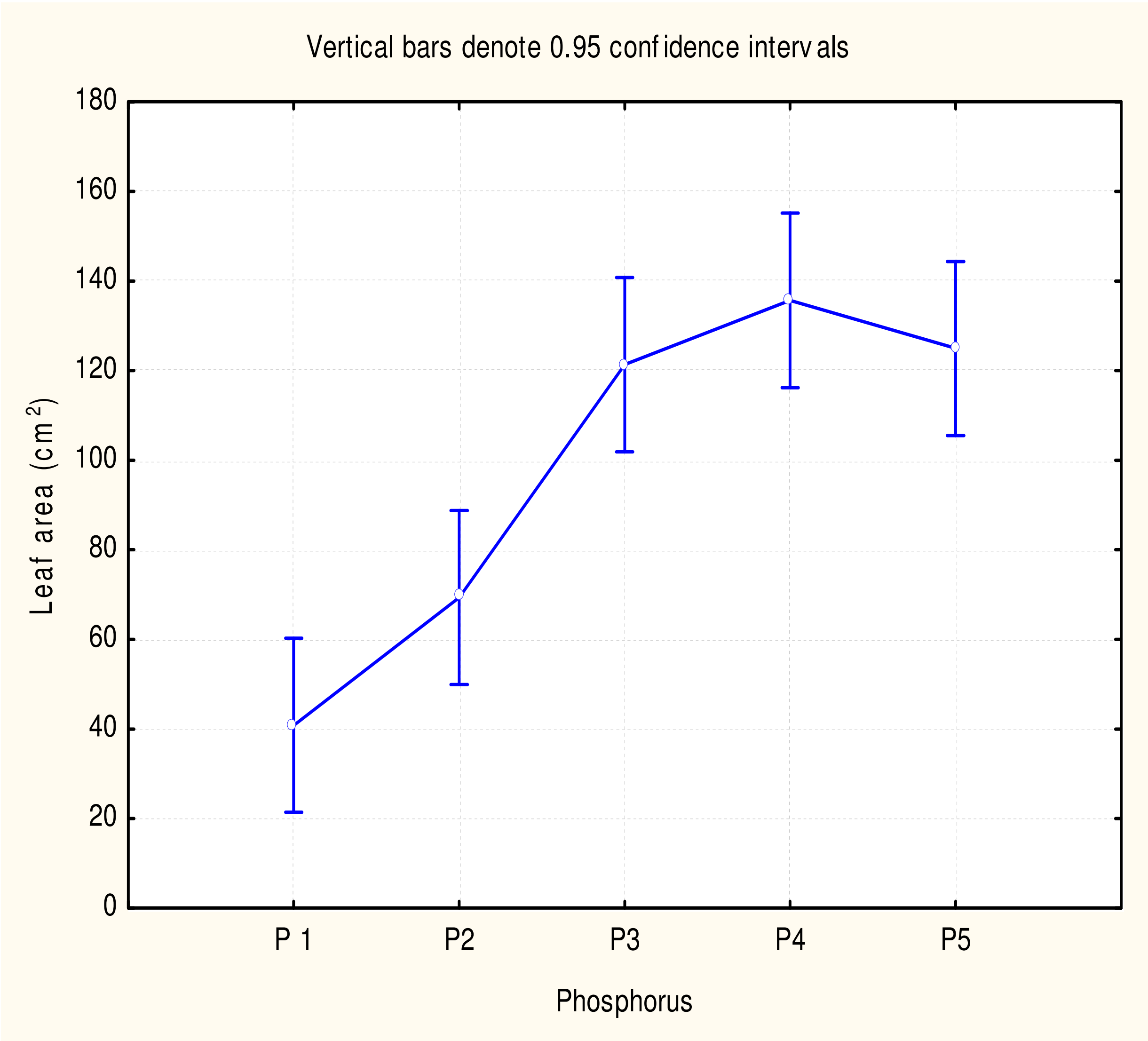

Figure 4.7 Effect of different P levels on leaf area (cm^2 $plant^{-1}$) of wheat plants at anthesis

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

The number of ears increased from 1.88 ears plant^{-1} with the application of 0 kg P ha^{-1} and reached a maximum of 3.0 ears plant^{-1} where 20 kg P ha^{-1} was applied (Figure 4.8). However, there was no significant difference between 20 kg P ha^{-1} (P_3), 30 kg P ha^{-1} (P_4) and 40 kg P ha^{-1} (P_5).

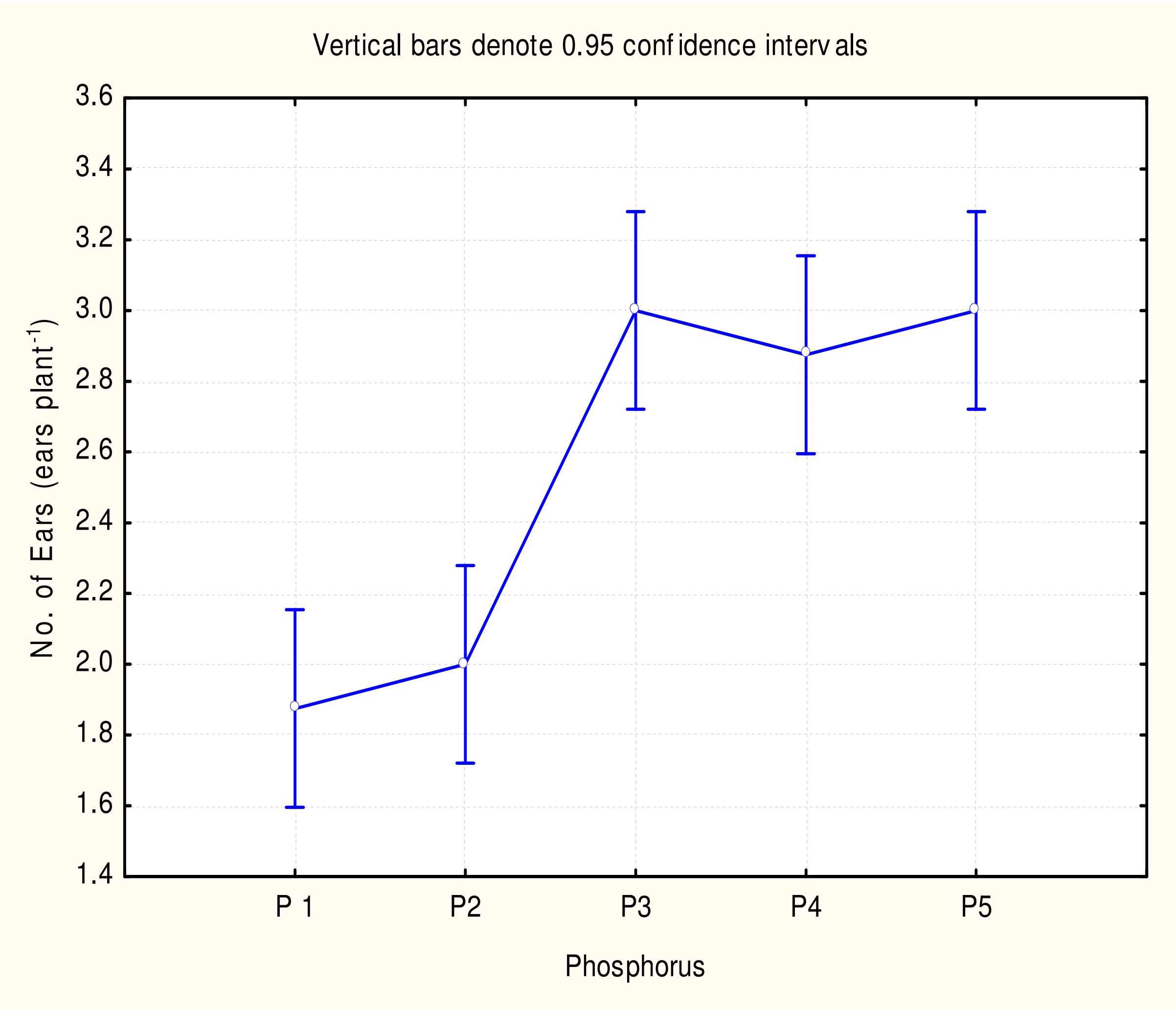

Figure 4.8 Effect of different P levels on number of ears (ears plant^{-1}) of wheat plants at anthesis

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

The results (Figure 4.9) showed an increase in root fresh mass with increasing P application levels from 2.96 g plant^{-1} at P_1 (0 kg P ha^{-1}) to 10.5 g plant^{-1} at P_5 (40 kg P ha^{-1}) without reaching a maximum. The root fresh mass at P_5 was however not statistically different from the root fresh mass produced with 30 kg P ha^{-1} (P_4). Jones *et al.* (2005) have previously found significant differences in root biomass when applications of 0 kg P ha^{-1} and 25 kg P ha^{-1} treatments were compared in soils with medium P contents (Olsen P = 7.6 mg kg^{-1}) and a wet soil with low (Olsen P = 2.5 mg kg^{-1}) P content. Phosphorous fertilization has also been previously found to significantly increase root growth under both water-stressed and non water-stressed conditions (Saneoka *et al.,* 1990; Al-Karaki *et al.,* 1995; Singh & Sale, 2000).

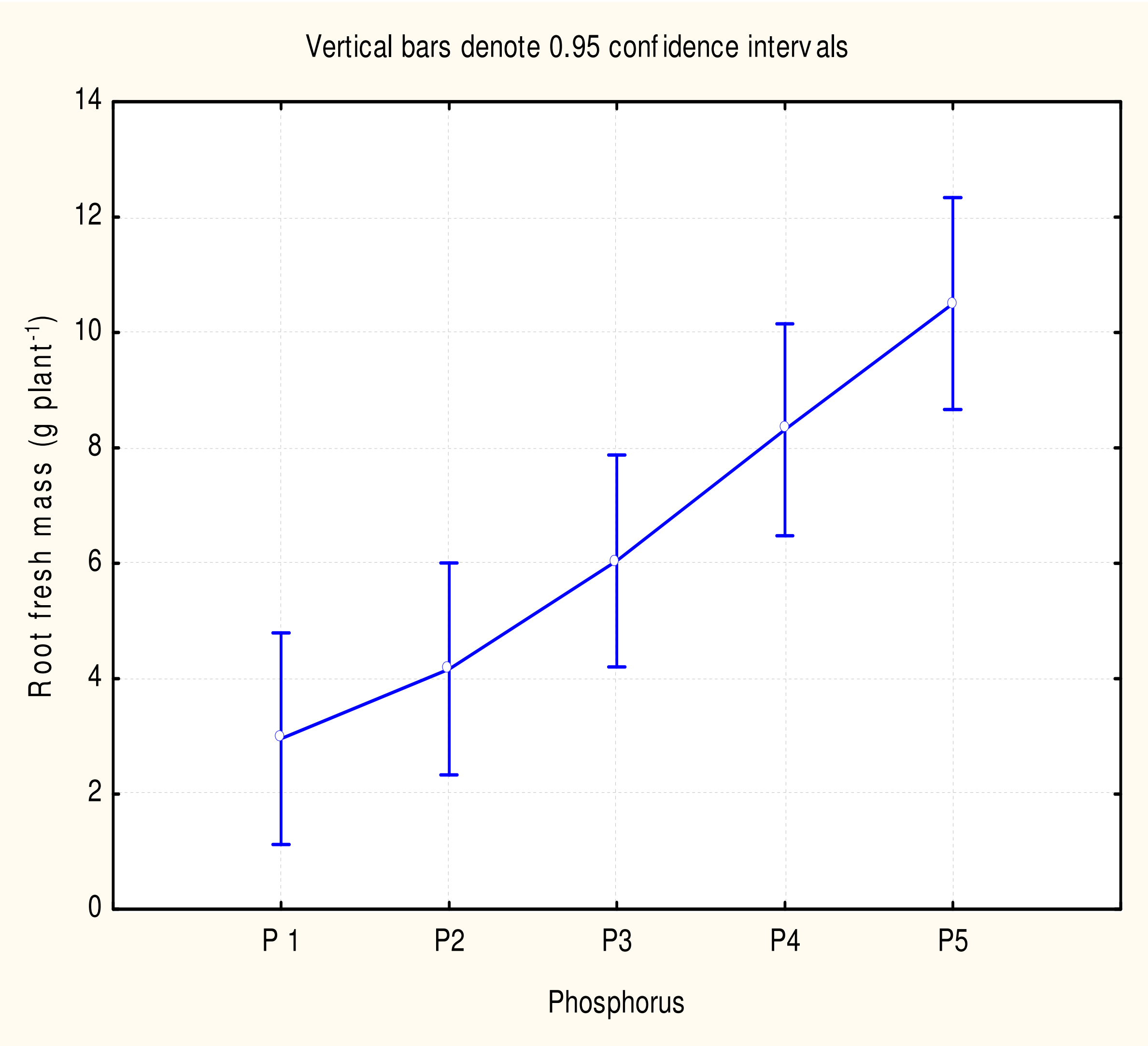

Figure 4.9 Effect of different P levels on root fresh mass (g plant^{-1}) of wheat plants at anthesis

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

The data on root dry mass (Figure 4.10) showed trends similar to root fresh mass (Figure 4.9). Root dry mass also increased with increasing P application levels from 0.681 g plant^{-1} with P_1, but reached a value of 2.25 g plant^{-1} at a P application level of 40 kg P ha^{-1}. This root dry mass was however not statistically different from root dry mass of the P_3 (1.417 g plant^{-1}) and P_4 (1.946 g plant^{-1}) treatments. Bagayoko *et al.,* (2000) reported an increase of root dry matter of 18-fold and root length density of 17-fold due to P application on a sandy and severely P (P-Bray = 4.10 mg kg^{-1}) deficient West African soil.

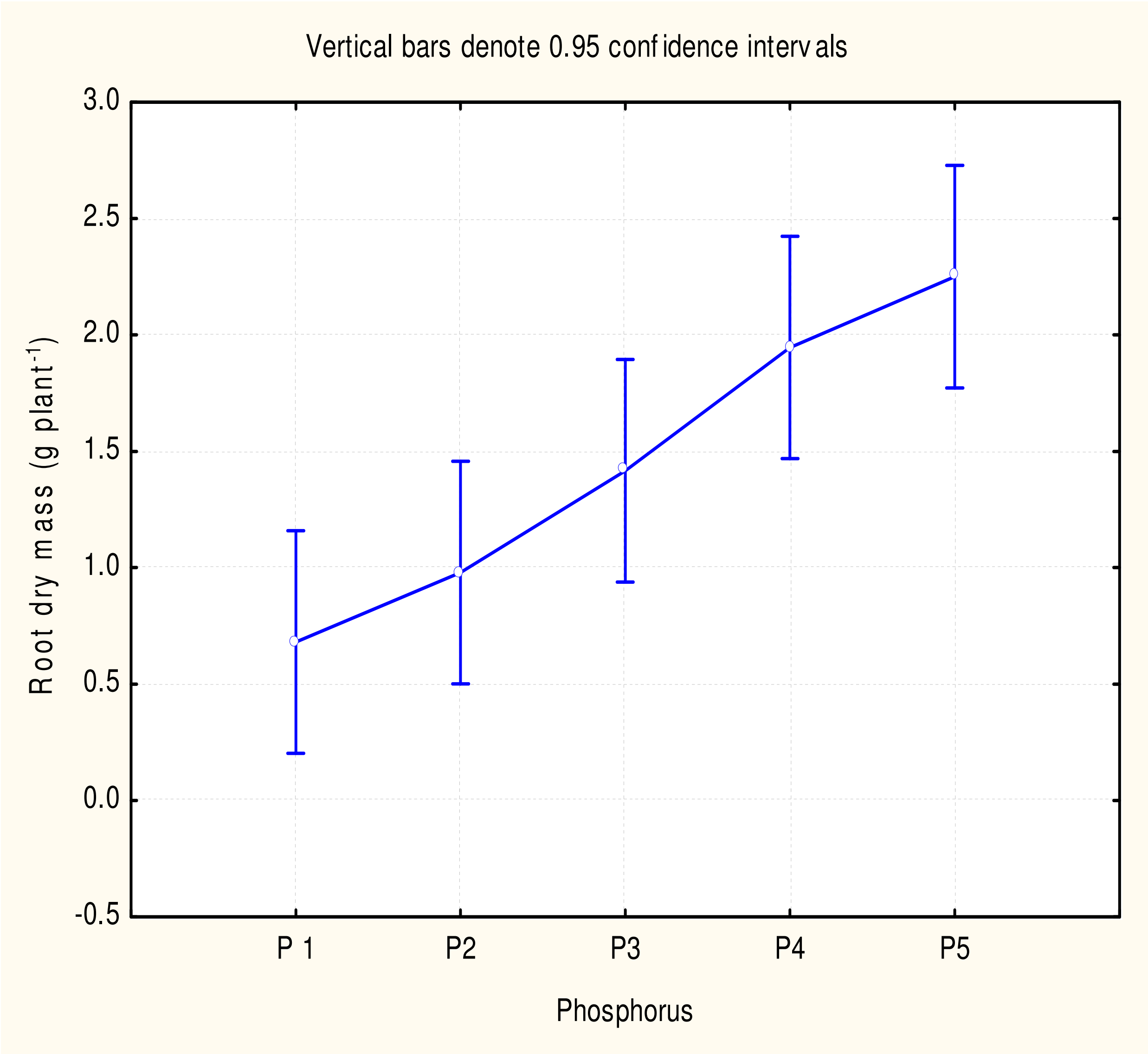

Figure 4.10 Effect of different P levels on root dry mass (g plant^{-1}) of wheat plants at anthesis

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Plant fresh mass was affected significantly with the application of phosphorus (Figure 4.11). A gradual increase in plant fresh mass was observed with increasing P levels. The highest plant fresh mass of 22.02 g plant^{-1} was produced at P_5 which was significantly higher than plant fresh mass produced by P_1 (7.896 g plant^{-1}) and P_2 (11.771 g plant^{-1}) but was not statistically different from 19.15 g plant^{-1} and 20.96 g plant^{-1} produced by P_3 and P_4

respectively. Plant dry mass showed similar results with a highest value of 6.592 g plant^{-1} at P_5 compared 2.508 g plant^{-1} where no P was applied (P_1) (Figure 4.12).

Abid *et al.* (2002) noted a significant effect on wheat straw yield with P levels. Similarly, Manchandra *et al.* (1982) and Niazi *et al.* (1990) reported an increase in straw yield of wheat with P application in saline soil conditions. Bagayoko *et al.* (2000) reported an increase of shoot dry mass of 24-fold due to P application on a sandy and severely P (P-Bray = 4.10 mg kg^{-1}) deficient West African soil, while a significant increase in straw yield by application of phosphorus was also reported by Ali & Yasin (1991); Sattar *et al.* (1991); Tanner *et al.* (1992) and Hussain (2007).

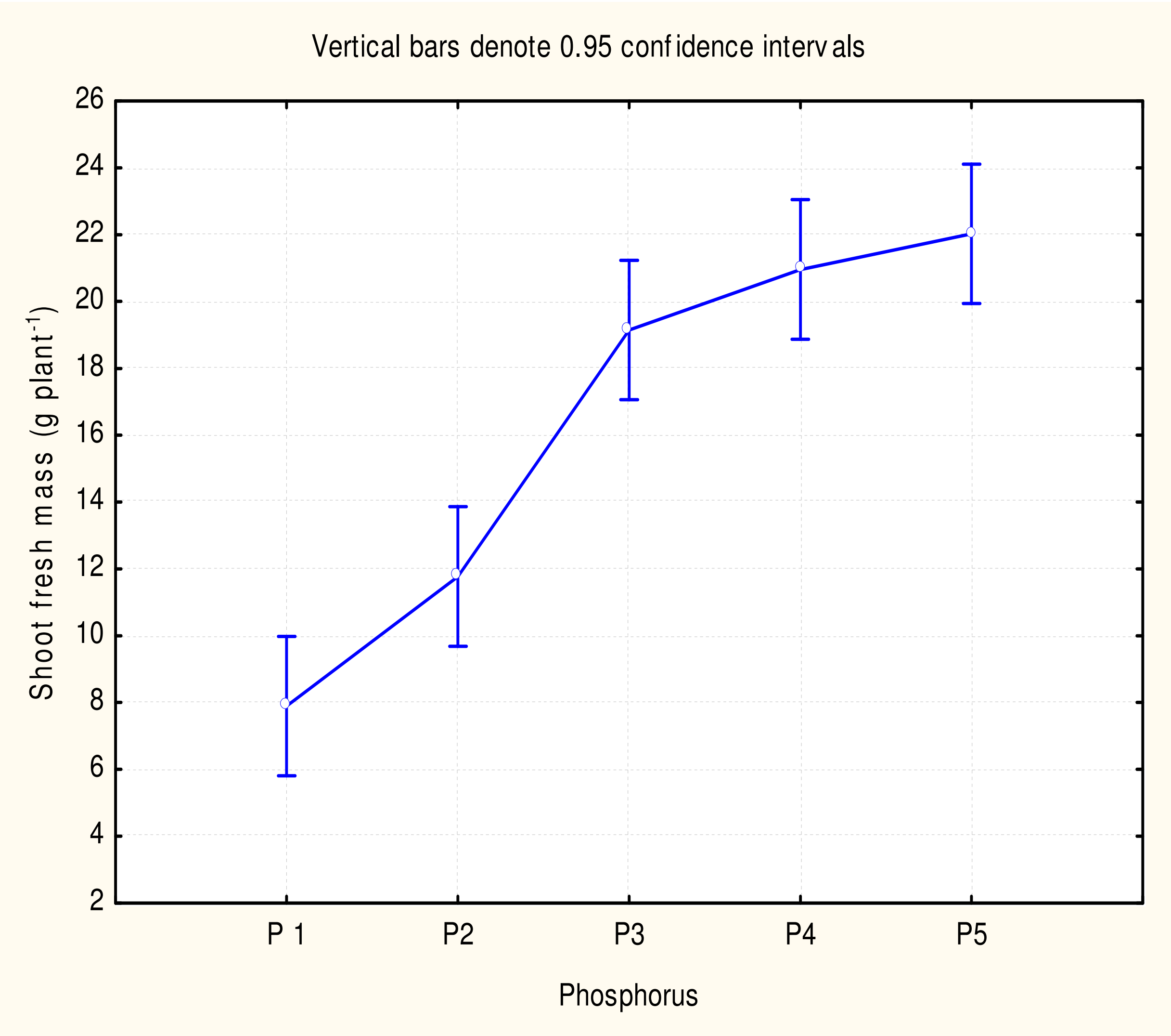

Figure 4.11 Effect of different P levels on shoot fresh mass (g plant^{-1}) of wheat plants at anthesis

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

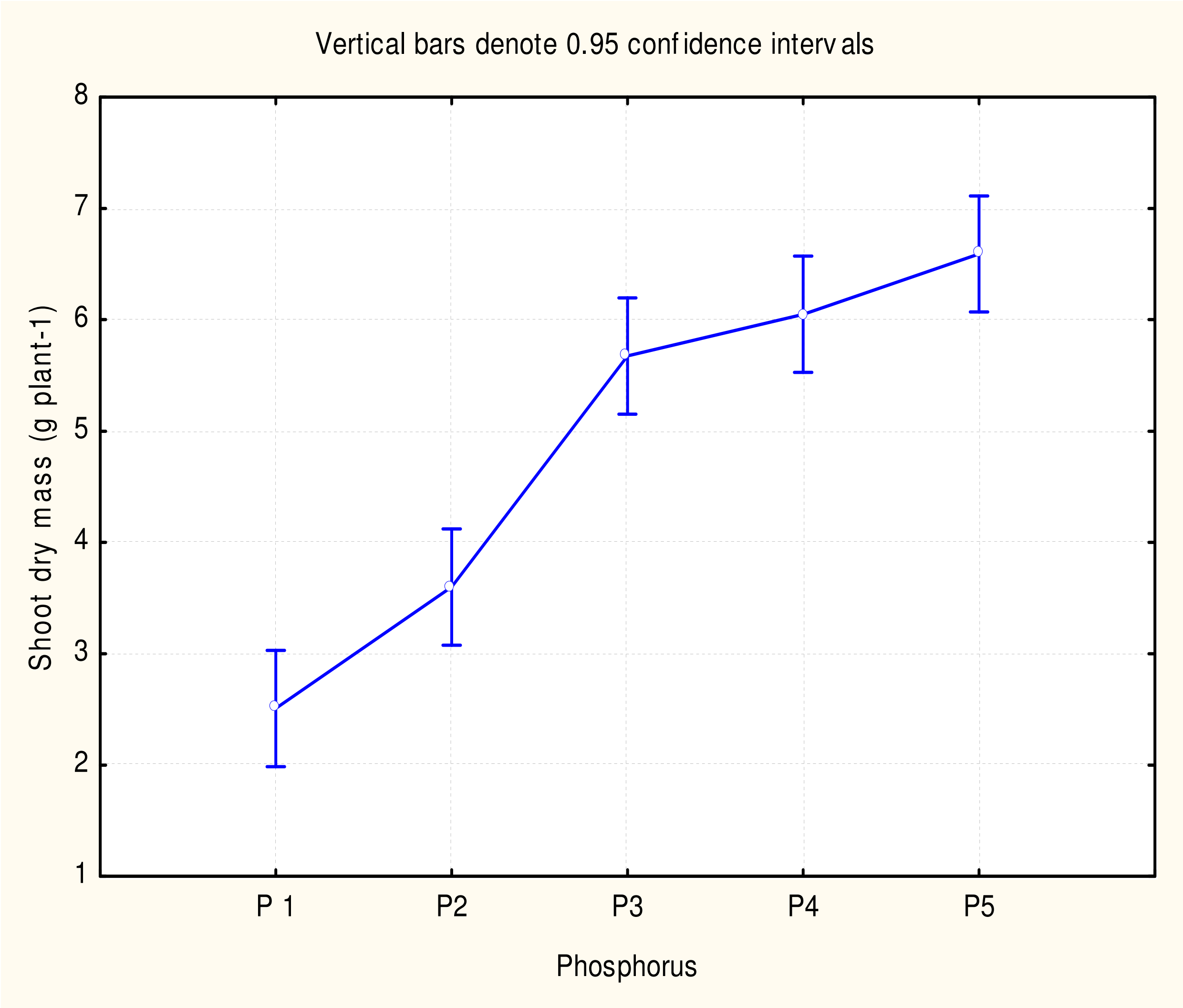

Figure 4.12 Effect of different P levels on shoot dry mass (g plant^{-1}) of wheat plants at anthesis

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results indicated that percentage plant moisture was significantly affected with phosphorus levels (Figure 4.13). Plants receiving 30 kg Pha^{-1} had the highest moisture content of 71.01%, but was not significantly different from the moisture content of either P_2 (69.13%), P_3 (70.26%) or P_5 plants (69.78%). However, plants which received no P (P_1) had the lowest moisture content of 68.06%.

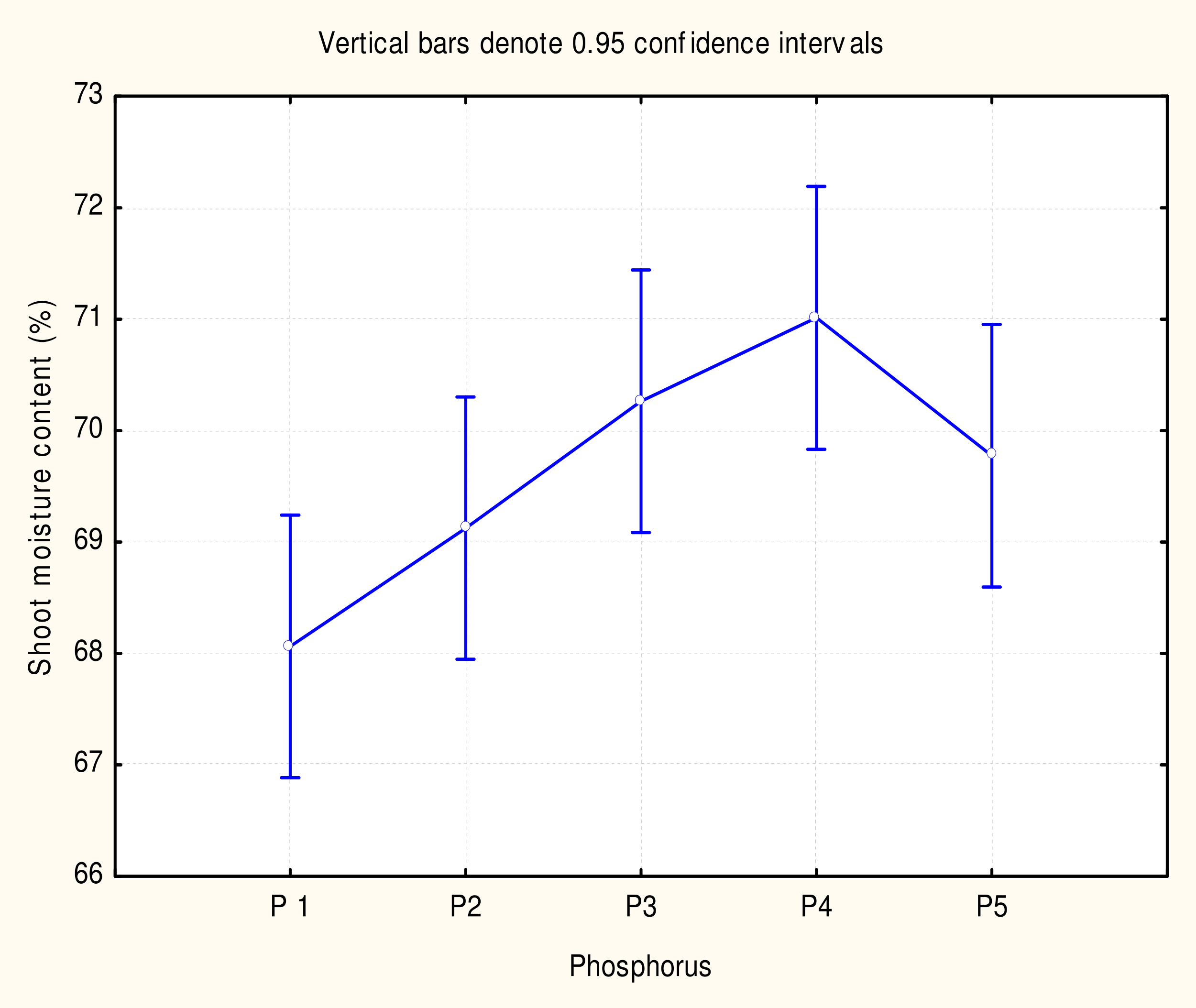

Figure 4.13 Effect of different P levels on above ground moisture content (%) of wheat plants at anthesis

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

The results (Tables 4.5) showed that seed inoculation with AM fungi had a significant effect on plant and root moisture content. Seed inoculation with AM fungi increased plant moisture content at anthesis from 69.11% to 70.19% but tended to decrease root moisture content from 78.40% to 74.66%. The root moisture content however showed a significant interaction between seed inoculation and P application level.

Table 4.5 Effect of mycorrhizal inoculation on growth of wheat plants at anthesis (90 DAP) expressed as mean values per plant

Treatments	Measured parameters							
	Leaf area (cm^2)	No. of ears	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
M_0	96.11a	2.60a	6.21a	1.35a	78.40a	16.44a	5.01a	69.11b
M_1	100.74a	2.50a	6.58a	1.56a	74.66b	16.28a	4.76a	70.19a

*Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1= seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application level

The significant interaction between the mycorrhizal seed treatment and the P application level with regard to root moisture at anthesis indicates that the mycorrhiza have colonized the roots of the wheat and started to affect the functioning of the roots which support earlier results which indicate that the effect of mycorrhizal inoculation becomes evident only after about one month (Khan, 1975; Owusu-Bennoah & Mosse, 1979; Bougher *et al.,* 1990). In this study mycorrhizal seed inoculation did show an effect on several plant parameters at tillering stage (50 DAP), but the absence of any significant interaction with P application level suggested a mycorrhizal effect other than improved root functioning.

From Figure 4.14 it became clear that in contrast to treatments where P were applied (P_2-P_5), moisture content (%) of roots from mycorrhiza inoculated plants were significantly less compared to root moisture content of uninoculated plants if no P was applied. This could be due to the fact that at very low P levels, mycorrhiza does get energy (sugars) from the plant but could not improve root functioning because of the low P content of the soil.

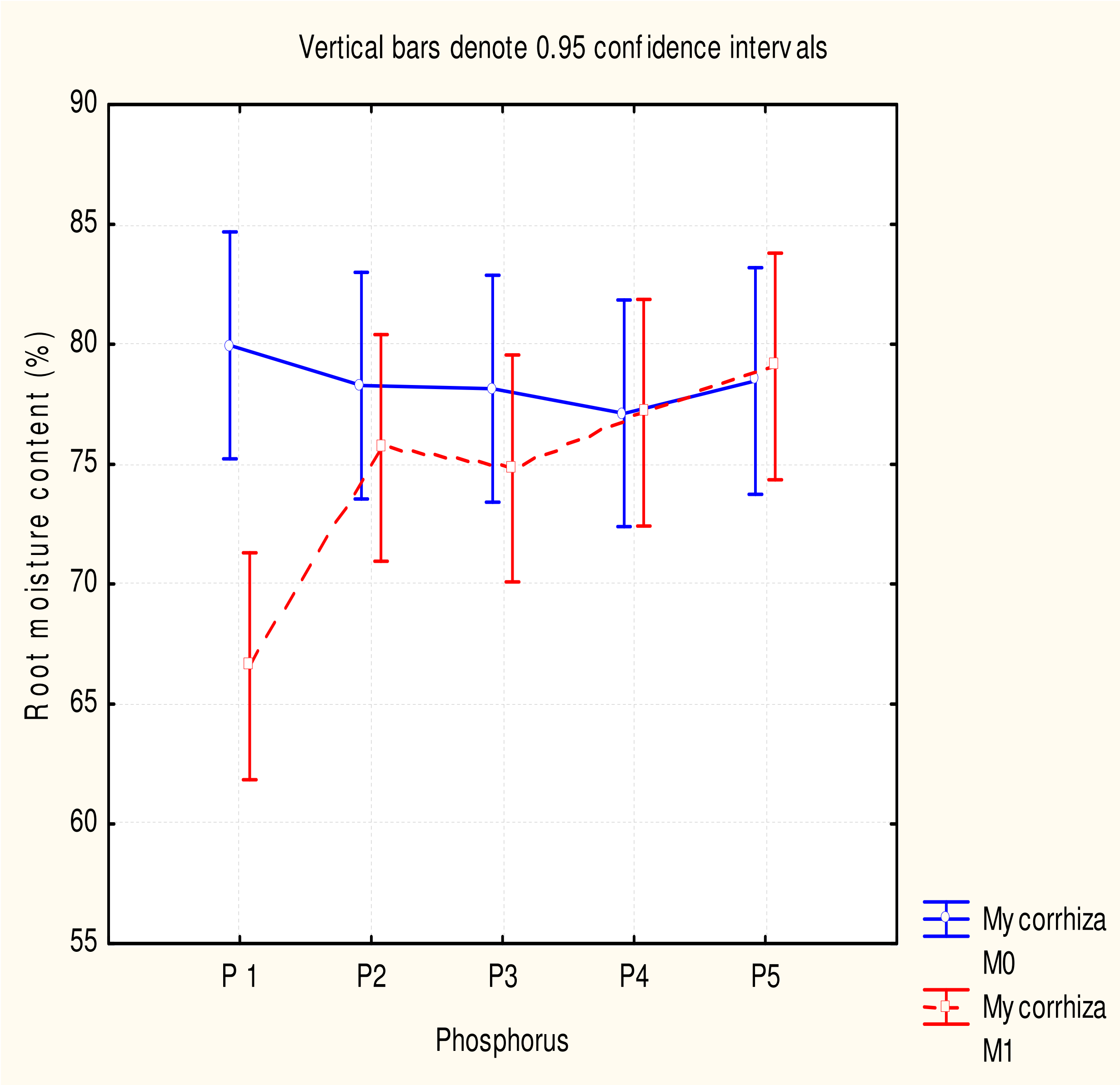

Figure 4.14 Effect of seed inoculation with mycorrhiza on the response of root moisture content (%) to different P application rates of wheat plants at anthesis

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

Maturity stage

Effect of phosphorus

At maturity stage, increasing P application levels had significant increasing effects on grain yield per plant, number of grains per plant, number of grains per ear, number of ears per plant, plant fresh mass, plant dry mass as well as the wheat protein content (Figures 4.15-4.18). Because of significant interactions between P application levels and mycorrhizal seed inoculation with regard to number of grains per plant, thousand kernel weight, plant fresh mass and plant dry mass (Table 4.3) the main effect of P on these parameters will not be discussed.

All measured parameters except for wheat protein content, showed an increase with an increase in P application levels, but tend to reach their maximum or at least level off at P_4 or P_5 treatments.

Wheat grain yield (Figure 4.15) increased from 1.604 g plant^{-1} where no P was applied (P_1) to 5.156 g plant^{-1} at a P level of 40 kg P ha^{-1} (P_5). No significant differences were however found between P_4 (30 kg P ha^{-1}) and $P_{5.}$ A gradual increase in grain yield with increasing P levels was also observed by several researchers under various conditions (Singh *et al.,* 2000; Abid *et al.,* 2002; Akhtar *et al.,* 2002; Slaton *et al.,* 2005; Hussain, 2007).

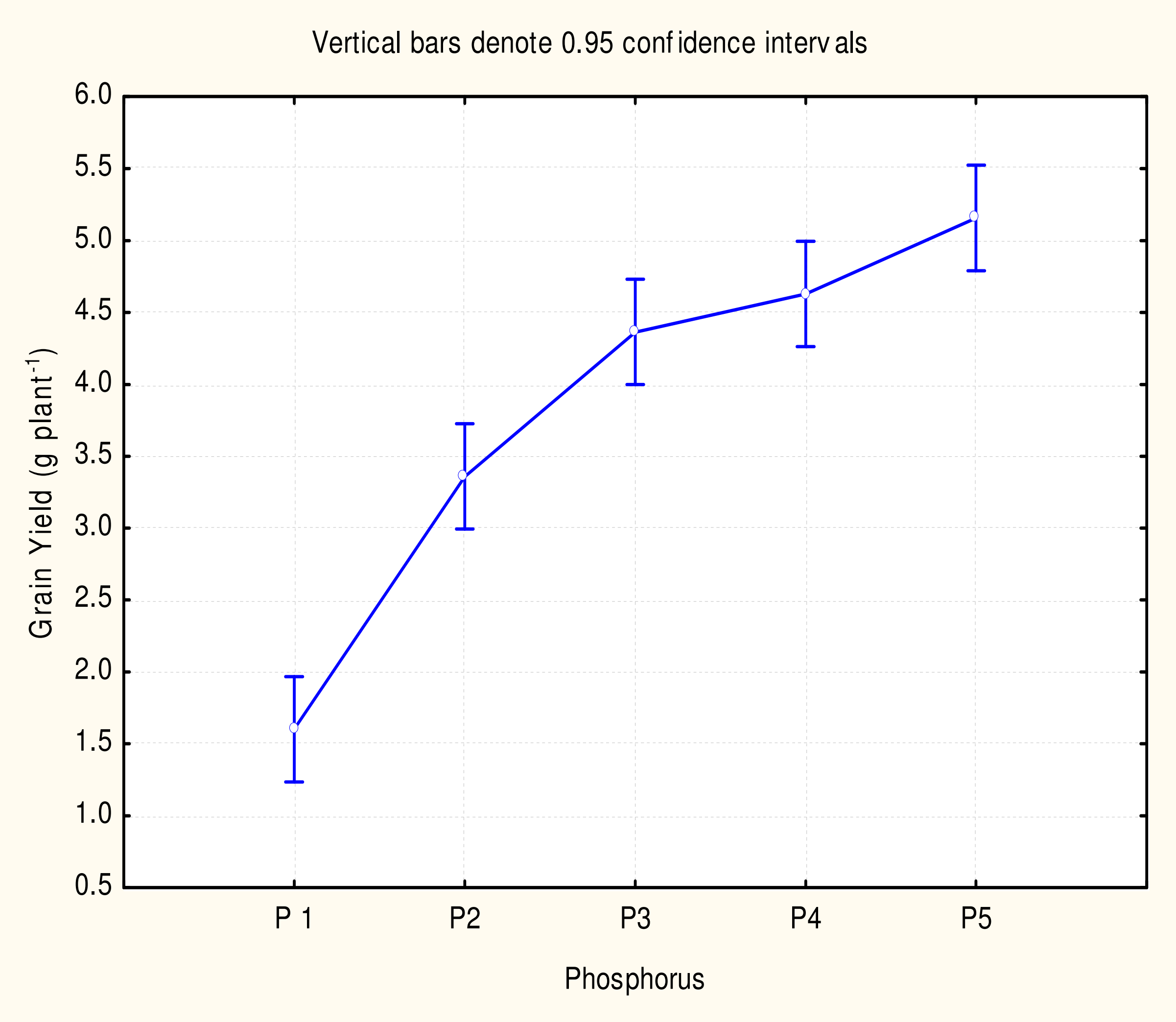

Figure 4.15 Effect of different P levels on grain yield (g plant^{-1}) of wheat plants at maturity

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The number of grains per ear increased with an increase in P application level. Application level of 20 kg P ha^{-1} (P_3) produced 38.63 grains ear^{-1} which was significantly more than 21.13 grains ear^{-1} produced by 0 kg P ha^{-1} (P_1) (Figure 4.16). Grains produced ear^{-1} by treatment P_3 were however not significantly higher than that of the P_2 (30.50 grains ear^{-1}), P_4 (37.75 grains ear^{-1}) or P_5 (36.50 grains ear^{-1}) treatments. The results obtained in this pot trial in sandy soil with a P content of 18 mg kg^{-1}, supported earlier studies (Singh *et al.,* 2000) in a rice-wheat rotation on a soil with 4.0 mg kg^{-1} 0.5 M $NaHCO^3$ extractable P (Olsen P), which showed a significant increase in wheat grain yield when P application levels were increased to 26 kg P ha^{-1}, but no increase in yield with higher application levels.

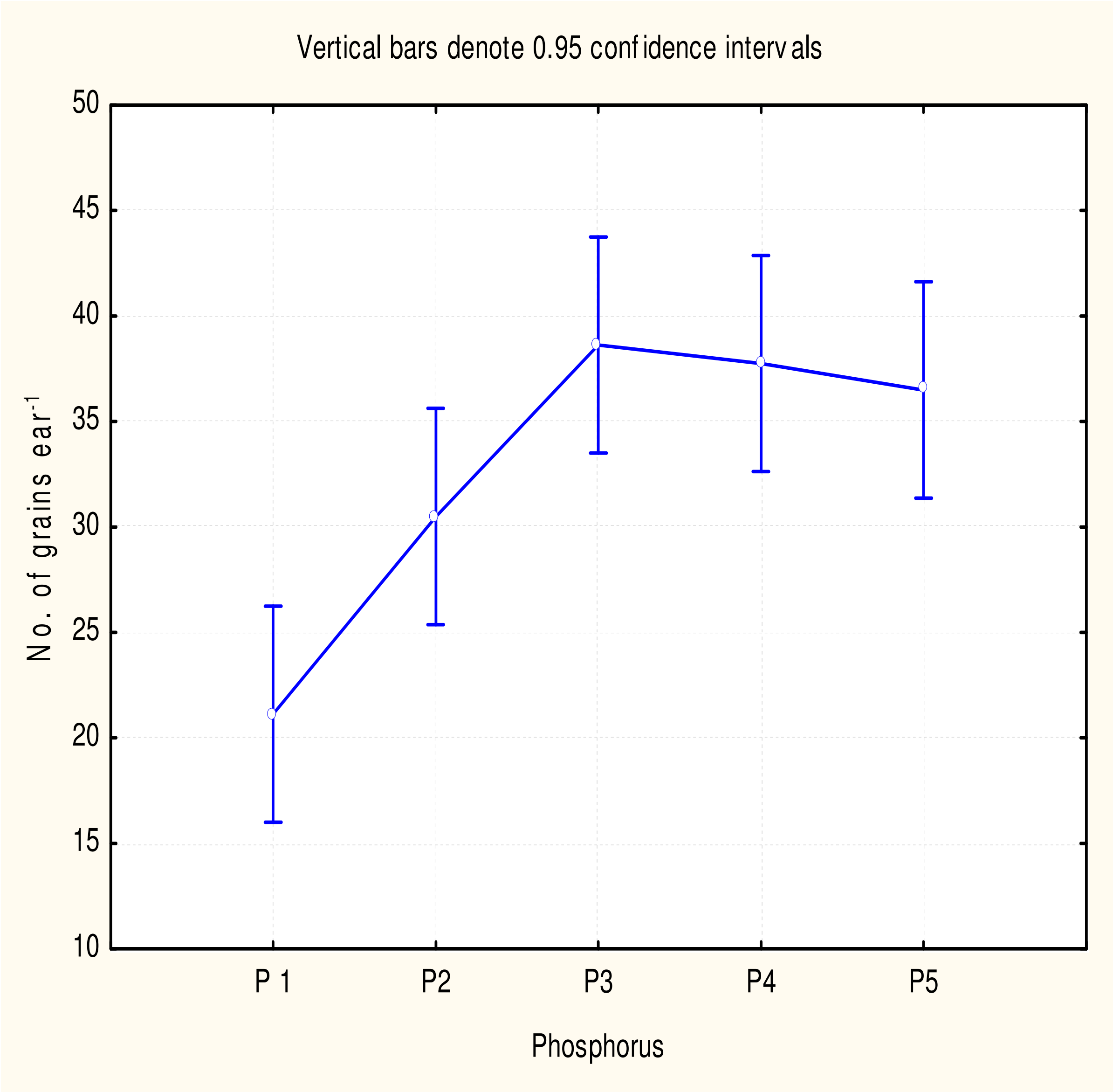

Figure 4.16 Effect of different P levels on number of grains per ear of wheat plants at maturity

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The number of ears per plant increased from 2.00 ears $plant^{-1}$ where no P was applied (P_1) to 3.88 ears $plant^{-1}$ where 40 kg P ha^{-1} (P_5) was applied (Figure 4.17). However, the ears produce per plant by treatment P_5 was not significantly higher than that of P_2 (3.13 ears $plant^{-1}$), P_3 (3.00 ears $plant^{-1}$) and P_4 (3.25 ears $plant^{-1}$).

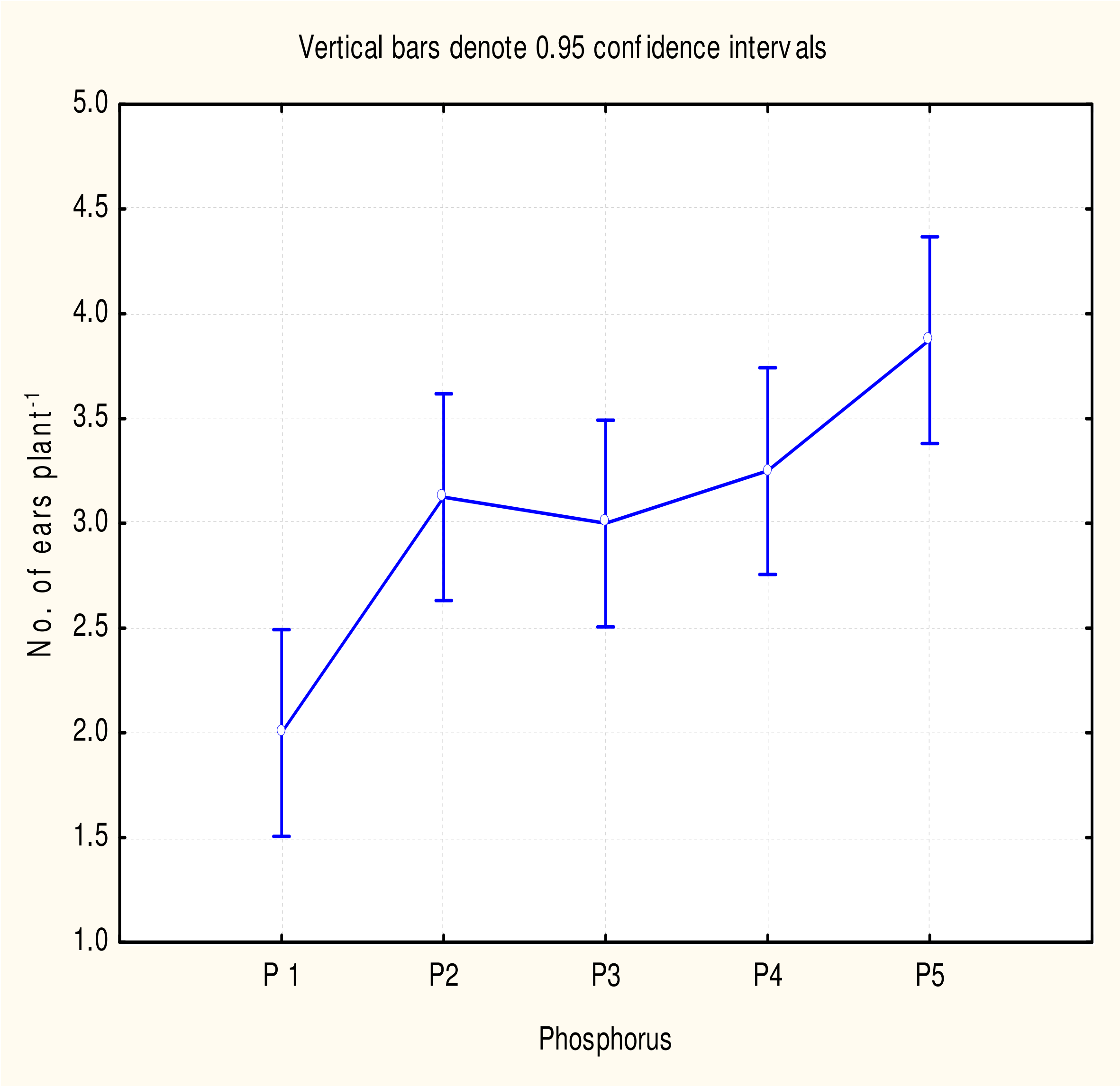

Figure 4.17 Effect of different P levels on number of ears per plant of wheat plants at maturity

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Increasing P levels initially decreased the wheat protein content between 0 kg P ha^{-1} (P_1) and 20 kg P ha^{-1} (P_3), but further increases in P levels between 30 kg P ha^{-1} (P_4) and 40 kg P ha^{-1} (P_5) resulted in an increase in wheat protein content (Figure 4.18). However, there was no significant difference between plants receiving no P (P_1) and 40 kg P ha^{-1} (P_5). Bogdevitch & Mikulich (2008) found that the maximum calculated protein content (14.5%) in wheat grain could be expected at mobile P content 253 mg kg^{-1} in soil and a further increase of soil P content will be accompanied by a reduction of grain protein content.

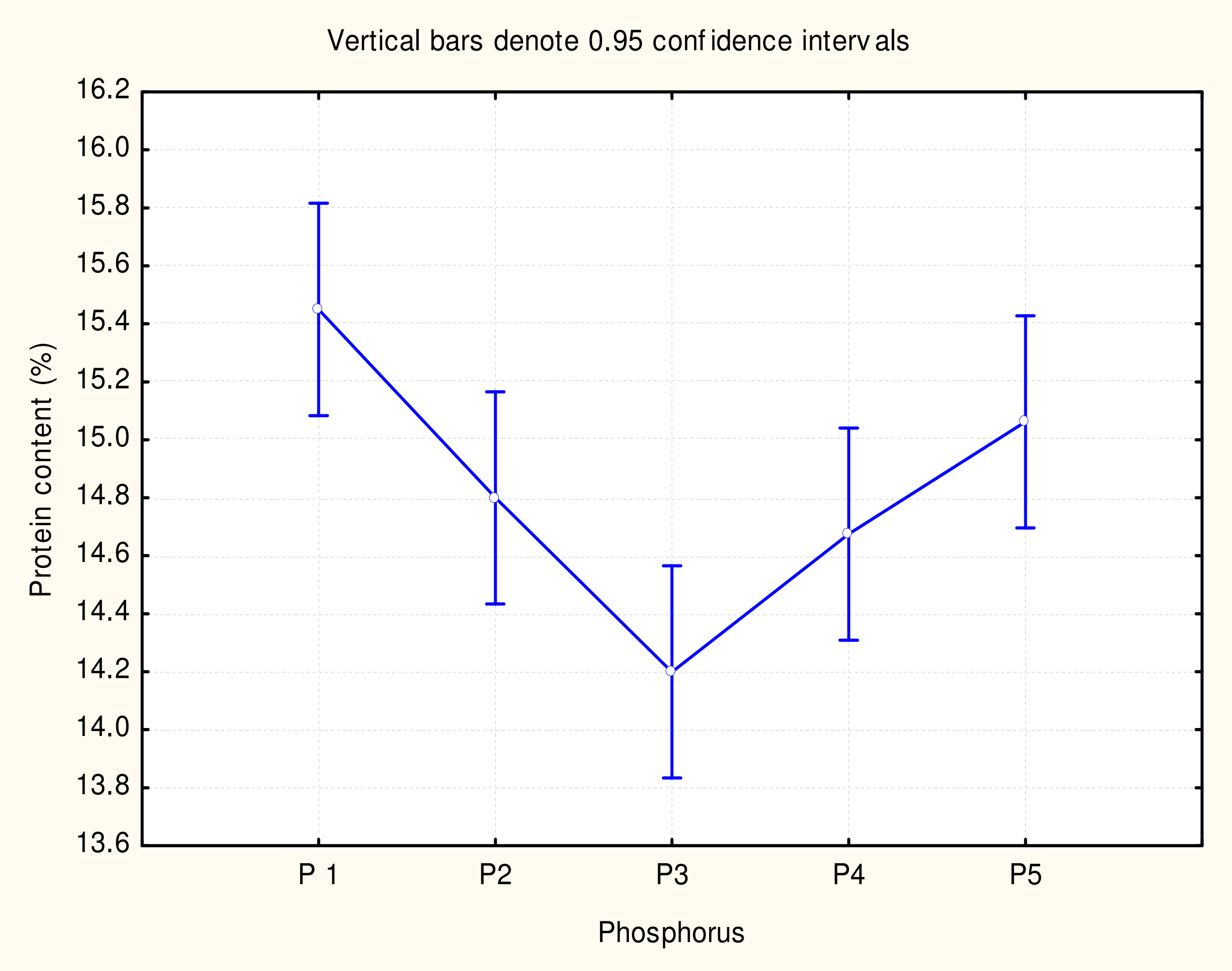

Figure 4.18 Effect of different P levels on wheat protein content (%) at maturity

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

The results showed that mycorrhizal seed inoculation resulted in a significant increase in grain yield (from 3.62 g plant^{-1} to 4.02 g plant^{-1}), thousand kernel weight (from 37.44 g to 38.93 g), plant fresh mass (13.47 g to 14.85 g) and plant dry mass (8.28 g to 9.44 g) (Table 4.6). A significant interaction between P and mycorrhizal treatments with regard to thousand kernel weight, plant fresh mass and plant dry mass however indicated that the response to seed inoculation with AM fungi was affected by P application levels. Babana & Antoun (2006) stated that on average, inoculation with AM fungus Gigaspora spp. caused significant increases in grain (0.49 t ha^{-1}) yield.

Table 4.6 Effect of mycorrhizal inoculation on growth and yield of wheat plants at maturity (120 days after planting) expressed as mean values per plant

Treatments	Measured parameters							
	Grain yield (g)	No. of grains	No. of grains ear^{-1}	No. of ears	1000 kernel weight (g)	Fresh mass (g)	Dry mass (g)	Protein content (%)
M_0	3.62b	96.35a	32.35a	2.95a	37.44b	13.47b	8.27b	14.99a
M_1	4.02a	104.75a	33.45a	3.15a	38.93a	14.85a	9.44a	14.69a

*Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1= seed inoculated with AM fungi

Effect of P application levels and mycorrhizal seed inoculation

The number of grains per plant, thousand kernel weight, plant fresh mass and plant dry mass per plant showed a significant interaction between P fertilization and mycorrhizal seed inoculation (Figures 4.19-4.22). In general all measured parameters with the exception of thousand kernel weight showed an increase with an increase in P application levels and tend to reach their maximum at P_4 and P_5 treatments, but responses to P treatment tend to be affected by mycorrhizal inoculation at low levels of P (0 kg P ha^{-1} and 10 kg P ha^{-1}).

Increase in P levels significantly increased the number of grains per plant in both non-mycorrhizal and mycorrhizal inoculated plants (Figure 4.19). However, at P_2 (10 kg P ha^{-1}) the mycorrhizal inoculated plants produced 107 grains $plant^{-1}$ which was significantly higher than the 75 grains $plant^{-1}$ produced by the non-mycorrhizal plants. Number of grains per plant also tend to be higher at a P application level of 30 kg ha^{-1}(P_4), but showed no differences when no P was added to the soil (P_1) or at high levels (P_5). McKenzie & Middleton (1997) stated that a significant proportion of inorganic P may be "biologically fixed" by microorganisms when soil P levels are low and in some cases, microorganisms may even compete with plants for P when soil P levels are low, while at high P levels plants may be able to take up sufficient P without the help of mycorrhiza.

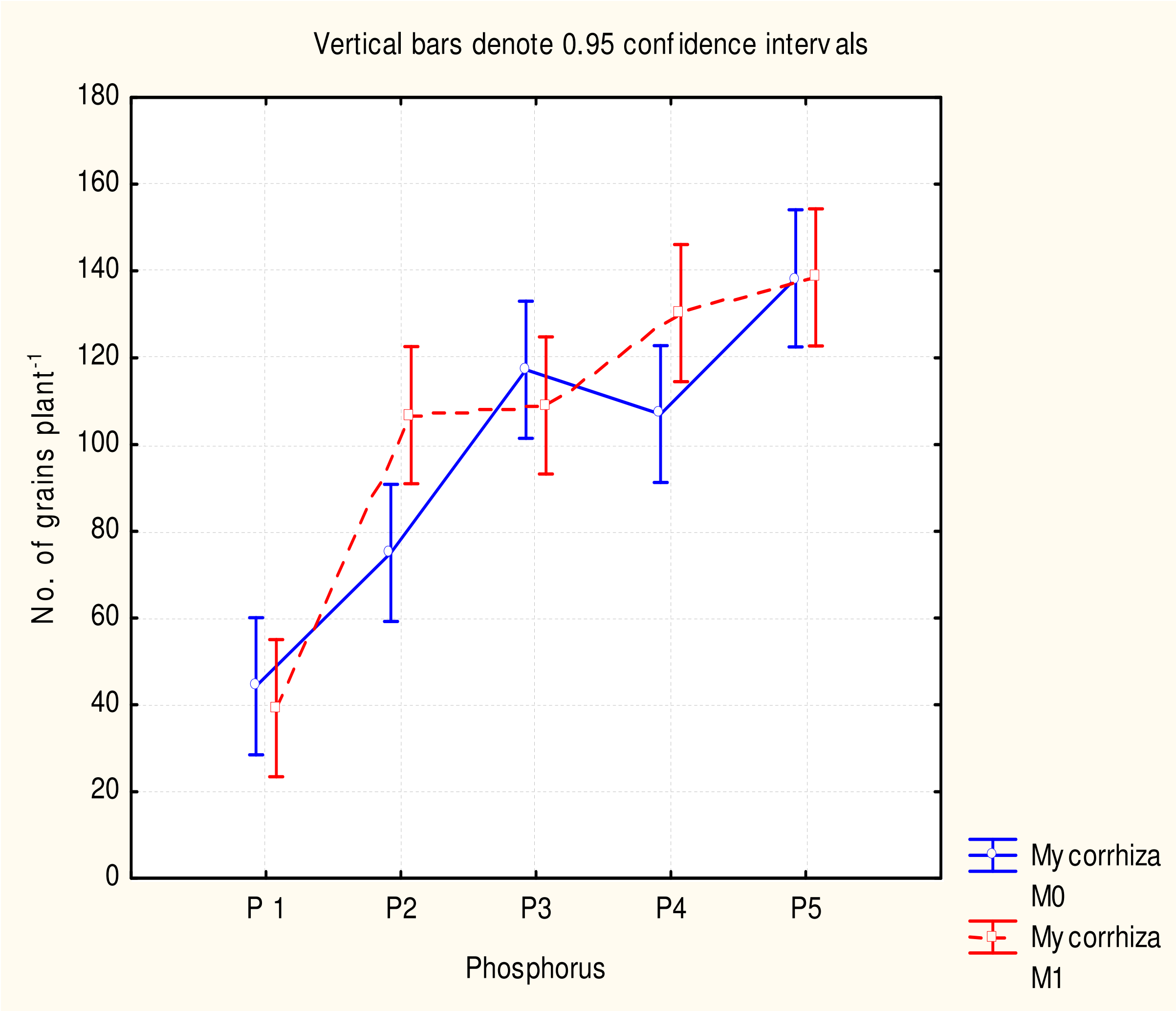

Figure 4.19 Effect of different P levels on the response of number of grains per plant of wheat at maturity to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

Increase in P levels increased thousand kernel weights in non-mychorrhizal plants, but not in the mycorrhizal inoculated plants (Figure 4.20). This resulted in a significantly higher (40.83 g plant^{-1}) thousand kernel weight in mycorrhizal inoculated plants compared to non-inoculated plants (36.25 g plant^{-1}) when no P was added (P_1) to the P deficient experimental soil. Khan (1972) also reported a positive response of wheat plants to mycorrhizal inoculation in a P deficient soil and the results again suggested larger effects when no or very little P was added.

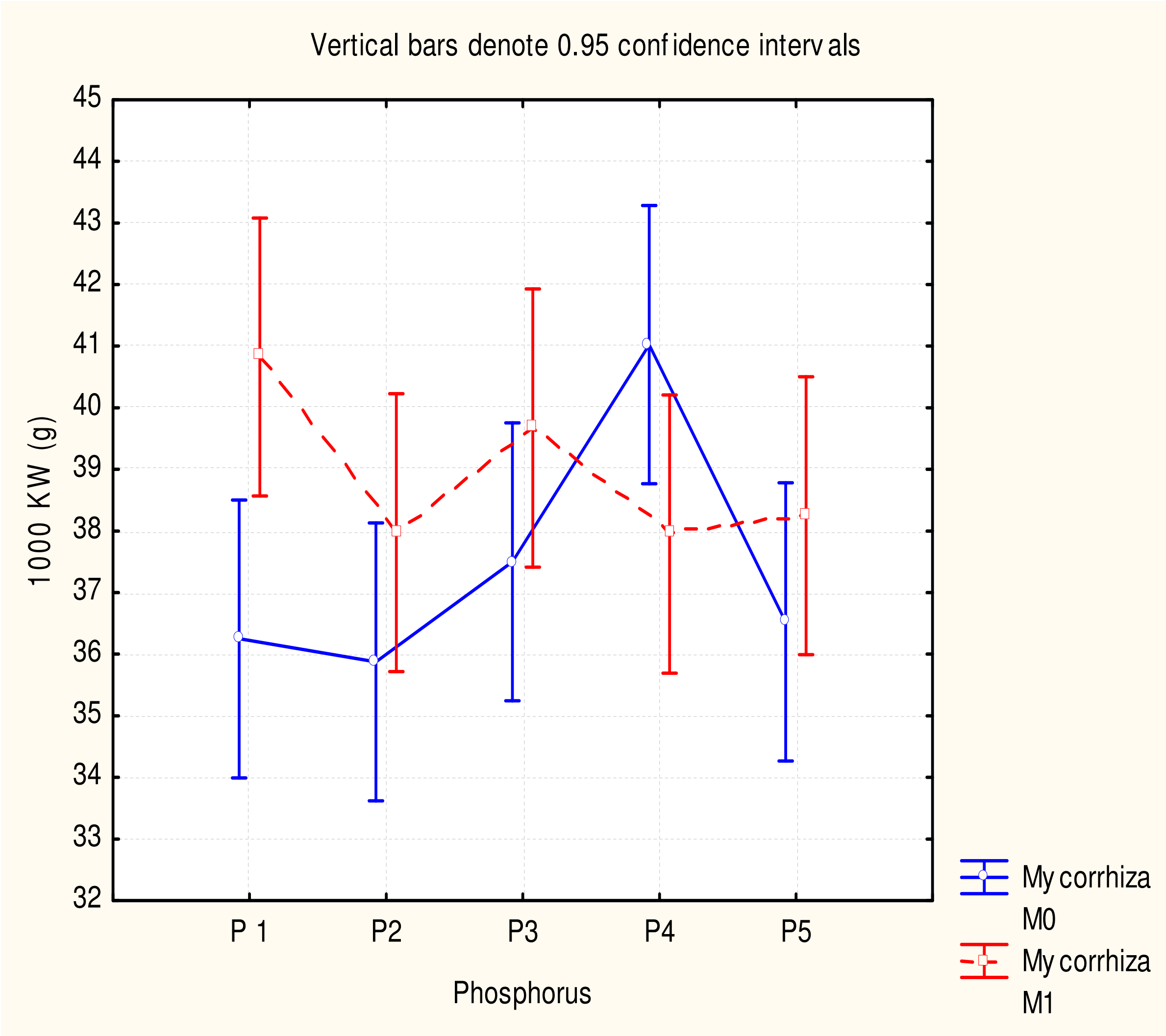

Figure 4.20 Effect of different P levels on the response of 1000 kernel weight (g) of wheat plants at maturity to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

Increase in P levels increased both fresh mass (Figure 4.21) and dry mass (Figure 4.22) per plant in non-inoculated and mycorrhizal inoculated plants. As also found with number of grains plant^{-1}, significant differences were observed at P_2 (10 kg P ha^{-1}) with mycorrhizal inoculated plants producing significantly higher fresh and dry mass plant^{-1} compared to non-mycorrhizal plants. Higher fresh and dry mass with mycorrhizal plants were also found at P_4, but seed inoculation with AM fungi did not have any beneficial effect at P_1, P_3 and P_5. Mycorrhizal inoculation therefore tended to increase fresh and dry mass production of wheat, but because wheat is not highly dependent on mycorrhiza (Seymour, 2009) the increase in dry mass production was not consistent. Grant *et al.* (2005) reported that the non-significant response of mycorrhizal plants to an increase in P levels could be due to the fact that mycorrhizal associations tend to decrease with increasing background soil P. Khan (1972) also reported that the difference in dry weight between mycorrhizal and non-mycorrhizal plants is not maintained after the addition of phosphorus, indicating that the fungus does not enhance wheat growth in soils containing enough available phosphorus.

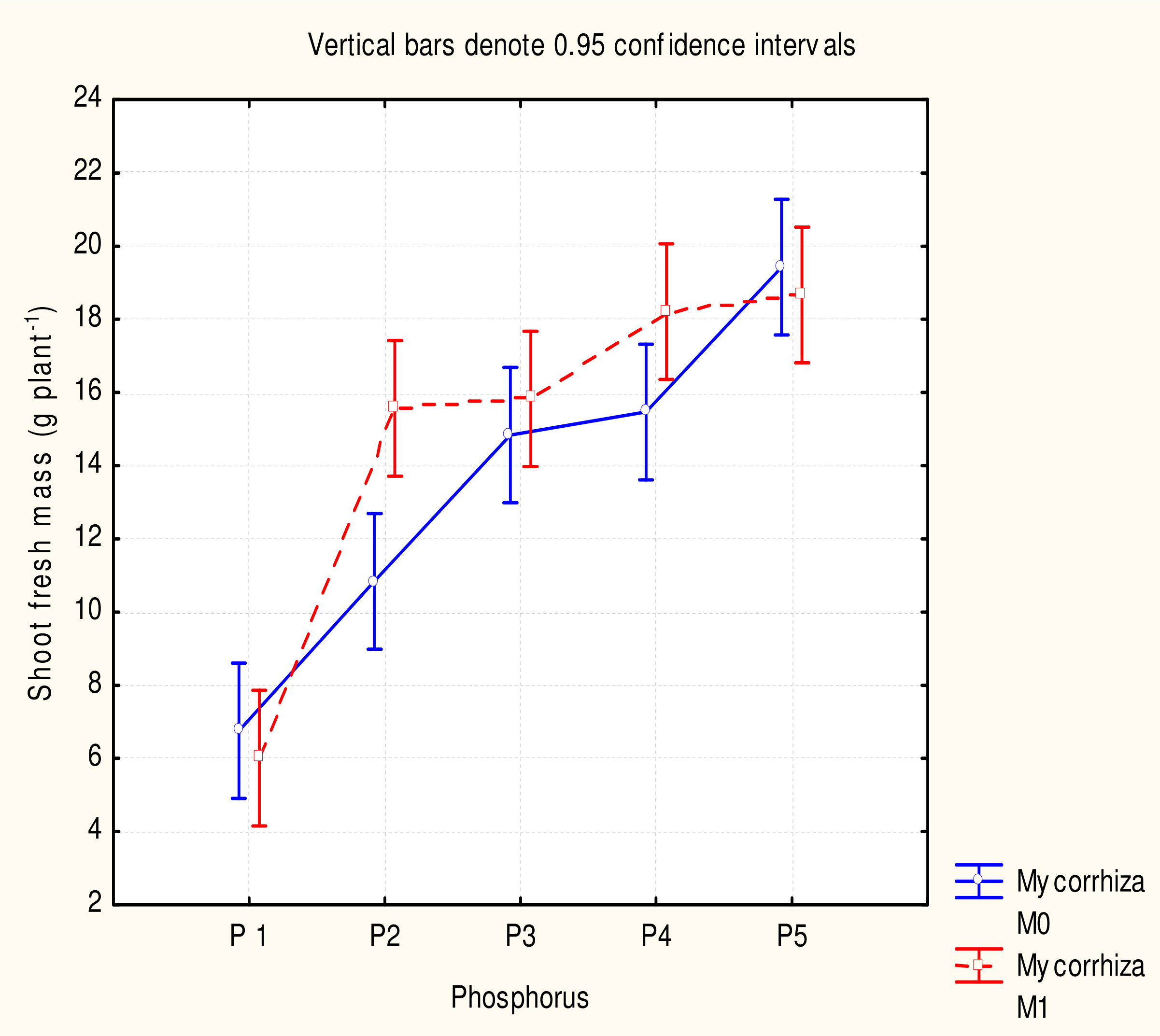

Figure 4.21 Effect of different P levels on the response of fresh mass (g plant^{-1}) of wheat at maturity to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

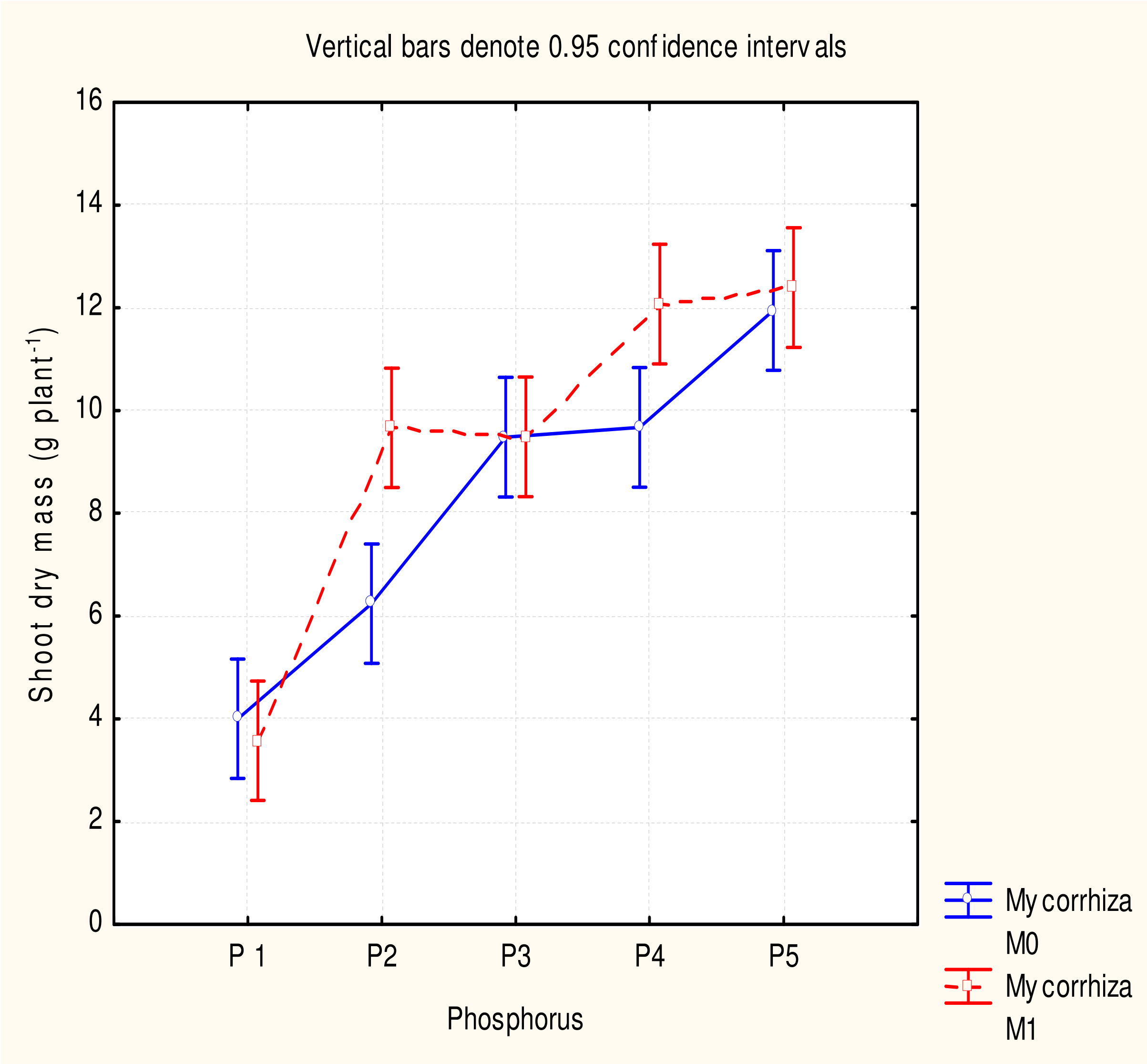

Figure 4.22 Effect of different P levels on the response of dry mass per plant (g) of wheat at maturity to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1= seed inoculated with AM fungi]

In this experiment wheat growth and yield were significantly enhanced by the application of different P levels and mycorrhizal seed inoculation in P deficient sandy soil. The results suggested that the optimum P application level for wheat in the sandy soil with P content of 18 mg kg^{-1} (citric acid) was between 30 and 40 kg P ha^{-1}, but some parameters tested showed no significant increase when P application levels were higher than 20 kg P ha^{-1}. The responses to mycorrhizal seed inoculation were also not consistent mainly due to the fact that wheat has a low mycorrhizal dependency (Seymour, 2009).

Mineral concentration of wheat straw

The effect of P application levels, seed inoculation with AM fungi and their interaction on nutrient content of wheat plants was determined at 120 days after planting. The results from analysis of variance are summarized in Table 4.7.

Phosphorus application levels had a significant effect on P, K, Mg, Fe, Cu, Zn, Mn, S and OC content. No significant effect due to mycorrhizal seed inoculation was found on any of the measured elements except for Mn. A significant interaction between P application level and mycorrhizal seed inoculation with regard to Zn and Mn content was found at 120 days after planting.

Only significant (P≤0.05) results will be discussed as follows.

Table 4.7 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on nutrient content of wheat plants at 120 days after planting

Treatments	NH4 N (%)	P (%)	K (%)	Mg (%)	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	S (%)	OC (%)
P	NS	*	*	*	NS	*	*	*	*	NS	NS	*	*
M	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
M*P	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS

- NS: not significant at P≤0.05
- *: significant at P≤0.05

Effect of phosphorus

Increasing P application levels had a significant increasing effect on P, K, Mg and S, but a decreasing effect on Fe, Cu, Zn, Mn and OC content of wheat plant at 120 DAP (Table 4.8). In the case of P, K, Mg and S, increasing P fertilizer levels from 0 to 30 kg P ha^{-1} resulted in significant increases, namely: P content from 0.09 to 0.12%; K from 2.27 to 3.16%; Mg from

0.12 to 0.15%, and S from 0.10% to 0.12% respectively. Increases of P application levels from 30(P_4) to 40(P_5) kg P ha^{-1}, had no or even a decreasing effect on the content of these nutrients. In contrast to this, increasing the P fertilizer levels from 0 to 40 kg P ha^{-1} resulted in a significant decrease in Fe content from 140.89 to 92.84 mg kg^{-1}; Cu from 5.29 to 4.03 mg kg^{-1}; Zn from 37.09 to 26.11 mg kg^{-1}, Mn from 60.16 to 19.34 mg kg^{-1}, and OC from 54.9 to 54.4%$^{-1}$ respectively.

The results of this study indicate that P application may interfere with the availability and uptake by wheat of these nutrient elements. The results are supported by Mulders' Chart of plant nutrient interactions which shows that higher P application reduces availability of Fe, Cu and Zn while high P levels can influence the uptake of Mg (Anonymous, 2009). Abid *et al.* (2002) reported an increase in P content of wheat straw with an increase in P application in different textured saline-sodic soils. It has also been reported that Zn content tend to decrease with P application levels and this agrees with the known phosphate induced Zn deficiency (Mengel & Kirkby, 1982). It has also been suggested that high P affect physiological Zn availability in plant tissues (DAR, 1995).

Table 4.8 Effect of different P levels on nutrient content of wheat plants at 120 days after planting

Treatments	P (%)	K (%)	Mg (%)	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	S (%)	OC (%)
P_1	0.09b	2.27b	0.12c	140.89a	5.29a	37.09a	60.16a	0.10b	54.9a
P_2	0.09b	2.49ab	0.12bc	115.36b	4.46b	30.53bc	31.20b	0.10b	54.8a
P_3	0.11ab	2.71ab	0.13bc	100.90bc	4.43b	32.91ab	26.04b	0.11ab	54.6ab
P_4	0.12a	3.16a	0.15a	96.09bc	4.17b	30.73bc	19.64b	0.12a	54.2b
P_5	0.11ab	2.91ab	0.14ab	92.84c	4.03b	26.11c	19.34b	0.12a	54.4ab

* Means with different letters within the same column are significantly different ($P \leq 0.05$).

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

Mycorrhizal seed inoculation resulted in a significant decrease in the content of Mn from 36.94 to 25.62 mg kg^{-1} (Table 4.9) in wheat plants at 120 DAP, which is in contrast to the results of Al-Karaki *et al*. (1998) who reported increased levels of several nutrients in plants as a result of mycorrhizal inoculation.

Table 4.9 Effect of mycorrhizal inoculation on nutrient content of wheat plants at 120 days after planting expressed as mean values per plant

	Measured parameters
Treatments	Mn (mg kg^{-1})
M_0	36.94a
M_1	25.62b

*Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application levels

Increase in P levels decreased Zn content in non-mycorrhizal and mycorrhizal inoculated plants except at 20 kg P ha^{-1} where an increase of Zn content with P application levels was observed on mycorrhizal plants (Figure 4.23). As a result, a significant difference was observed with mycorrhizal plants producing significantly higher Zn content (37.23 mg kg^{-1}) compared to non-mycorrhizal plants (28.58 mg kg^{-1}).

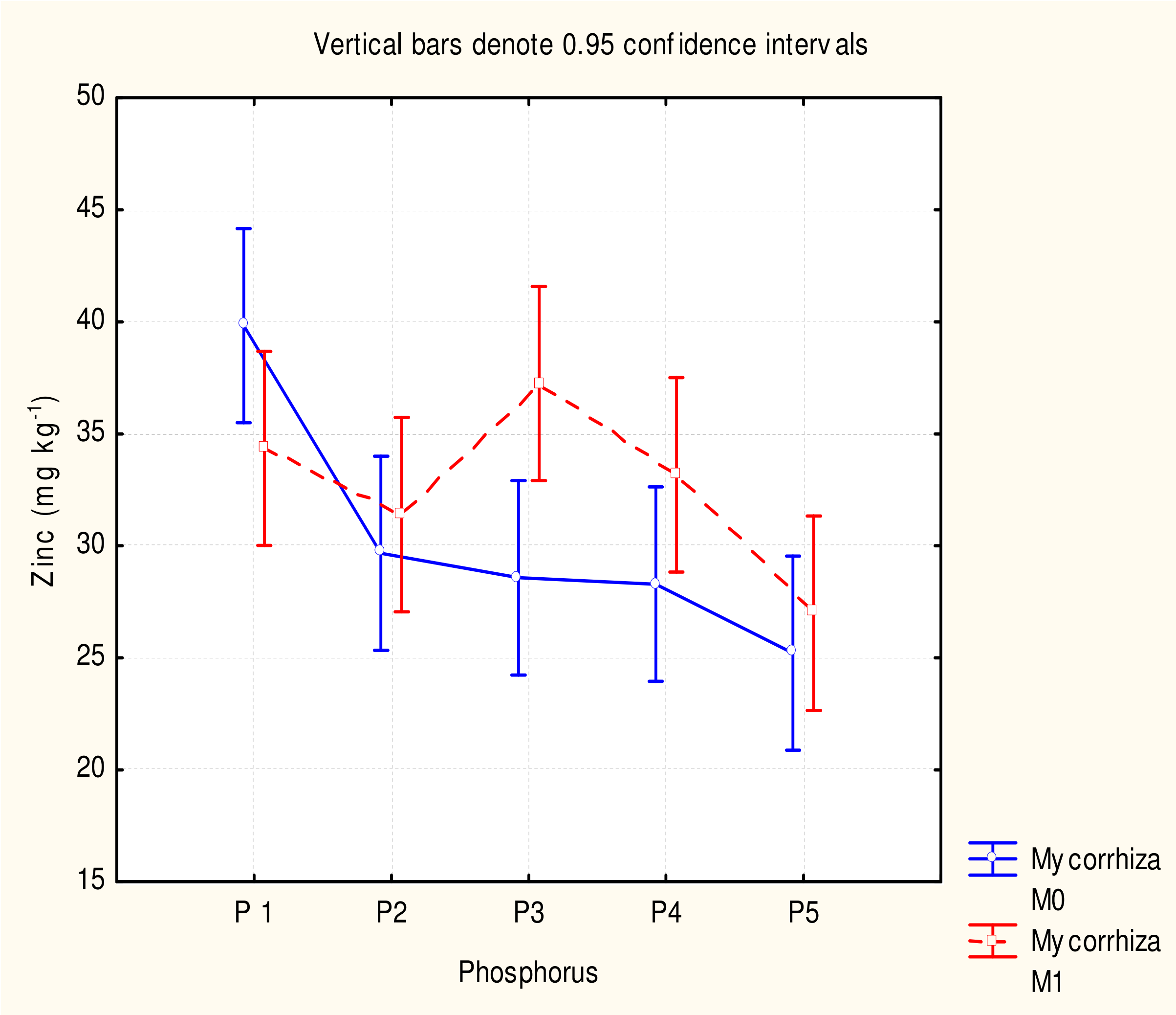

Figure 4.23 Effect of different P levels on the response of zinc content of wheat plants at 120 days after planting to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

The application of P fertilizer decreased Mn content of wheat in both non-mycorrhizal and the mycorrhizal inoculated plants (Figure 4.24). However, non-mycorrhizal plants displayed significantly higher (79.11 mg kg^{-1}) Mn contents compared to mycorrhizal plants (41.22 mg kg^{-1}) where no P was applied.

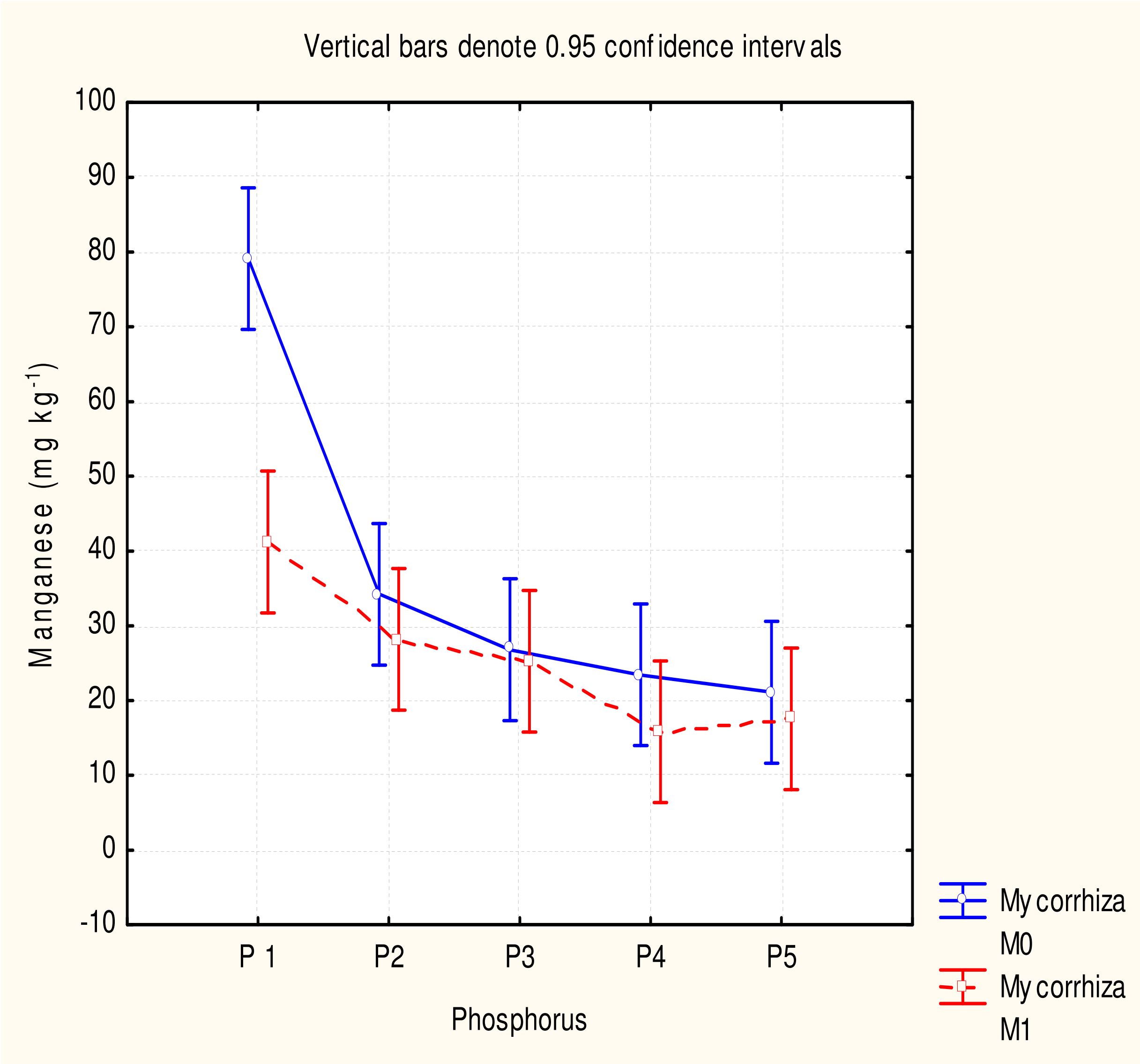

Figure 4.24 Effect of different P levels on the response of manganese content of wheat plants at 120 days after planting to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Nutrient uptake by wheat plants

The effect of P application levels, seed inoculation with AM fungi and their interaction on nutrient uptake of wheat was determined at 120 days after planting. Results from analysis of variance are summarized in Table 4.10.

Phosphorus application levels had a significant effect on all the nutrient elements except for Mg. A significant effect due to mycorrhizal seed inoculation was found on NH_4-N, P, Mg, Fe, Cu, Zn, S and OC content. A significant interaction between P application levels and mycorrhizal seed inoculation with regard to NH_4-N, K, Mg, Zn, Mn, Al, S and OC uptake was found at 120 days after planting.

Only significant ($P \le 0.05$) results will be discussed as follows.

Table 4.10 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on nutrient uptake of wheat plants at 120 days after planting

Treatments	NH4 N (g)	P (g)	K (g)	Mg (g)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Mn (mg)	B (mg)	Al (mg)	S (g)	OC (g)
P	*	*	*	*	*	*	*	*	NS	*	*	*	*
M	*	*	NS	*	NS	*	*	*	NS	NS	NS	*	*
M*P	*	NS	*	*	NS	NS	NS	*	*	NS	*	*	*

- NS: not significant at $P \le 0.05$
- *: significant at $P \le 0.05$

Effect of phosphate

Increasing P application levels had a significant effect on of NH_4-N, P, K, Mg, Na, Fe, Cu, Zn, B, Al, S and OC uptake of wheat plants at 120 DAP (Table 4.11). All the measured elements showed an increase with an increase in P application levels, but tended to reach their maximum or at least level off at P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}). The application of P fertilizer increased P from 0.004 to 0.014 g $plant^{-1}$, Na from 0.31 to 1.02 mg $plant^{-1}$, Fe from 0.53 to 1.14 mg $plant^{-1}$, Cu from 0.020 to 0.049 mg $plant^{-1}$ and B from 0.024 to 0.066 mg $plant^{-1}$, where no P was applied (P_1) compared to a P level of 40 kg P ha^{-1} (P_5) respectively.

The results of this study indicate that P application increased the uptake of these nutrient elements by wheat plants. Abid *et al.* (2002) reported a significant effect on N and P content of wheat straw with P levels over control in saline-sodic silty clay loam and silt loam soils. Similar results were observed by Rabie *et al.* (1985) and Niazi *et al.* (1990) and reported that P uptake by wheat plants was more at higher P levels than at lower P levels.

Table 4.11 Effect of different P levels on nutrient uptake per plant of wheat plants at 120 days after planting

Treatments	NH4 N (g)	P (g)	K (g)	Mg (g)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	B (mg)	Al (mg)	S (g)	OC (g)
P_1	0.07d	0.004c	0.09d	0.0005d	0.31b	0.53b	0.020c	0.14c	0.024b	0.07b	0.004d	2.08d
P_2	0.14c	0.008b	0.20c	0.0009c	0.65ab	0.93a	0.036b	0.25b	0.054a	0.15ab	0.008c	4.36c
P_3	0.17bc	0.010b	0.26bc	0.0012b	0.86a	0.97a	0.042ab	0.31ab	0.060a	0.21a	0.010b	5.18bc
P_4	0.20ab	0.013a	0.34ab	0.0016a	1.05a	1.05a	0.046a	0.34a	0.072a	0.23a	0.013a	5.90ab
P_5	0.21a	0.014a	0.35a	0.0017a	1.02a	1.14a	0.049a	0.32ab	0.066a	0.16ab	0.014a	6.62a

* Means with different letters within the same column are significantly different ($P \leq 0.05$).

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

In this pot trial with P deficient sandy soil, mycorrhizal seed inoculation resulted in a significant increase in the uptake of P (from 0.009 to 0.010 g plant$^{-1)}$, Fe (from 0.81 to 1.03 mg plant^{-1}) and Cu (from 0.035 to 0.042 mg plant^{-1}) by wheat plants (Table 4.12). Al-Karaki *et al.* (1998) reported higher shoot contents of P, Zn, Cu and Fe in mycorrhizal plants than non-mycorrhizal plants in a greenhouse experiment.

Table 4.12 Effect of mycorrhizal inoculation on nutrient uptake per plant of wheat plants at 120 days after planting

Treatments	NH4 N (g)	P (g)	Mg (g)	Fe (mg)	Cu (mg)	Zn (mg)	S (g)	OC (g)
M_0	0.14b	0.009b	0.0011b	0.81b	0.035b	0.24b	0.009b	4.51b
M_1	0.17a	0.010a	0.0013a	1.03a	0.042a	0.30a	0.010a	5.15a

*Means with different letters within the same column are significantly different ($P \leq 0.05$).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application levels

The NH_4-N, K, Mg, Zn, Mn, Al, S and OC uptake showed a significant interaction between P fertilization and mycorrhizal seed inoculation (Figures 4.25-4.32). In general all measured elements except for Mn, showed an increase with an increase in P application level and tend to reach their maximum at P_4 and P_5 treatments, but responses to P treatment tend to be affected by mycorrhizal inoculation at low and high levels of P (10 kg P ha^{-1} and 30 kg P ha^{-1}) respectively.

Increase in P levels increased the uptake of NH_4-N, K, Mg, Zn, Al, S and OC content in both non-inoculated and mycorrhizal inoculated plants (Figure 4.25-4.31). However, significant differences were observed at P_2 (10 kg P ha^{-1}) with mycorrhizal inoculated plants producing significantly higher nutrient uptake compared to non-mycorrhizal plants. Higher nutrient uptake with mycorrhizal plants was also found at P_4, but seed inoculation with AM fungi did not have any beneficial effect at P_1, P_3 and P_5.

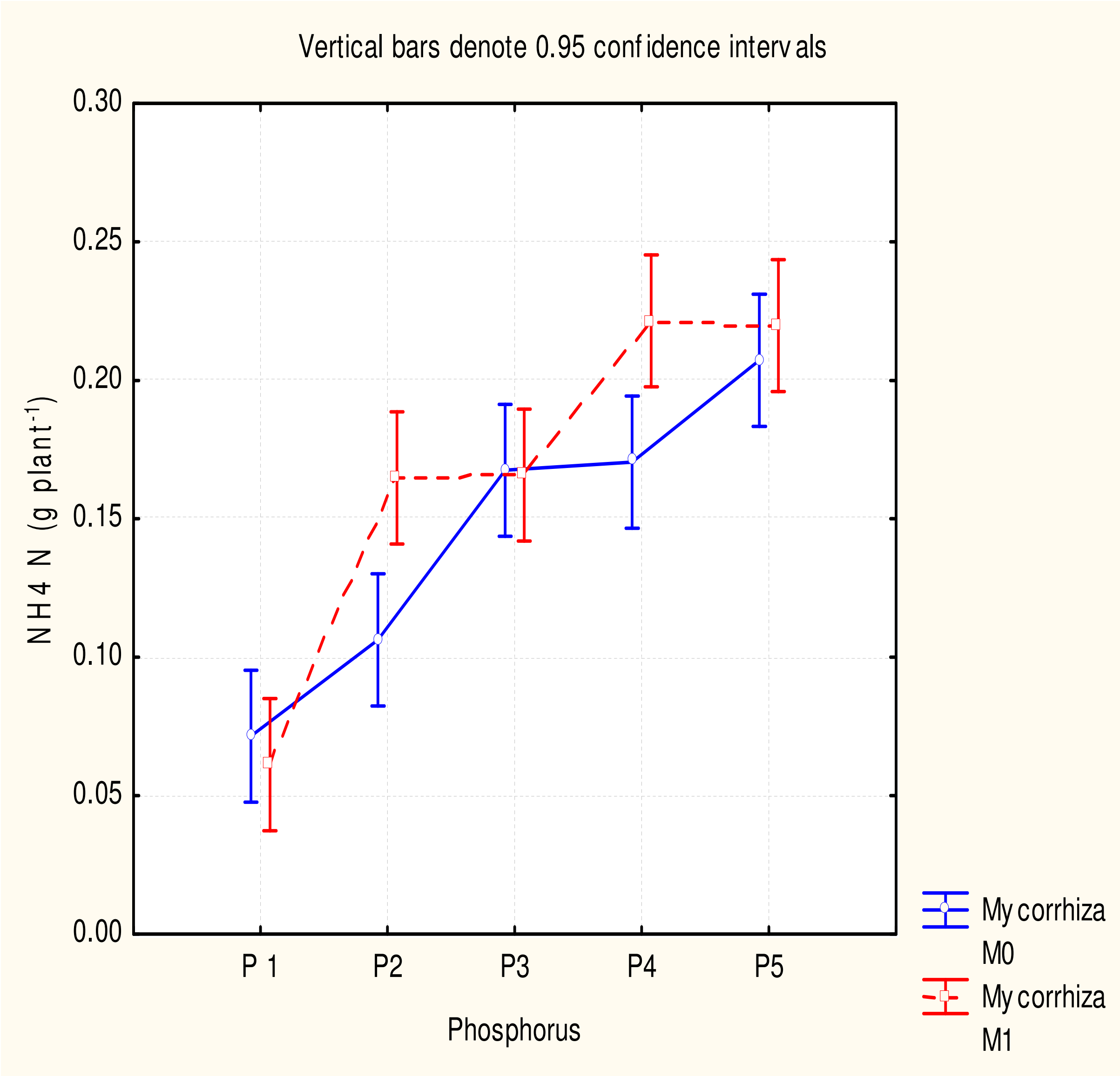

Figure 4.25 Effect of mycorrhizal seed inoculation on the nitrogen content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

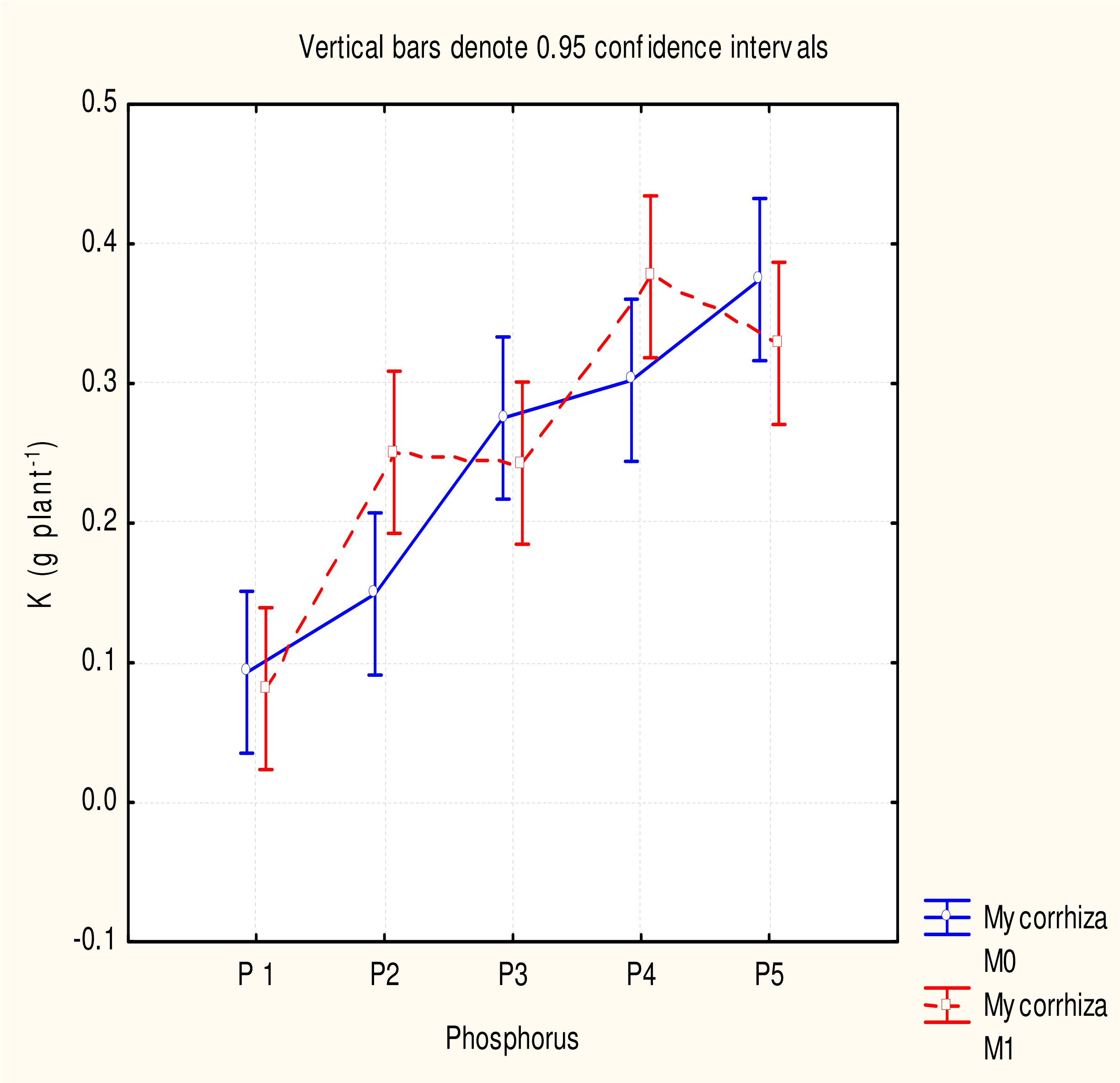

Figure 4.26 Effect of mycorrhizal seed inoculation on the potassium content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

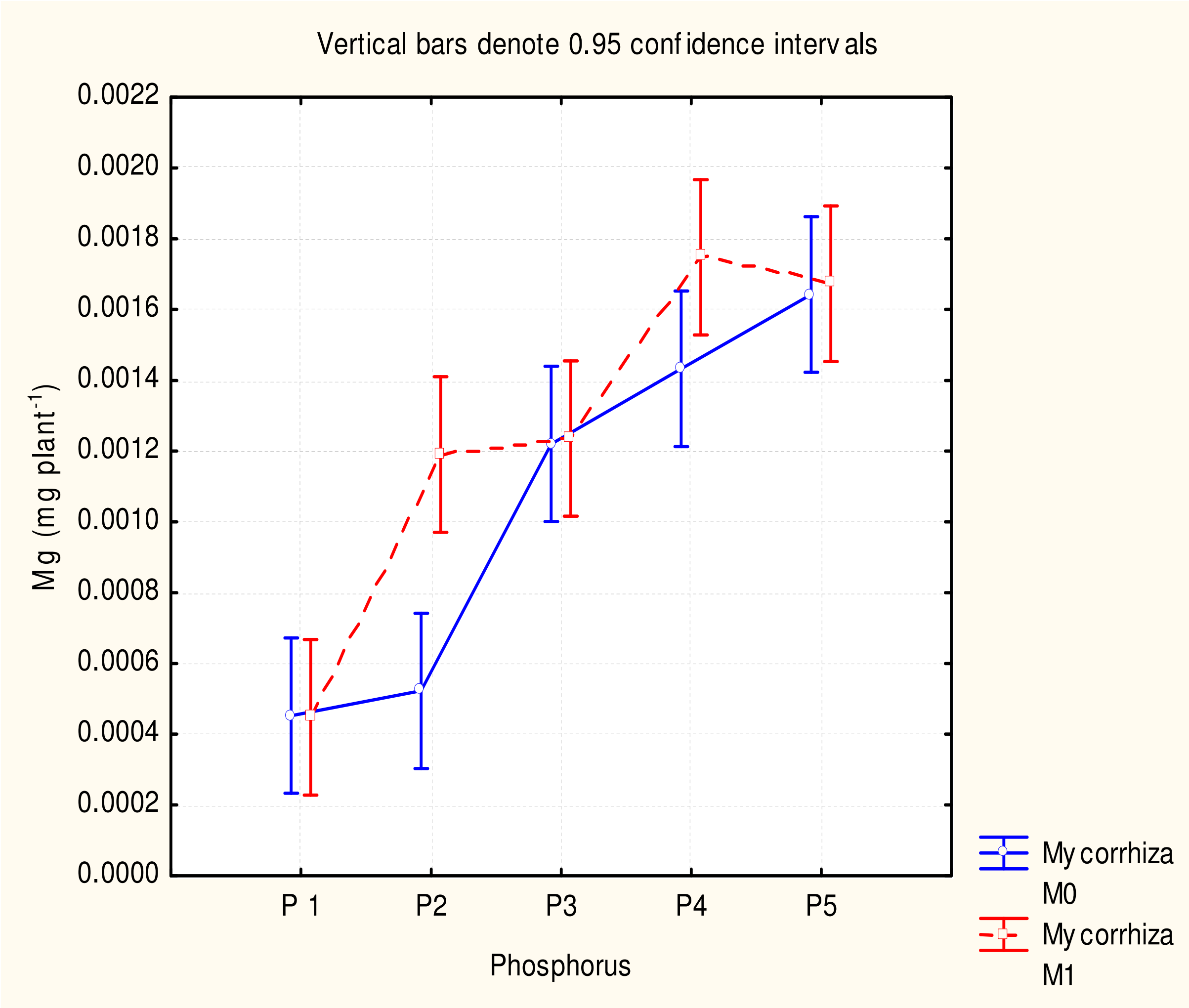

Figure 4.27 Effect of mycorrhizal seed inoculation on the magnesium content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

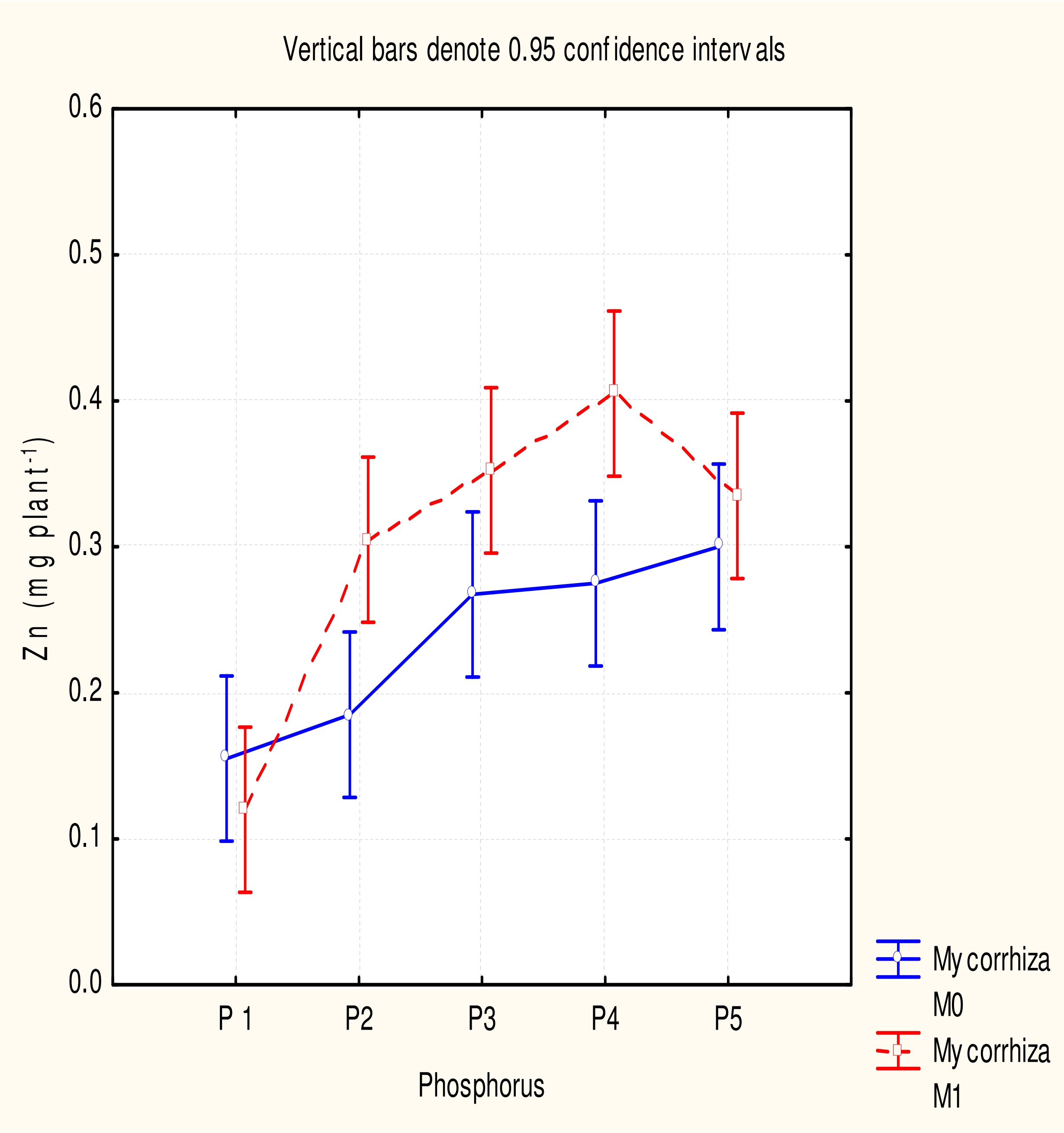

Figure 4.28 Effect of mycorrhizal seed inoculation on the zinc content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

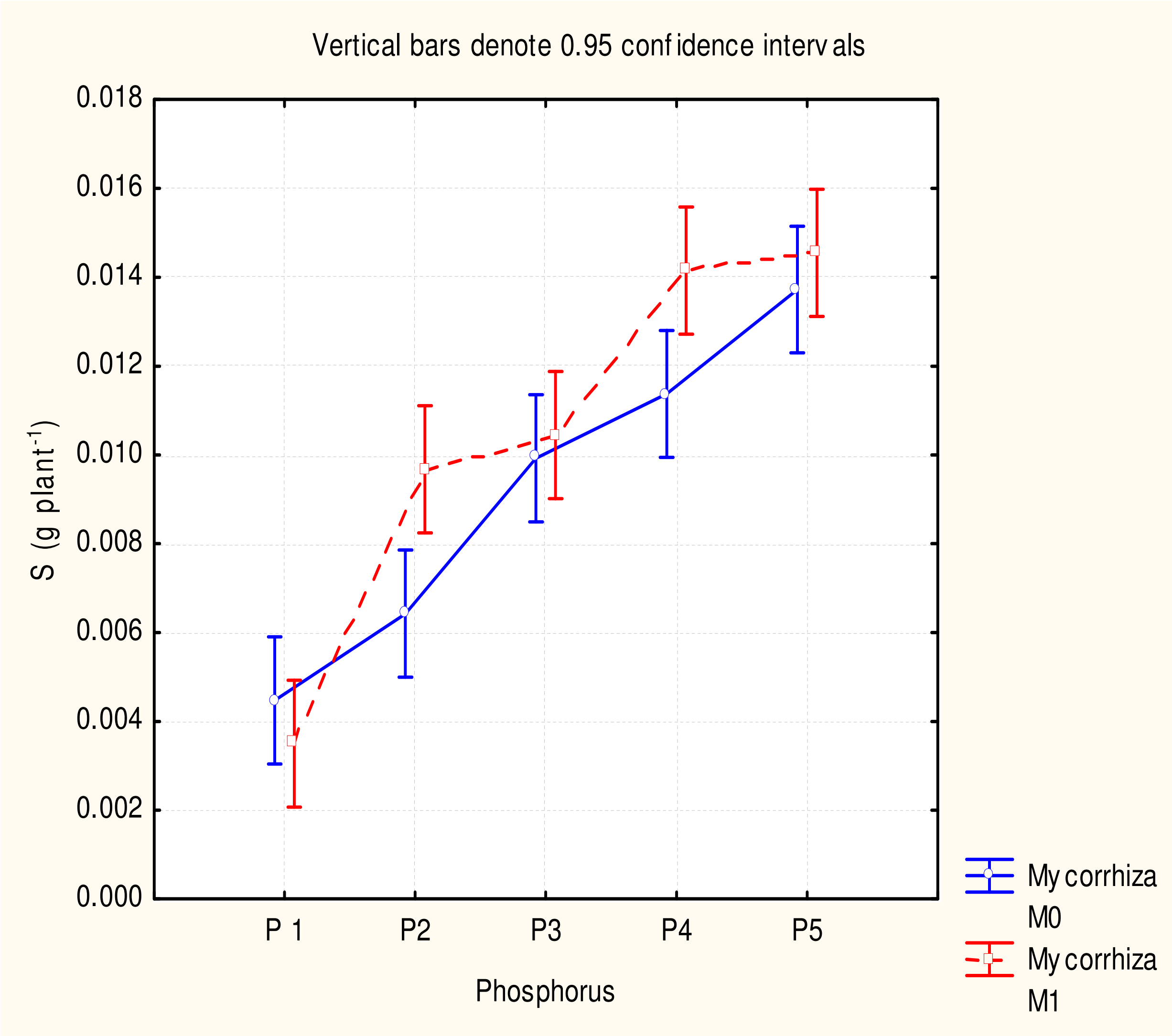

Figure 4.29 Effect of mycorrhizal seed inoculation on the sulphur content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

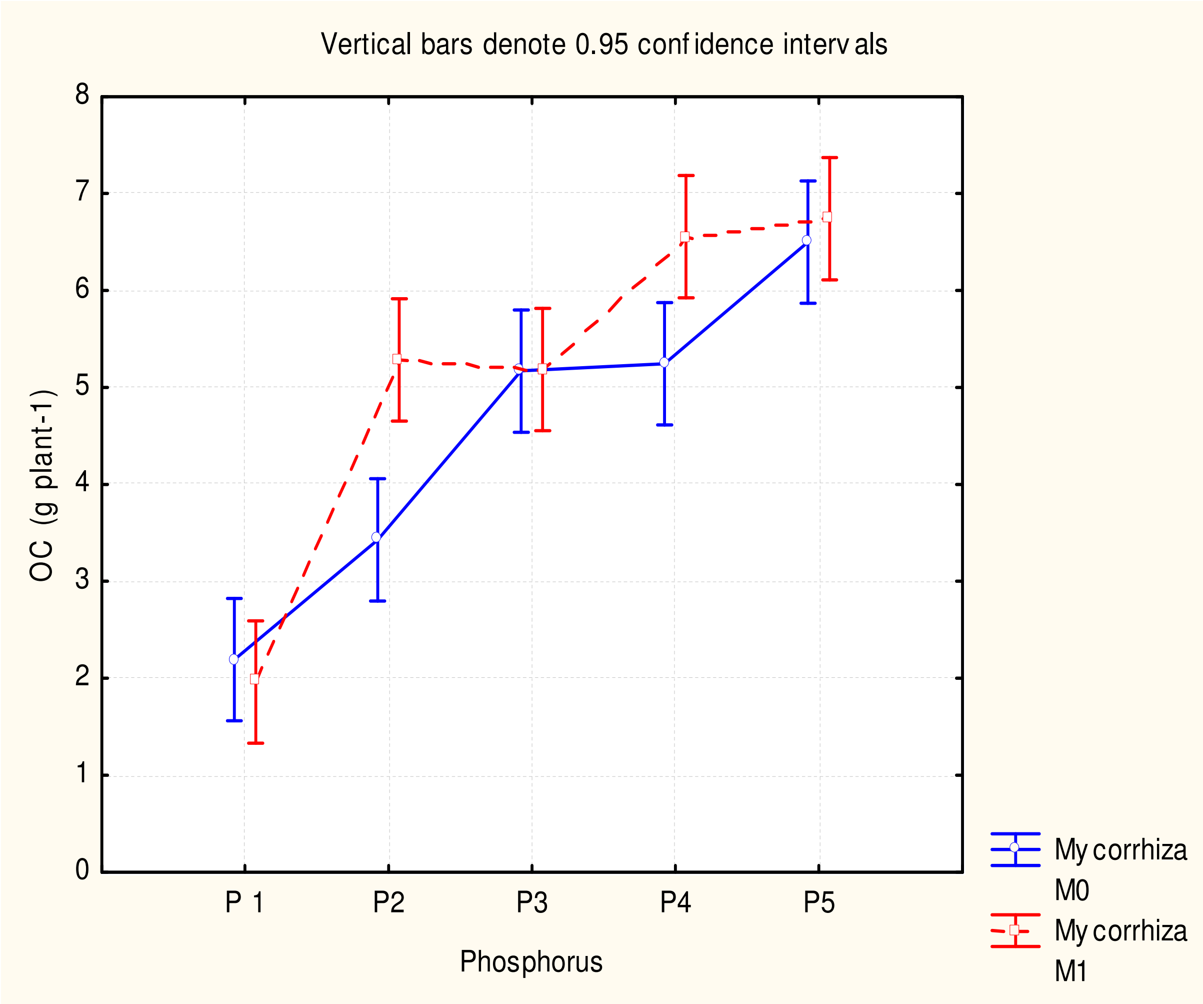

Figure 4.30 Effect of mycorrhizal seed inoculation on the organic carbon content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

The Al content of wheat plants showed a significant interaction between P fertilization levels and mycorrhizal seed inoculation (Figure 4.31). In general Al contents showed an increase with an increase in P application levels and tend to reach their maximum values at P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}) treatments, but responses to P treatment tend to be affected by mycorrhizal inoculation at a P level of 40 kg P ha^{-1}. At this P application level, non-mycorrhizal plants produced significantly higher Al content (0.326 mg plant^{-1}) compared to mycorrhizal inoculated plants (0.0003 mg plant^{-1}).

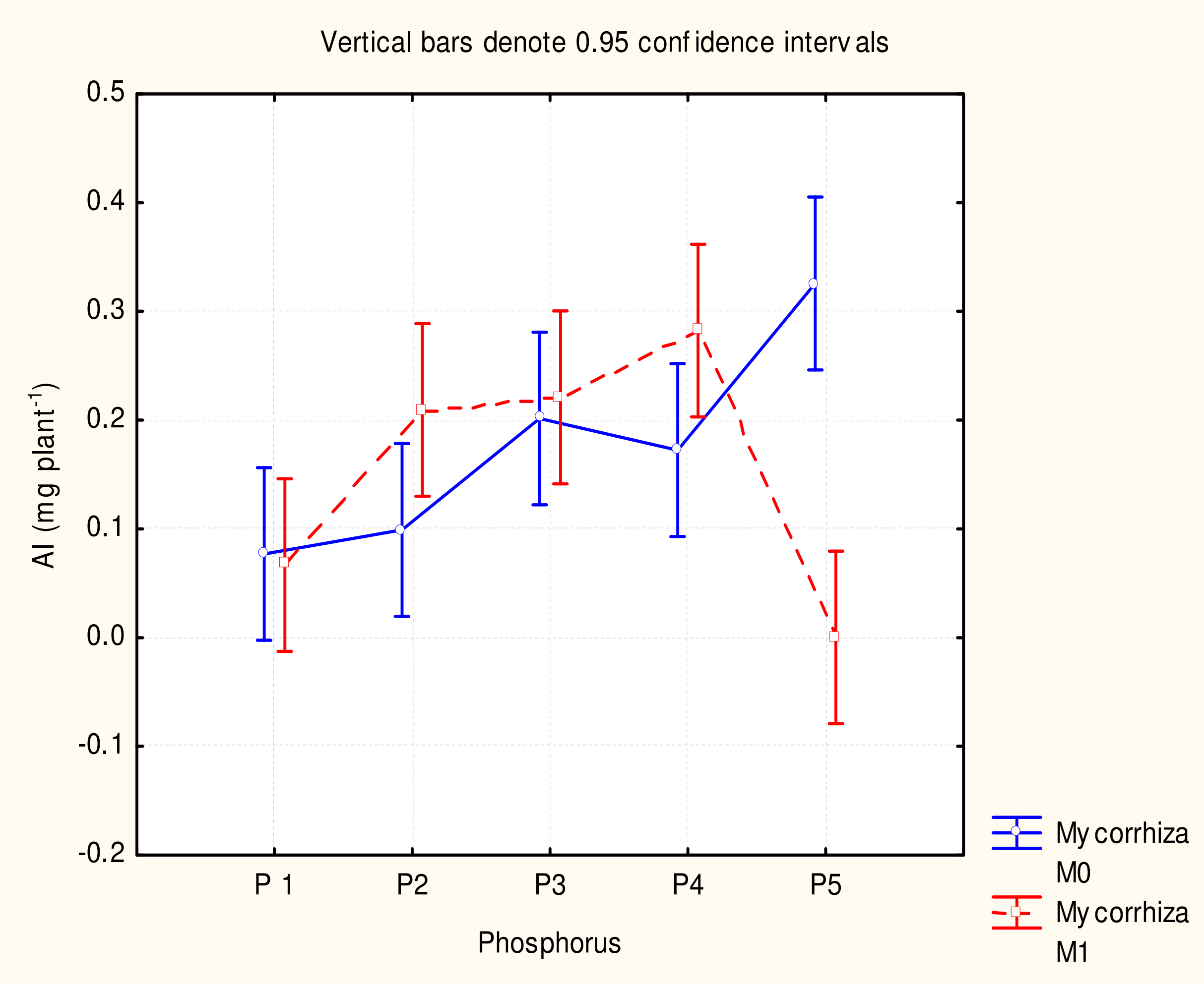

Figure 4.31 Effect of mycorrhizal seed inoculation on aluminium content of wheat plants in response to different P application rates at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Increase in P levels decreased Mn content in non-mycorrhizal plants, but not in the mycorrhizal inoculated plants (Figure 4.32). However, non-mycorrhizal plants produced significantly higher (0.305 mg $plant^{-1}$) Mn content compared to mycorrhizal plants (0.150 mg $plant^{-1}$) when no P was added (P_1) to the P deficient experimental soil.

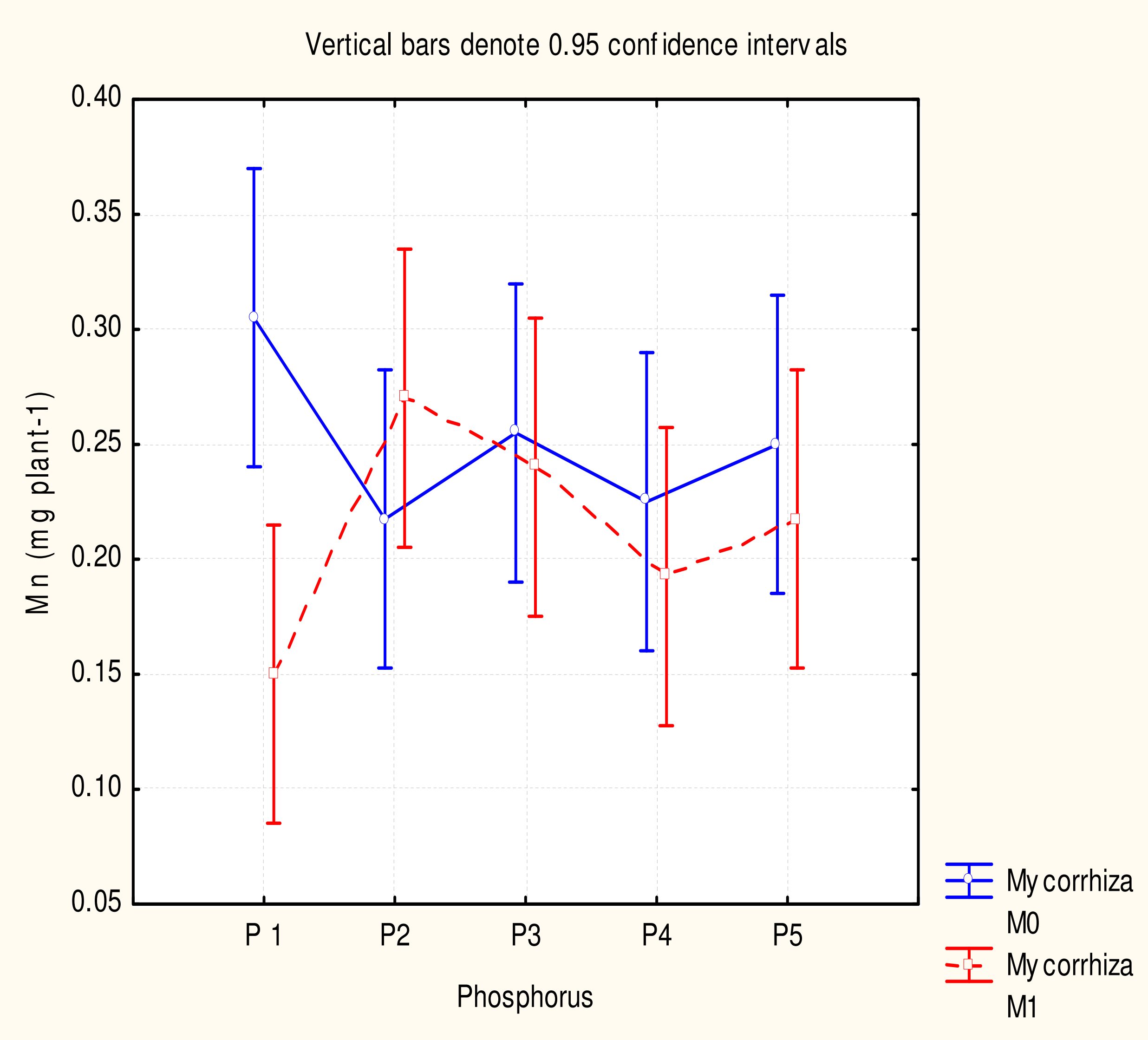

Figure 4.32 Effect of mycorrhizal seed inoculation on the manganese content of wheat plants to P application at 120 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

In this experiment wheat nutrient content was significantly enhanced by the application of different P levels and mycorrhizal seed inoculation in a P deficient sandy soil. The results suggested that the optimum P application level for wheat in the sandy soil with P content of 18 mg kg^{-1} (citric acid) was between 30 and 40 kg P ha^{-1}, but some elements measured showed no significant increase when P application levels were higher than 20 kg P ha^{-1}. Higher nutrient uptake due to mycorrhizal inoculation was also observed in this experiment.

A significant interaction between P application level and mycorrhizal inoculation was observed at P level of 10 kg P ha^{-1} and 30 kg P ha^{-1}, but seed inoculation with AM fungi did not have any beneficial effect at P_1, P_3 and P_5.

The results of this experiment showed that the total amount of nutrients in wheat above ground parts of mycorrhizal plants may be greater than that of non-mycorrhizal plants due to increased above ground growth. Thus, increased amounts of nutrients in above ground parts of mycorrhizal plants can often be explained by higher nutrient demand due to enhanced plant P uptake.

Maize

The effect of P application levels, seed inoculation with AM fungi and their interaction on the growth of maize were measured at 30, 45 and 60 days after planting respectively. Results from the analysis of variance are summarized in Table 4.13.

At 30 days after planting, P application levels had a significant increasing effect on all the measured parameters except for percentage plant moisture (Table 4.13). At 45 days after planting, P application levels had a significant increasing effect on all parameters, while at 60 days after planting, P application levels had a significant increasing effect on all parameters except for percentage plant moisture. These results clearly illustrated the P deficiency in the soil used in this study.

The effect of seed inoculation with AM fungi varied between different maize growth stages (Table 4.13). At 30 days after planting, mycorrhizal seed inoculation had no significant effect on the measured parameters. At 45 days after planting, mycorrhizal seed inoculation had a significant increasing effect on stem diameter, root fresh mass and percentage root moisture, while at 60 days after planting all measured parameters except stem diameter and plant moisture were significantly affected by mycorrhizal seed treatment. These results indicated that the mycorrhizal effect became stronger as the plant became more mature.

A significant interaction between P application levels and mycorrhizal seed inoculation with regard to plant height was found at 30 days after planting, while no interaction were found at 45 days after planting (Table 4.13). At 60 days after planting, significant interaction were found with regard to fresh and dry mass per plant.

Only significant (P≤0.05) results will be discussed as follows.

Table 4.13 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on growth of maize plants at different growth stages

Sampling stage	Treatments	Leaf area (cm^2)	Stem diameter (mm)	Plant height (cm)	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
30 days after planting	P	*	*	*	*	*	*	*	*	NS
	M	NS	NS	NS	NS	NS	NS	NS	NS	NS
	M*P	NS	NS	*	NS	NS	NS	NS	NS	NS
45 days after planting	P	*	*	*	*	*	*	*	*	*
	M	NS	*	NS	*	NS	*	NS	NS	NS
	M*P	NS	NS	NS	NS	NS	NS	NS	NS	NS
60 days after planting	P	*	*	*	*	*	*	*	*	NS
	M	*	NS	*	*	*	*	*	*	NS
	M*P	NS	NS	NS	NS	NS	NS	*	*	NS

- NS: not significant at P≤0.05
- *: significant at P≤0.05

30 days after planting

Effect of phosphorus

At 30 days after planting, increasing P application levels had a significant increasing effected on leaf area, stem diameter, root fresh mass, root dry mass, percent root moisture as well as fresh mass and dry mass per plant (Figure 4.33-4.39). All the measured parameters except for root moisture showed an increase with an increase in P application level, but tended to reach their maximum or at least level off at P_4 to P_5.

The application of P fertilizer increased leaf area from 154.3 cm^2 $plant^{-1}$ where no P was applied (P_1) to 349.12 cm^2 $plant^{-1}$ at a P level of 40 kg P ha^{-1} (P_5) (Figure 4.33). No significant differences were however found between P_3 (20 kg P ha^{-1}) and P_5 levels. Rasheed *et al.* (2004) reported a maximum flag leaf area of 27.41 cm^2 $plant^{-1}$ at P level of 60 kg ha^{-1} while the lowest value of 21.45 cm^2 $plant^{-1}$ was recorded in plots where no P was applied under irrigated conditions. These results are in conformity to Bakhsh (1997) and Nawab *et al.* (1999) who also reported a significant increase in flag leaf area when P was applied to maize in P deficient soil.

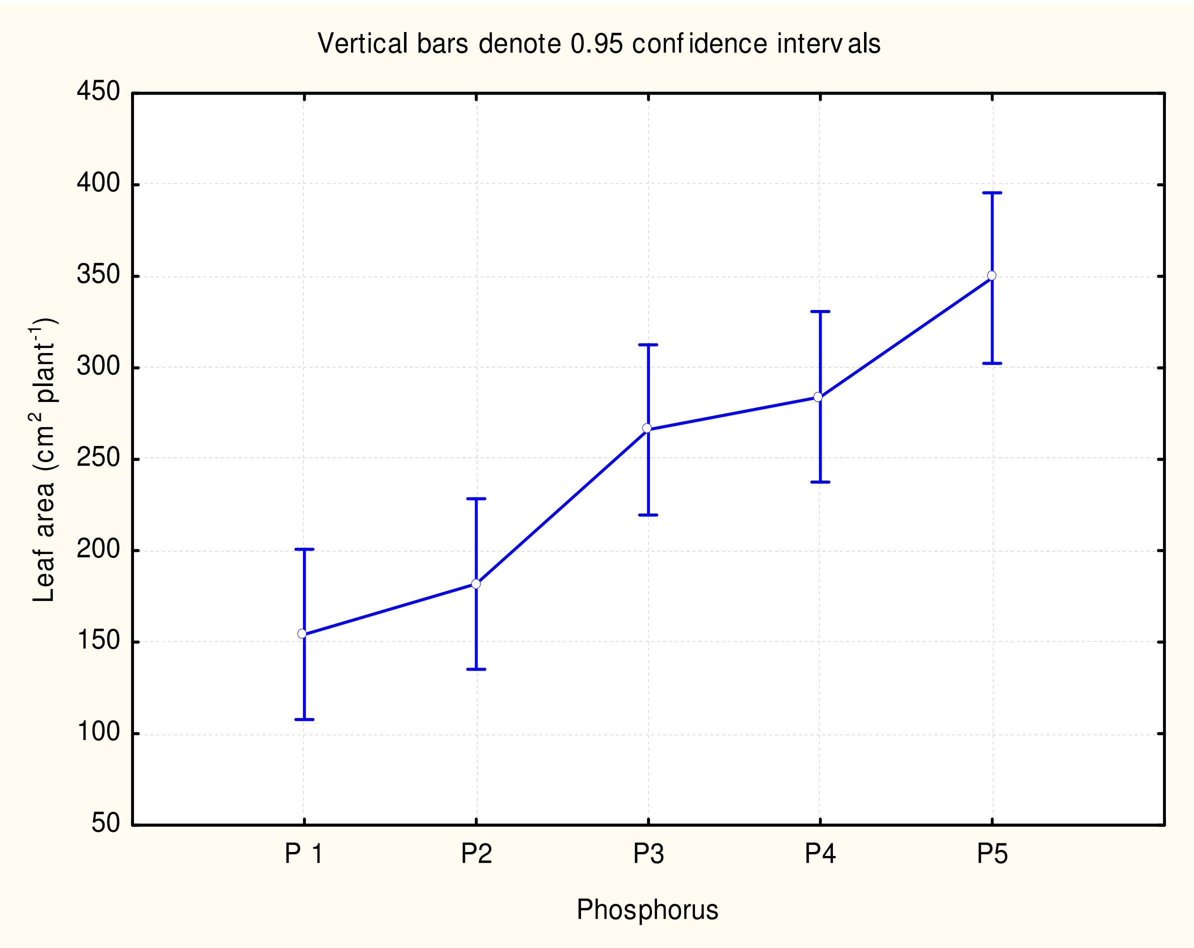

Figure 4.33 Effect of different P levels on leaf area (cm^2 $plant^{-1}$) of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Plant stem diameter increased with P application levels from 7.19 mm at P_1 (0 kg P ha^{-1}) to11.31 mm at a P application level of 40 kg P ha^{-1} (P_5) without reaching a maximum (Figure 4.34). This stem diameter was statistically different from the stem diameters produced by all the other P levels (P_1= 11.31 mm; P_2 = 7.56 mm; P_3= 9.13 mm and P_4= 9.19 mm. Ayub *et al.* (2002) also showed that plant stem diameter responded positively to N and P application under climatic conditions of Faisalabad in Pakistan.

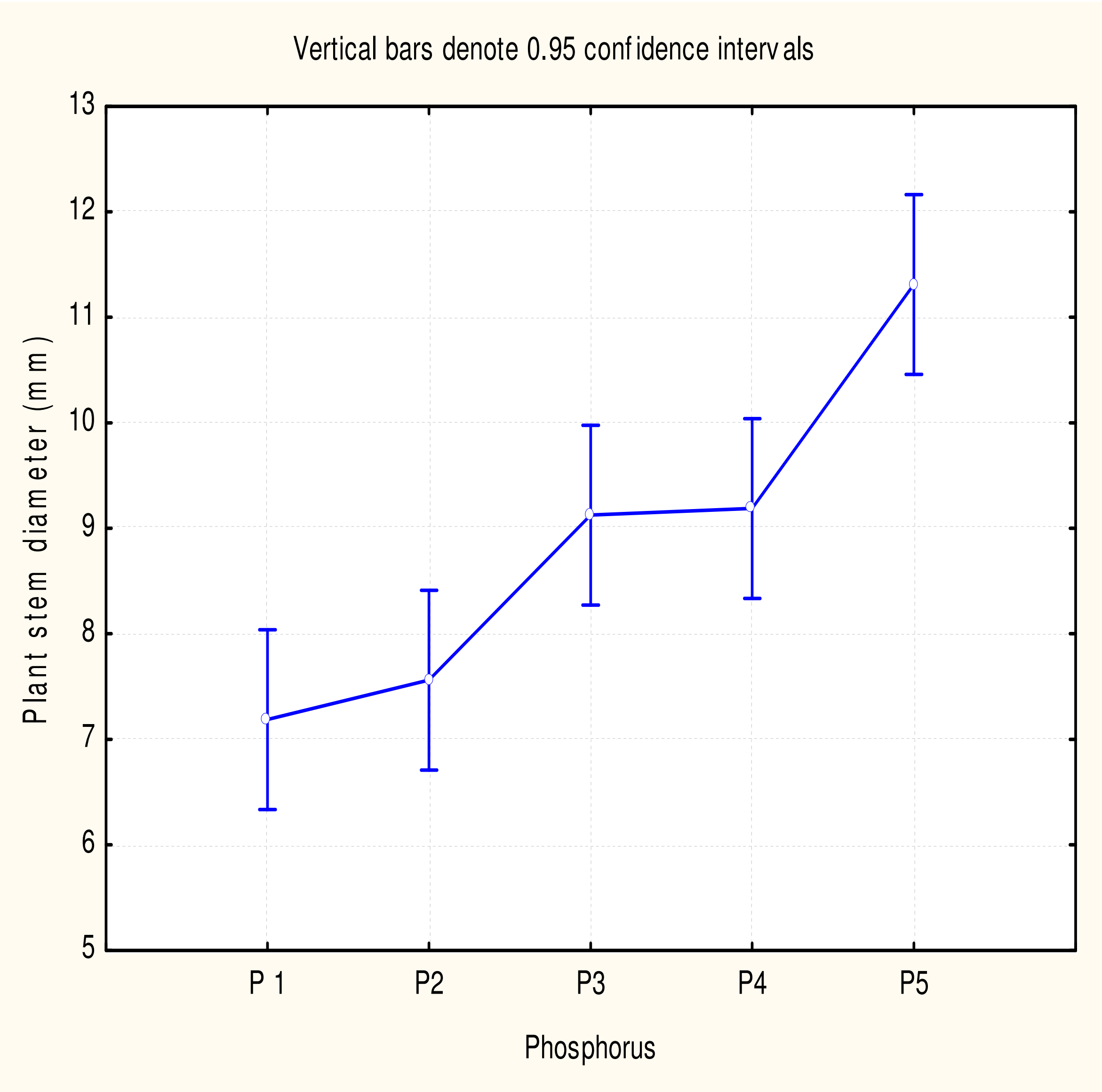

Figure 4.34 Effect of different P levels on stem diameter (mm) of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results (Figure 4.35) showed an increase in root fresh mass with increasing P application levels from 7.44 g plant^{-1} at 0 kg P ha^{-1} (P_1) to 11.50 g plant^{-1} (P_5) without reaching a maximum. The root fresh mass at P_5 was however not statistically different from the root fresh mass produced with 20 kg P ha^{-1} (P_3) and 30 kg P ha^{-1} (P_4).

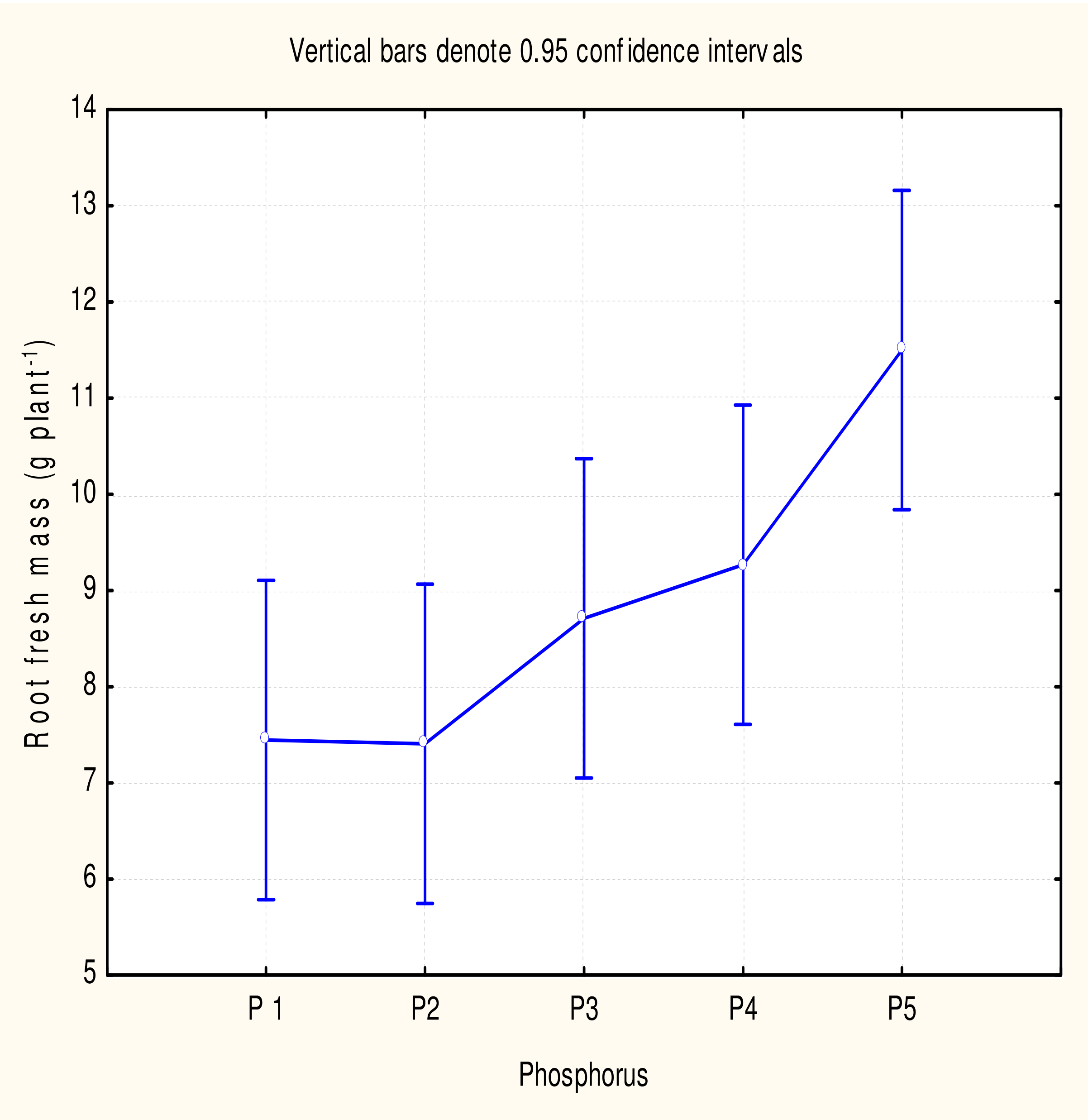

Figure 4.35 Effect of different P levels on root fresh mass of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The data on root dry mass (Figure 4.36) showed trends similar to root fresh mass (Figure 4.35). Root dry mass also increased with increasing P application levels from 0.656 g plant^{-1} with P_1, but reached the highest value of 1.281 g plant^{-1} at P_5. This root dry mass was however not statistically different from root dry mass of P_3 (0.969 g plant^{-1}) and P_4 (0.956 g plant^{-1}). Increased root dry mass due to P application could be due to the direct effect of P on root growth and its development (Purushottam *et al.,* 1995; Srivastava & Ahlawat, 1995). Anonymous (2008) found that a crop which has access to sufficient P gets a headstart right from seedling stage, produces deeper and more proliferous roots which enable it to feed on a bigger soil volume for water and nutrients.

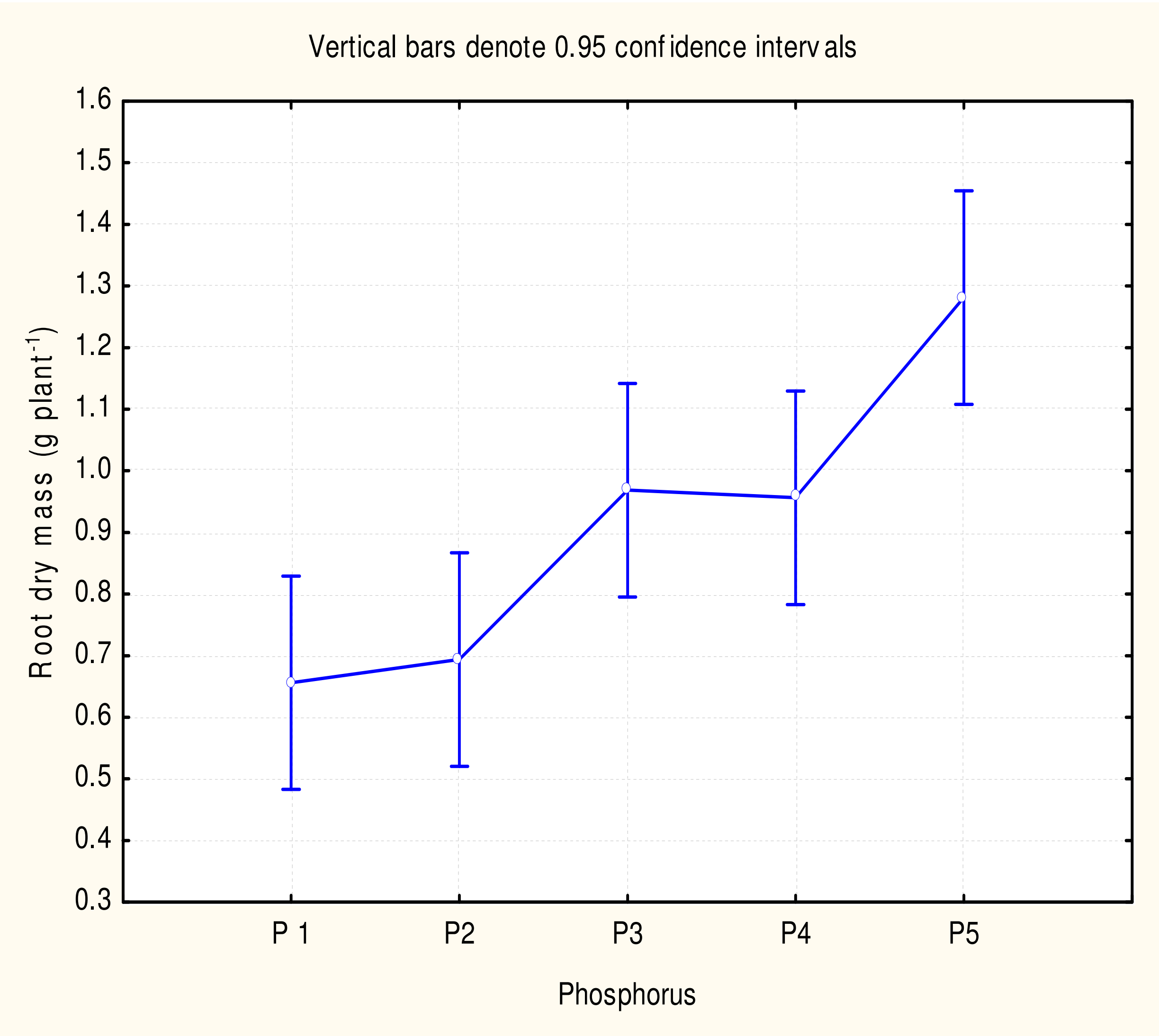

Figure 4.36 Effect of different P levels on root dry mass of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Increasing P application levels decreased the maize root moisture content between 0 kg P ha^{-1} (P_1) and 40 kg P ha^{-1} (P_5) (Figure 4.37). The highest root moisture content of 91.24% was measured in plants receiving 0 kg P ha^{-1}. This value was significantly different from the moisture content of all the other P application levels except for 10 kg P ha^{-1} (P_2) (90.63%).

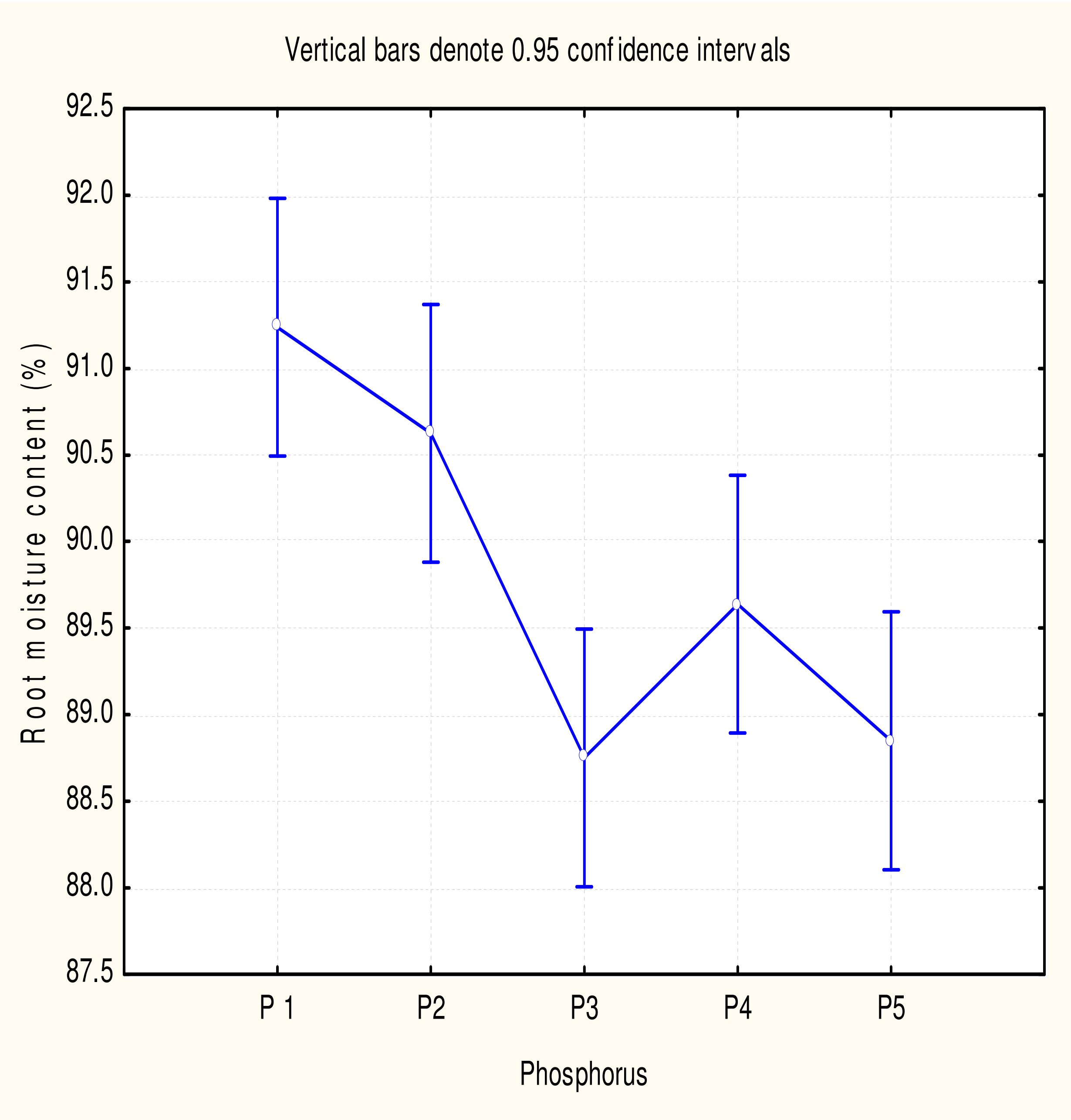

Figure 4.37 Effect of different P levels on root moisture of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results (Figure 4.38) showed an increase in plant fresh mass with increasing P application levels from 7.719 g plant^{-1} at P_1 (0 kg P ha^{-1}) to 19.750 g plant^{-1} at P_5 (40 kg P ha^{-1}) without reaching a maximum, indicating that higher P application levels will be needed in these sandy P deficient soil to satisfy the P requirements of maize. The fresh mass per plant at P_5 was significantly higher than the fresh mass per plant produced with all the other P application levels.

Plant dry mass showed similar results with a highest value of 2.006 g plant^{-1} at P_5 compared to 0.769 g plant^{-1} where no P was applied (P_1) (Figure 4.39).

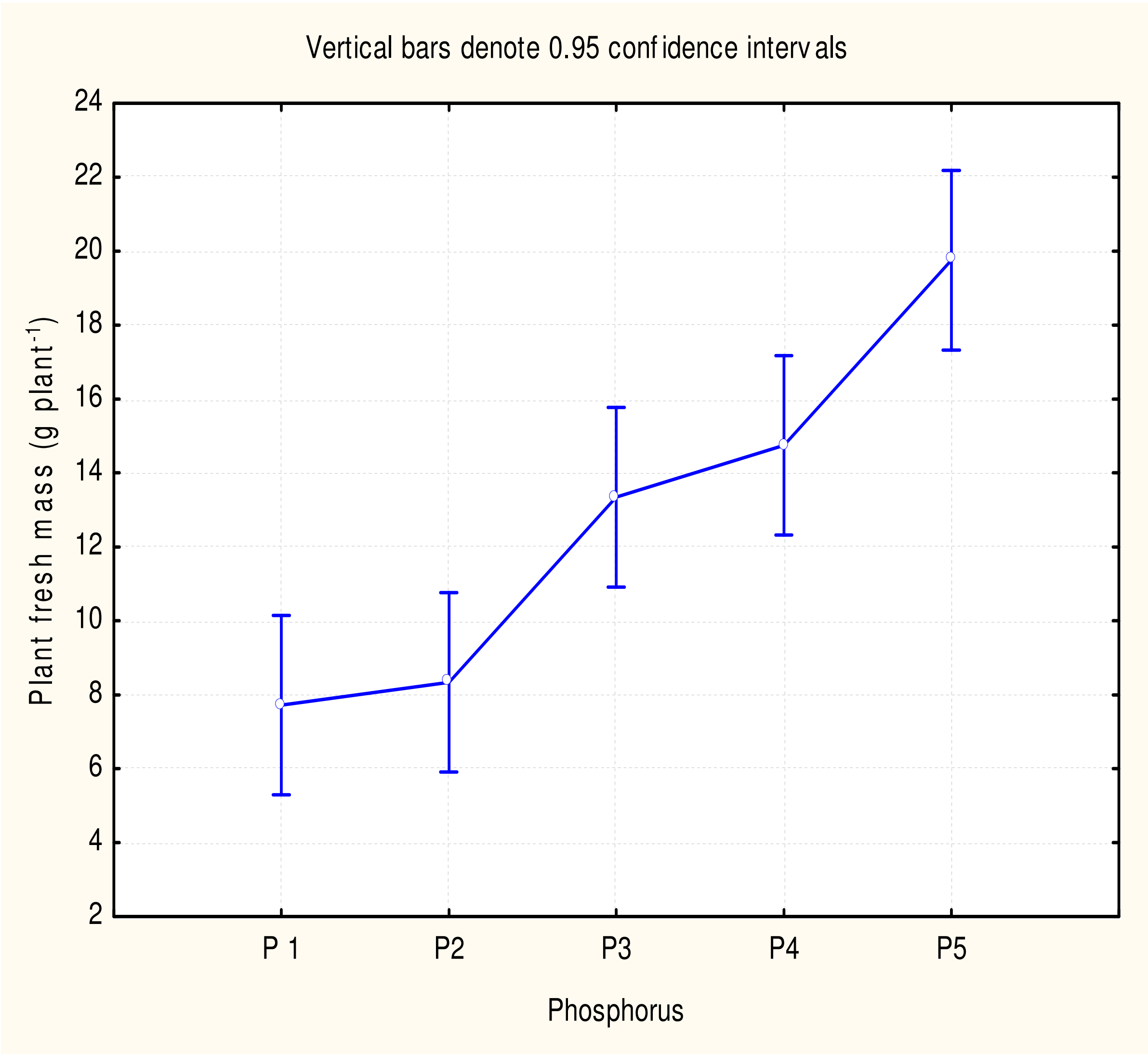

Figure 4.38 Effect of different P levels on fresh mass of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

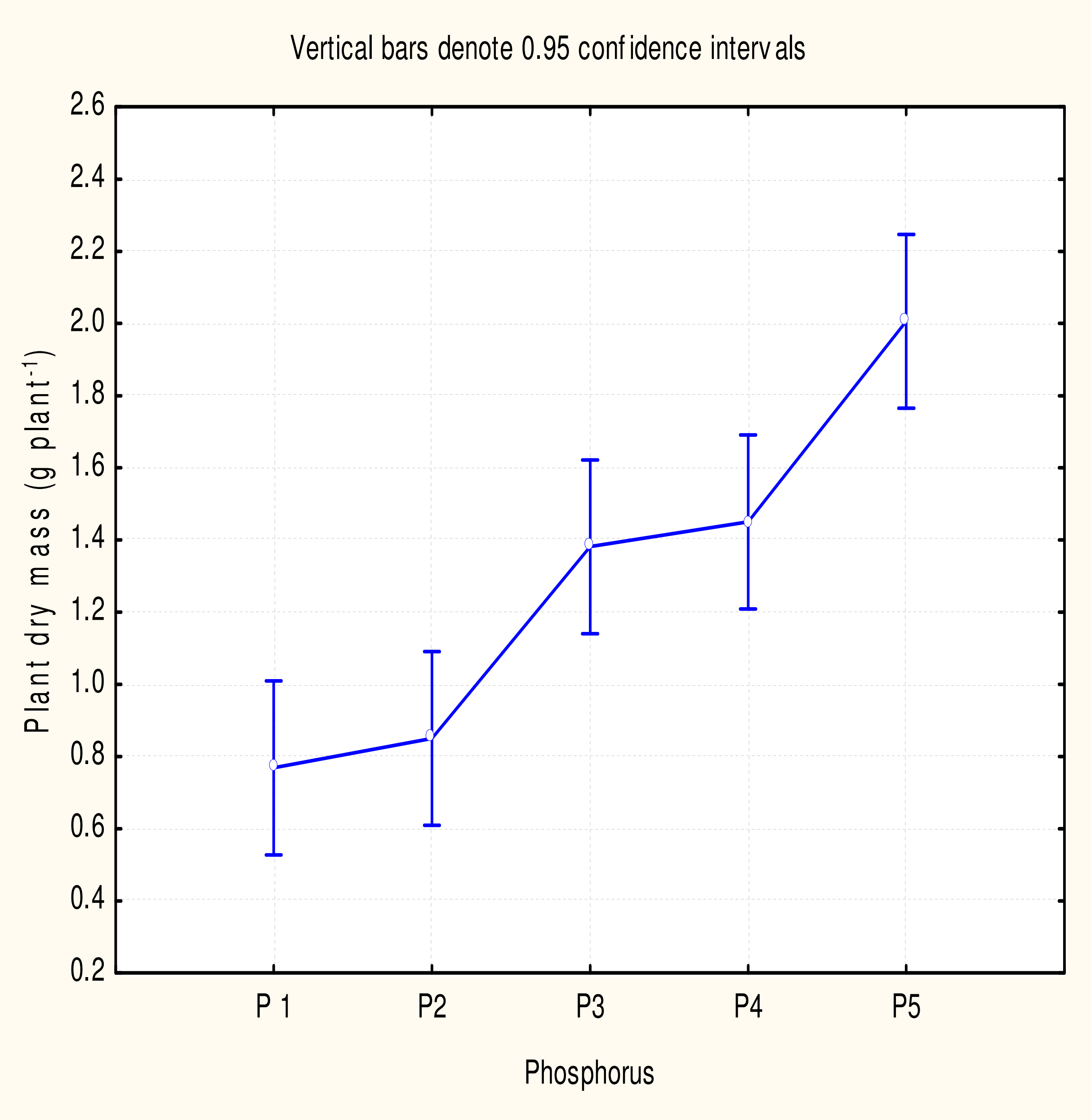

Figure 4.39 Effect of different P levels on dry mass of maize plants at 30 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

These results were expected since the results on growth attributes showed that P application levels significantly increased plant height, stem diameter and leaf area. Khaliq & Sanders (1997) have also reported a significantly higher dry matter production with P application in pots placed under field conditions. The results of this study indicate that maize plants were able to access phosphorus at an early growth stage hence the improvement in growth attributes and dry matter yield.

Effect of mycorrhizal inoculation

At 30 days after planting, mycorrhizal seed inoculation did not have a significant effect on maize growth. The absence of any positive response in mycorrhizal inoculated plants could be due to the fact that at this early stage of maize growth the mycorrhiza was still forming an association with maize roots and did not yet improve the nutrient uptake of inoculated plants (Khan, 1975). Bougher *et al.* (1990) stated that at low levels of soil P, height of seedlings from mycorrhizal inoculated seed was similar to that of uninoculated seedlings till about 35 days after planting, but started to accelerate after this initial period of growth. This observation supports earlier results (Khan, 1975; Owusu-Bennoah & Mosse, 1979) that suggested that the positive effect of mycorrhizal inoculation becomes evident only after about one month of growth.

Table 4.14 Effect of mycorrhizal inoculation on growth of maize plants at 30 days after planting expressed as mean values per plant

	Measured parameters								
Treatments	Leaf area (cm^2)	Stem diameter (mm)	Plant height (mm)	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
M_0	249.36a	8.80a	486.37a	8.55a	0.88a	89.84a	13.06a	1.31a	89.96a
M_1	244.88a	8.95a	495.02a	9.18a	0.95a	89.80a	12.50a	1.27a	89.79a

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application level

The application of P fertilizer increased plant height in non-mycorrhizal plants, but not in the mycorrhizal inoculated plants (Figure 4.40). This resulted in a significantly higher (475.13 mm $plant^{-1}$) plant height in mycorrhizal plants compared to non-mycorrhizal plants (338.13 mm $plant^{-1}$) where no P was applied. Although unlikely because of the absence of a positive mycorrhizal effect on root growth at this stage, increased plant heights in mycorrhizal plants may be due to improved nutrient uptake.

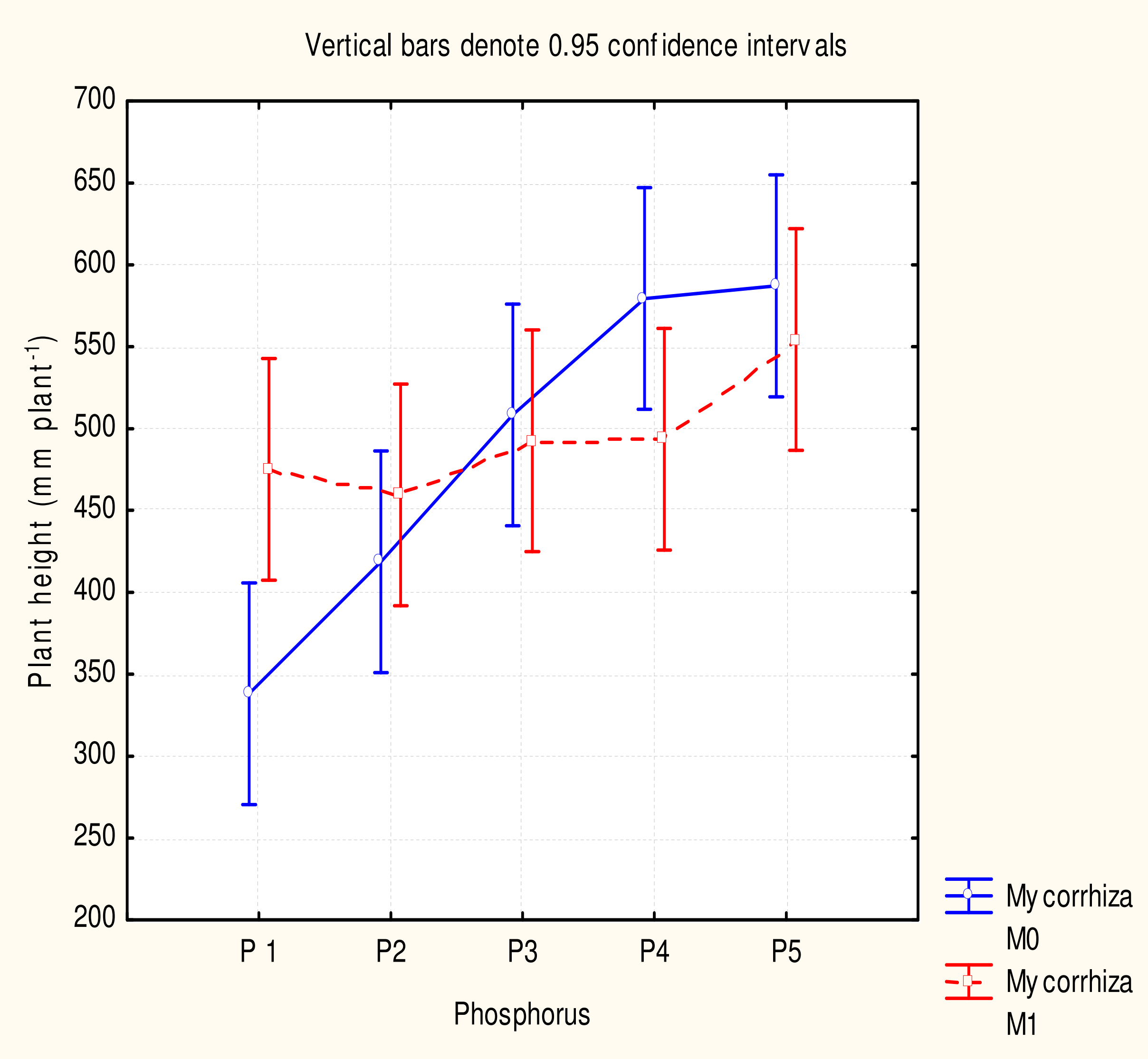

Figure 4.40 Effect of seed inoculation with mycorrhiza on the plant height response of maize at 30 days after planting to P applications.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

45 days after planting

Effect of phosphorus

At 45 days after planting, increasing P application levels had a significant increasing effect on all the measured parameters (Figures 4.41-4.49). All measured parameters except for percent plant moisture showed an increase with an increase in P application levels, but tend to reach their maximum or at least level off at P_3 to P_5 treatments.

Leaf area increased from 233.88 cm^2 plant^{-1} where no P was applied (P_1) to 741.04 cm^2 plant^{-1} at a P level of 40 kg P ha^{-1} (P_5) (Figure 4.41). No significant differences were however found between P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}).

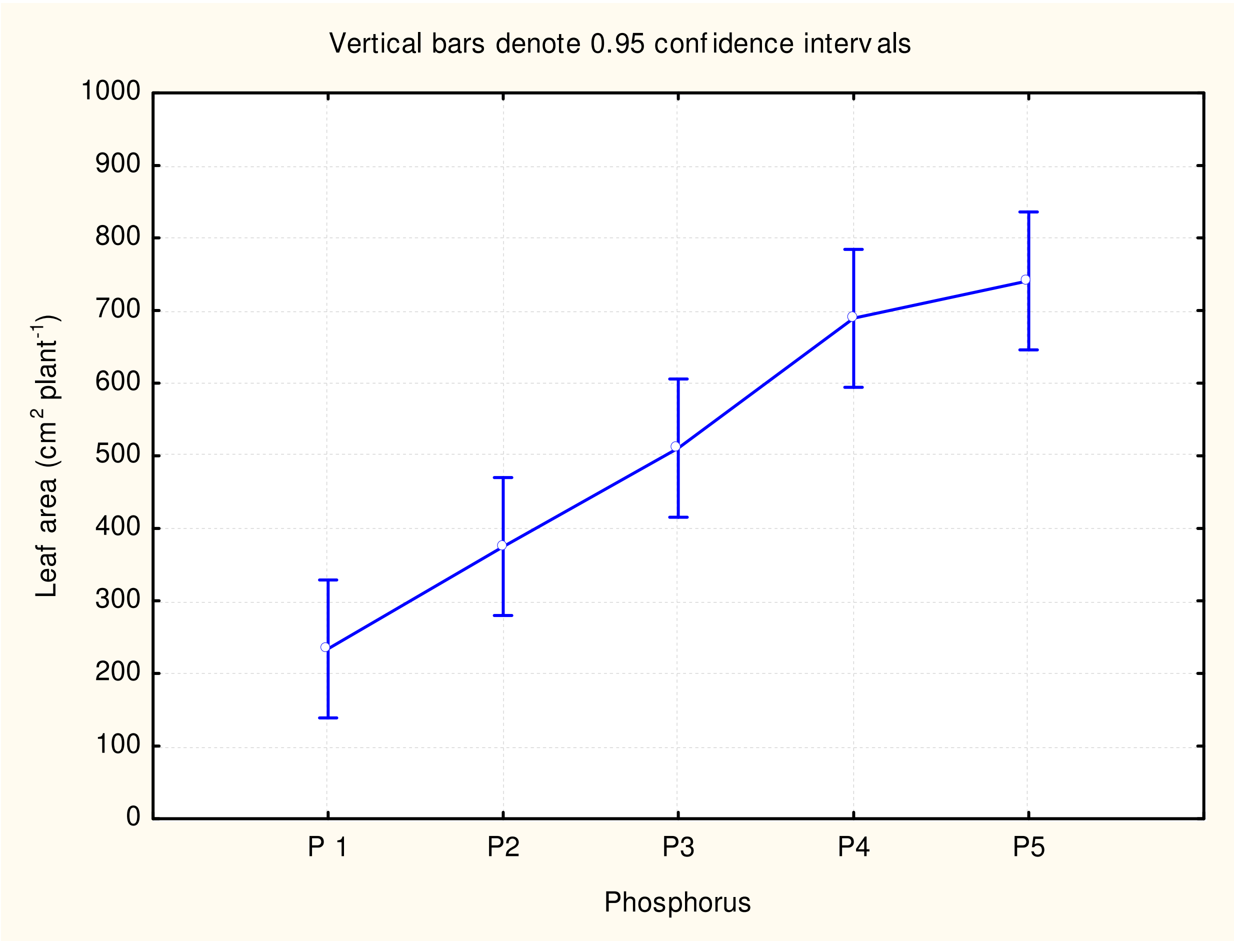

Figure 4.41 Effect of different P levels on leaf area of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Plant stem diameter increased with increasing P application levels from 7.63 mm where no P was applied (P_1) to 13.25 mm where 40 kg P ha^{-1} (P_5) was applied (Figure 4.42), but the stem diameter of plants produced with treatment P_5 was not significantly higher than that of treatment P_4 (11.96 mm). Bukvic´ *et al.* (2003) have previously found that P fertilization increased stalk diameter of maize plants grown in pots containing Eutric Cambisol soil.

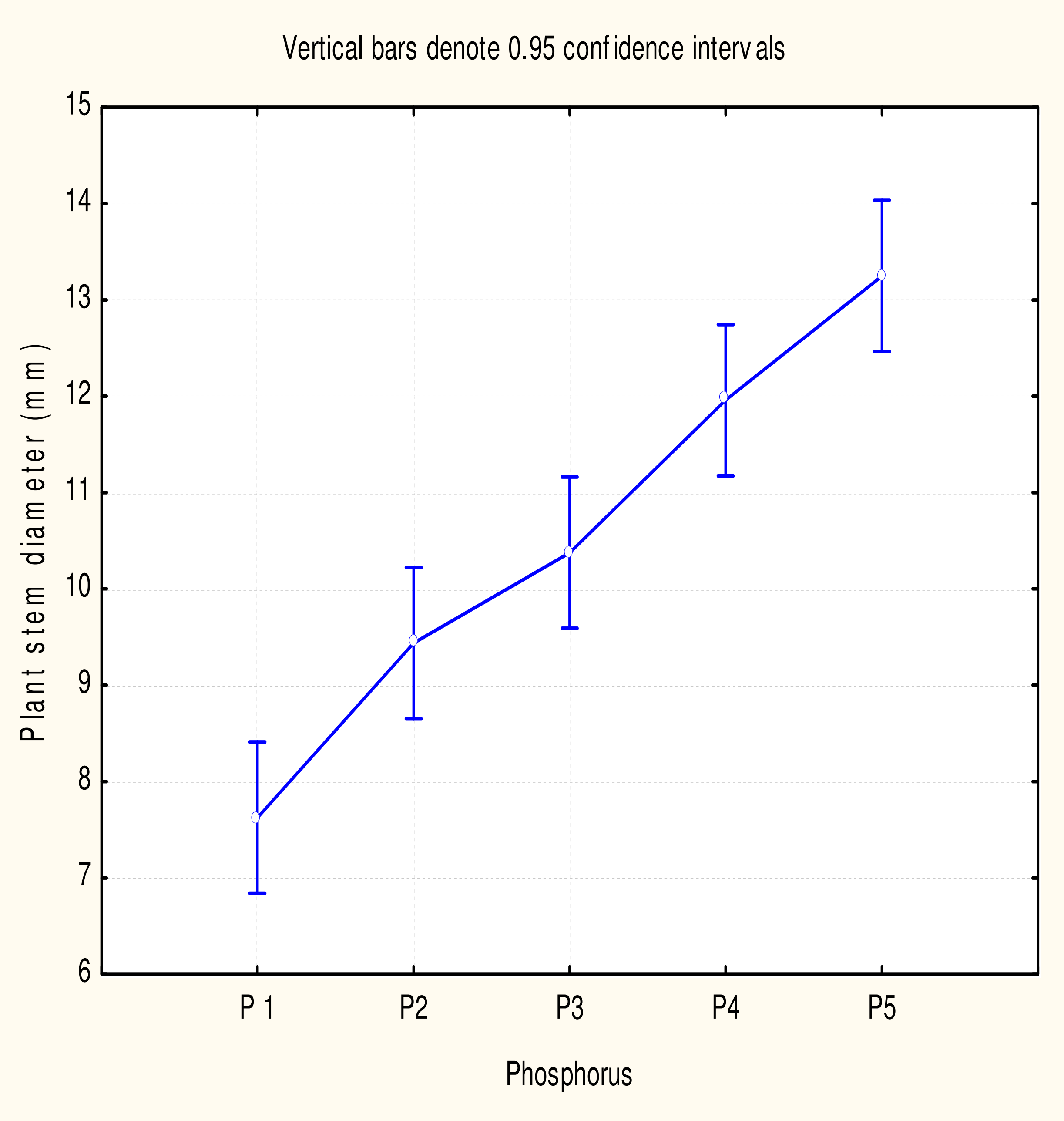

Figure 4.42 Effect of different P levels on stem diameter of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The plant height was influenced significantly by different levels of P (Figure 4.43). Plant height increased from 735.44 mm where no P was applied (P_1) to 1017.50 mm at a P level of 40 kg P ha^{-1} (P_5). No significant differences were however found between P_3 (1004.10 mm) and P_5 treatments. Phosphorus fertilization has also been previously found to increase height of maize plants grown in pots containing Eutric Cambisol soil (Bukvic´ *et al.* 2003). Materechera & Morutse (2009) also reported an increase in maize plant height with each increment of P for dry land maize production in a soil with very low inherent P levels.

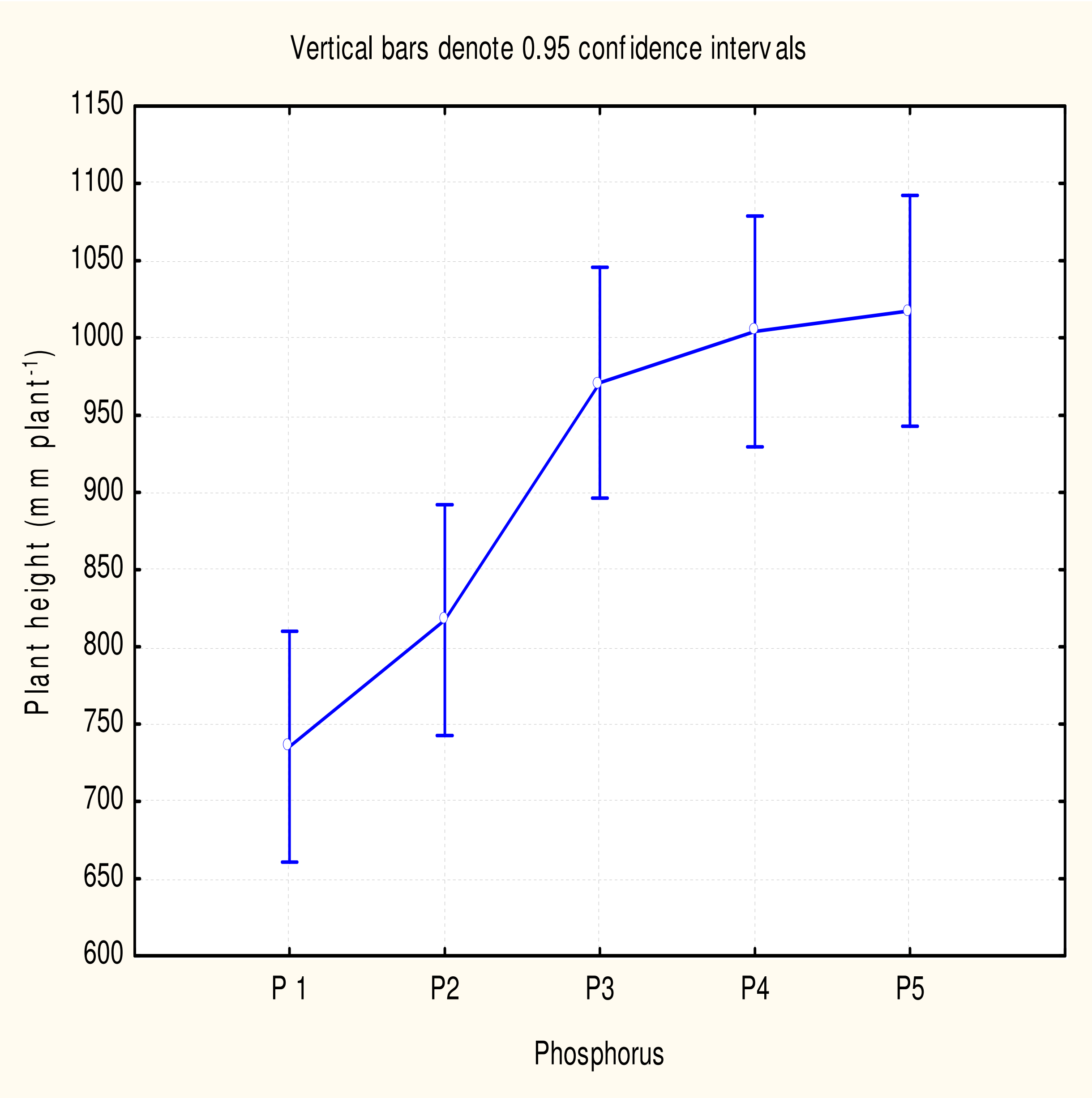

Figure4. 43 Effect of different P levels on height of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results (Figure 4.44) showed an increase in root fresh mass with increasing P application levels from 4.30 g $plant^{-1}$ at P_1 (0 kg P ha^{-1}) to 17.11 g $plant^{-1}$ at P_5 (40 kg P ha^{-1}) without reaching a maximum. The root fresh mass at P_5 was however not statistically different from the root fresh mass produced with 30 kg P ha^{-1} (P_4).

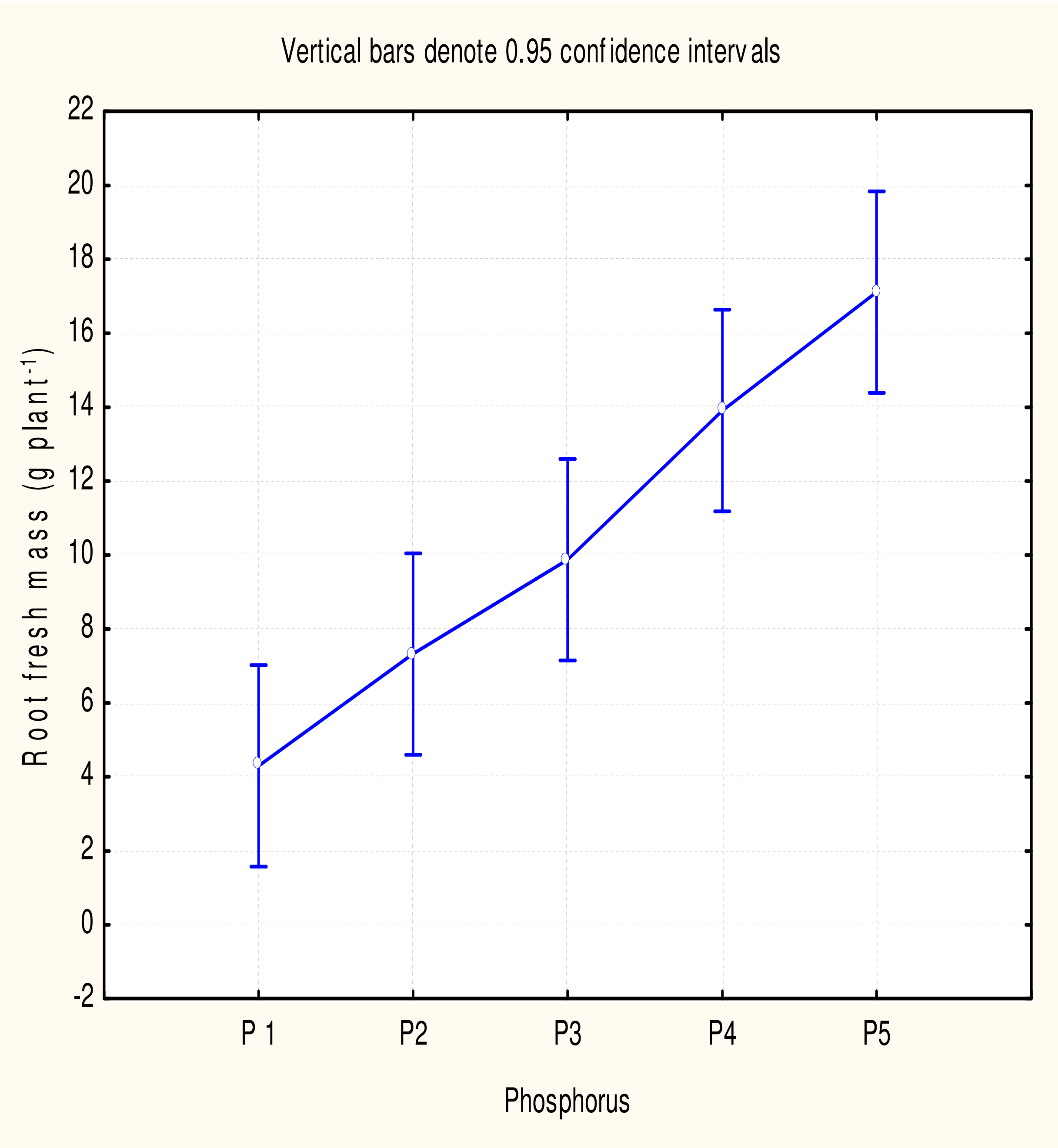

Figure 4.44 Effect of different P levels on root fresh mass of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Root dry mass showed similar results to that of root fresh mass with a highest value of 2.906 g plant^{-1} at P_5 (Figure 4.45) compared to 0.950 g plant^{-1} where no P was applied (P_1). This root dry mass was however not statistically higher than the root dry mass of P_3 (1.863 g plant^{-1}) and P_4 (2.556 g plant^{-1}). The increase in root mass can be ascribed to the direct effect of P on root growth and development (Price, 2006), because Fageria *et al.* (2006) found that increasing P levels stimulated root growth.

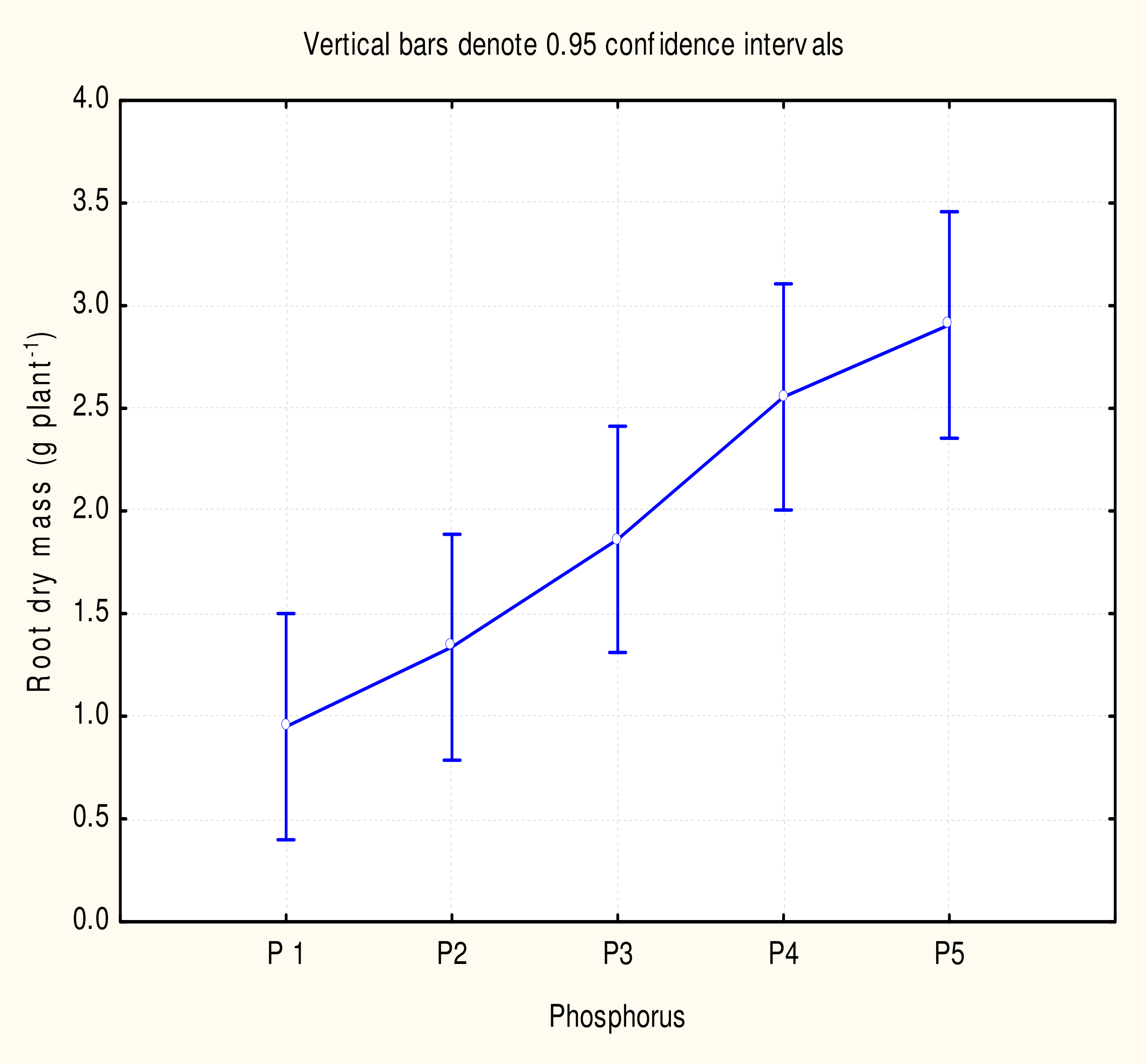

Figure 4.45 Effect of different P levels on root dry mass of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results indicated that percentage root moisture was significantly affected with P levels (Figure 4.46). Plants receiving 40 kg Pha^{-1} had the highest moisture content of 83.14%, but were not significantly different from the moisture content of either P_2 plants (80.56%), P_3 plants (80.40%) or P_4 plants (80.98%). However, plants which received no P (P_1) had the lowest moisture content of 77.24%.

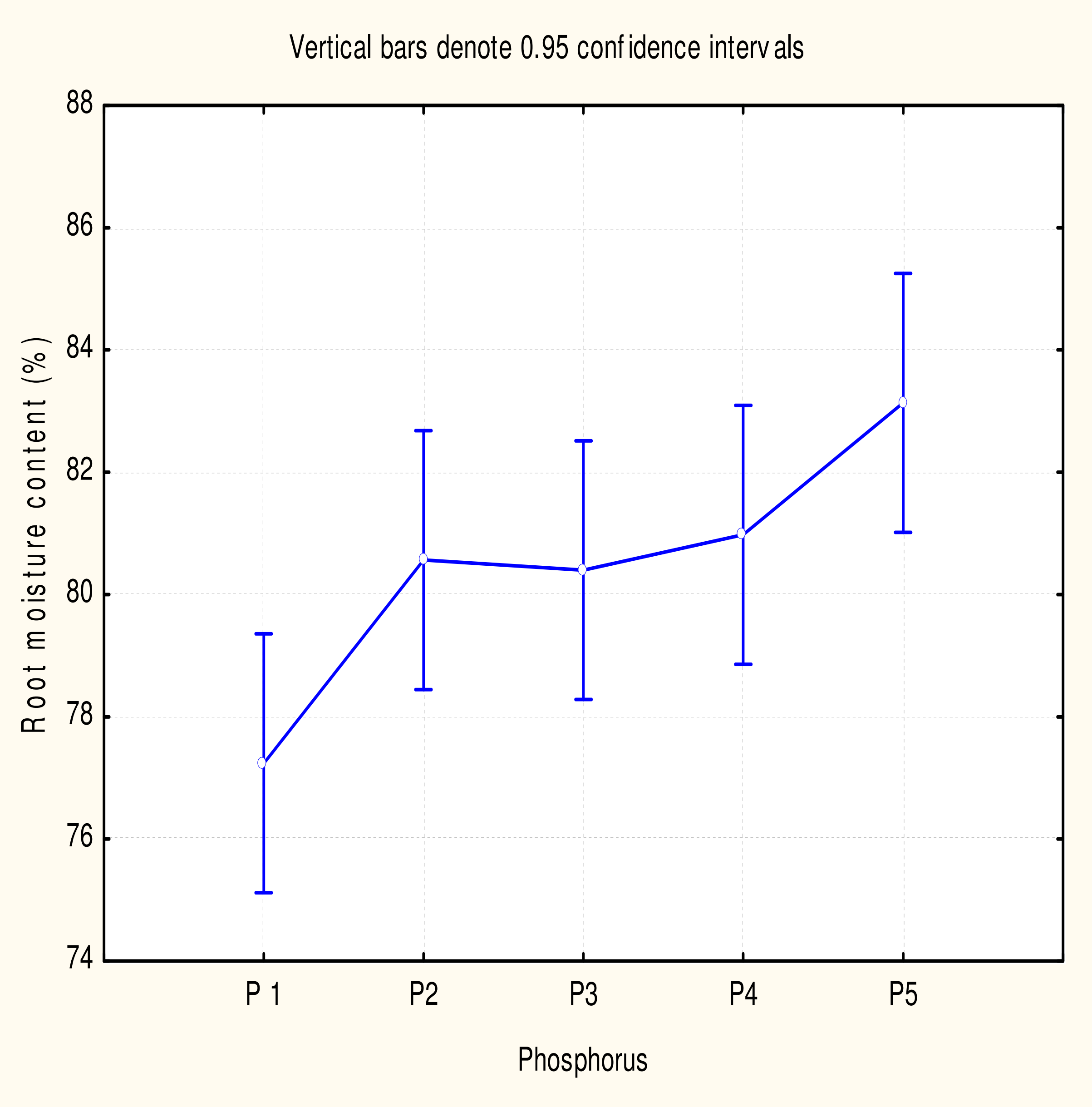

Figure 4.46 Effect of different P levels on root moisture of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Plant fresh mass was affected significantly with the application of P (Figure 4.47). A gradual increase in plant fresh mass was observed with increasing P levels. The highest plant fresh mass of 52.238 g plant^{-1} was produced at P_5 which was significantly higher than plant fresh mass produced by all the other P levels. Plant dry mass showed similar results with a highest value of 6.663 g plant^{-1} at P_5 compared 1.813 g plant^{-1} where no P was applied (P_1) (Figure 4.48). The increase in plant mass can be ascribed to the higher leaf area, plant height and stem diameter recorded in this study. A general increase of biomass with increased levels of P has been shown by Materechera & Morutse (2009). Bukvic´ *et al.* (2003) have also found that P fertilization increased total dry matter biomass of maize plants grown in pots containing Eutric Cambisol soil.

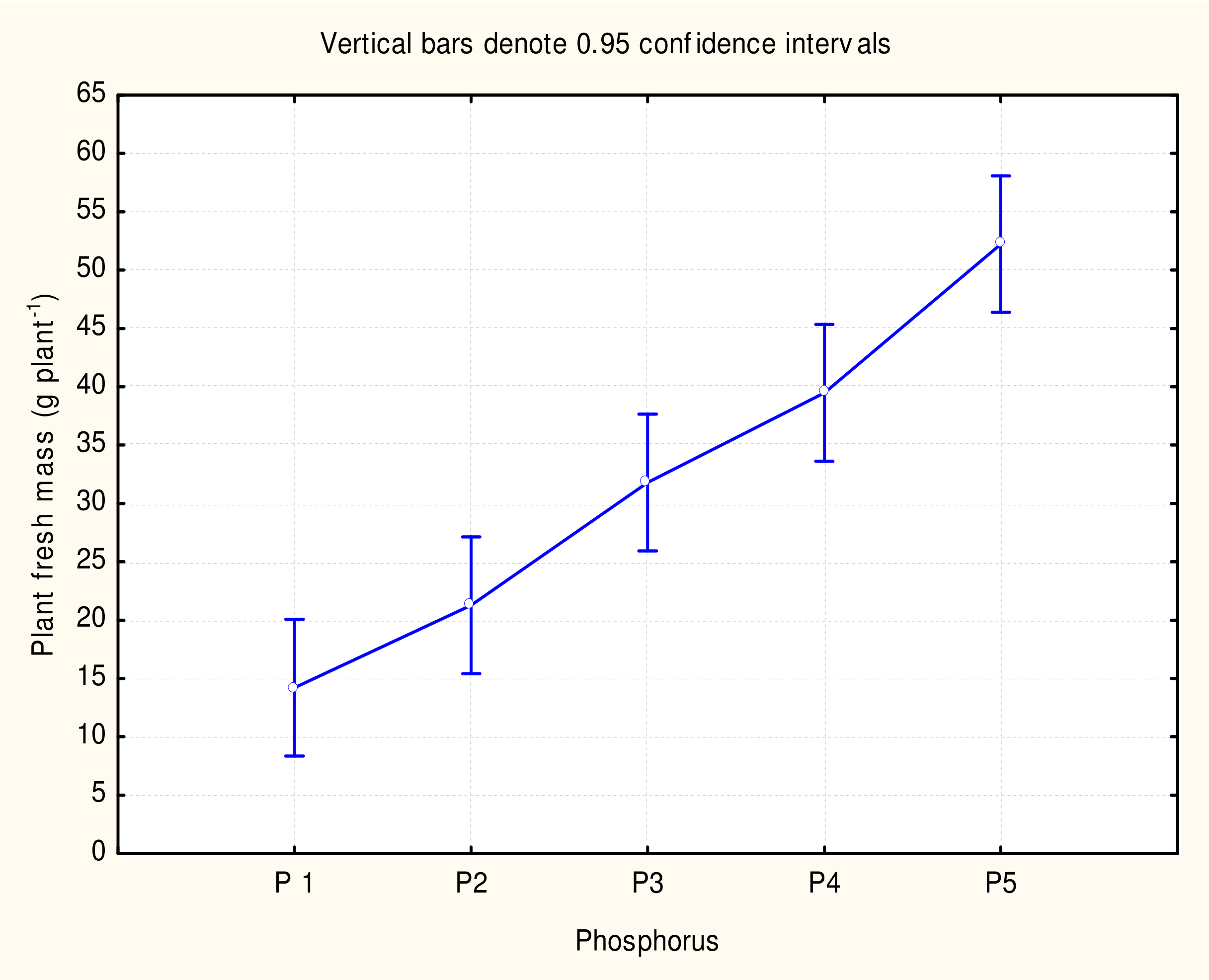

Figure 4.47 Effect of different P levels on plant fresh mass of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

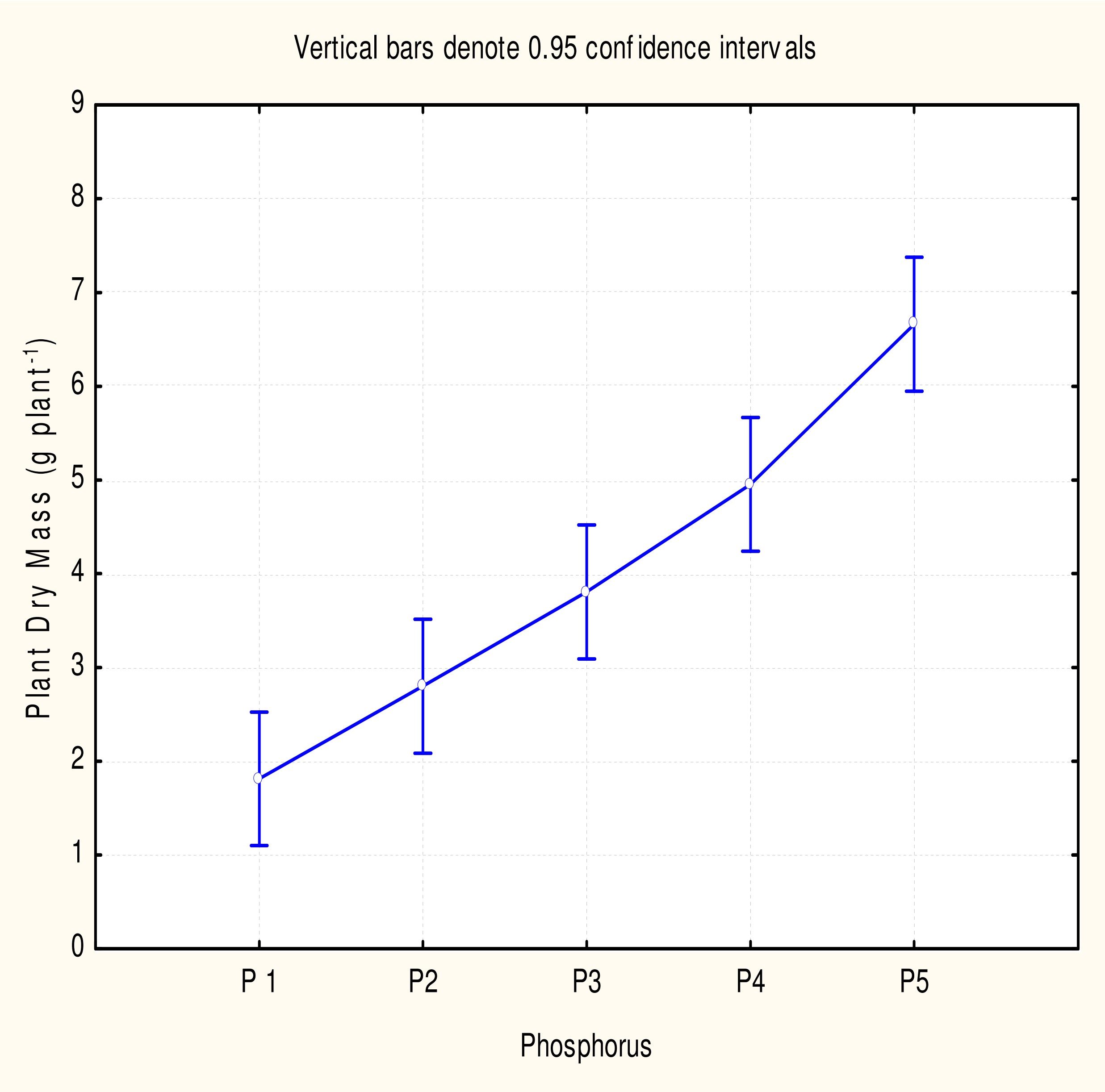

Figure 4.48 Effect of different P levels on plant dry mass of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Increasing P levels from 0 kg P ha^{-1} (P_1) to 10 kg P ha^{-1} (P_2) resulted in a decrease in plant moisture content, but further increases in P level to 20 kg P ha^{-1} (P_3) resulted in an increase in plant moisture content to a maximum moisture content of 87.89% kg P ha^{-1} (Figure 4.49). This value was however not significantly different from the moisture content of either the P_1 plants (87.25%), P_4 plants (87.50%) or the P_5 plants (87.18%).

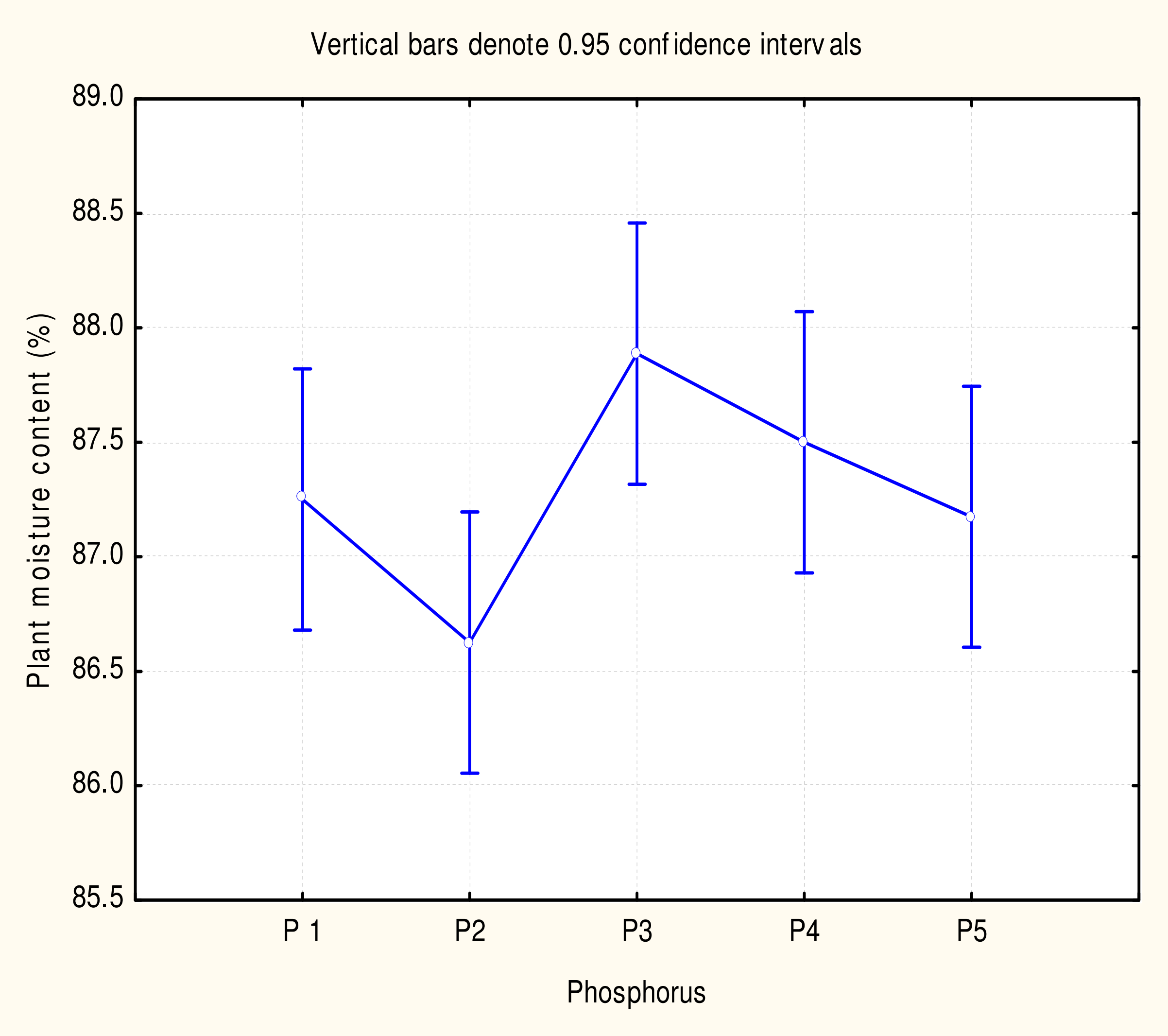

Figure 4.49 Effect of different P levels on moisture content of maize plants at 45 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

The results showed that seed inoculation with AM fungi had a significant effect on stem diameter, root fresh mass and percentage root moisture (Table 4.15). Seed inoculation with AM fungi increased plant stem diameter at 45 days after planting from 10.18 mm to 10.88 mm, root fresh mass from 8.66 g to 12.35 g plant^{-1} and root moisture from 78.08% to 82.85%. This response indicates that at 45 days after planting, the mycorrhiza have formed an association with plant roots and was now beginning to increase nutrient uptake by plants in exchange for the carbohydrates received from the plants as also found by Khan (1972) in steam-sterilized sand.

Table 4.15 Effect of mycorrhizal inoculation on growth of maize plants at 45 days after planting expressed as mean values per plant

	Measured parameters								
Treatments	Leaf area (cm^2)	Stem diameter (mm)	Plant height (mm)	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
M_0	479.48a	10.18b	917.95a	8.66b	1.79a	78.08b	31.13a	3.89a	87.33a
M_1	540.45a	10.88a	900.20a	12.35a	2.05a	82.85a	32.49a	4.13a	87.25a

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

In this study with maize no significant interaction between P fertilization and mycorrhizal inoculation was found at 45 days after planting.

60 days after planting

Effect of phosphorus

At 60 days after planting, increasing P application levels had significant effects on leaf area per plant, plant stem diameter, plant height, root fresh mass, root dry mass, percentage root moisture as well as the fresh and dry mass per plant (Figures 4.50-4.55). Because of significant interactions between P application levels and mycorrhizal seed inoculation with regard to plant fresh mass and plant dry mass (Table 4.16) the main effect of P on these parameters will not be discussed. All measured parameters showed an increase with an increase in P application levels, but tend to reach their maximum or at least level off at P_4 or P_5 treatments.

Leaf area showed an increasing trend from 255.88 cm^2 $plant^{-1}$ where no P was applied (P_1) to 1126.90 cm^2 $plant^{-1}$ at a P level of 40 kg P ha^{-1} (P_5) (Figure 4.50) without reaching a maximum. The leaf area at P_5 was however not statistically different from the leaf area produced with P_4 (30 kg P ha^{-1}).

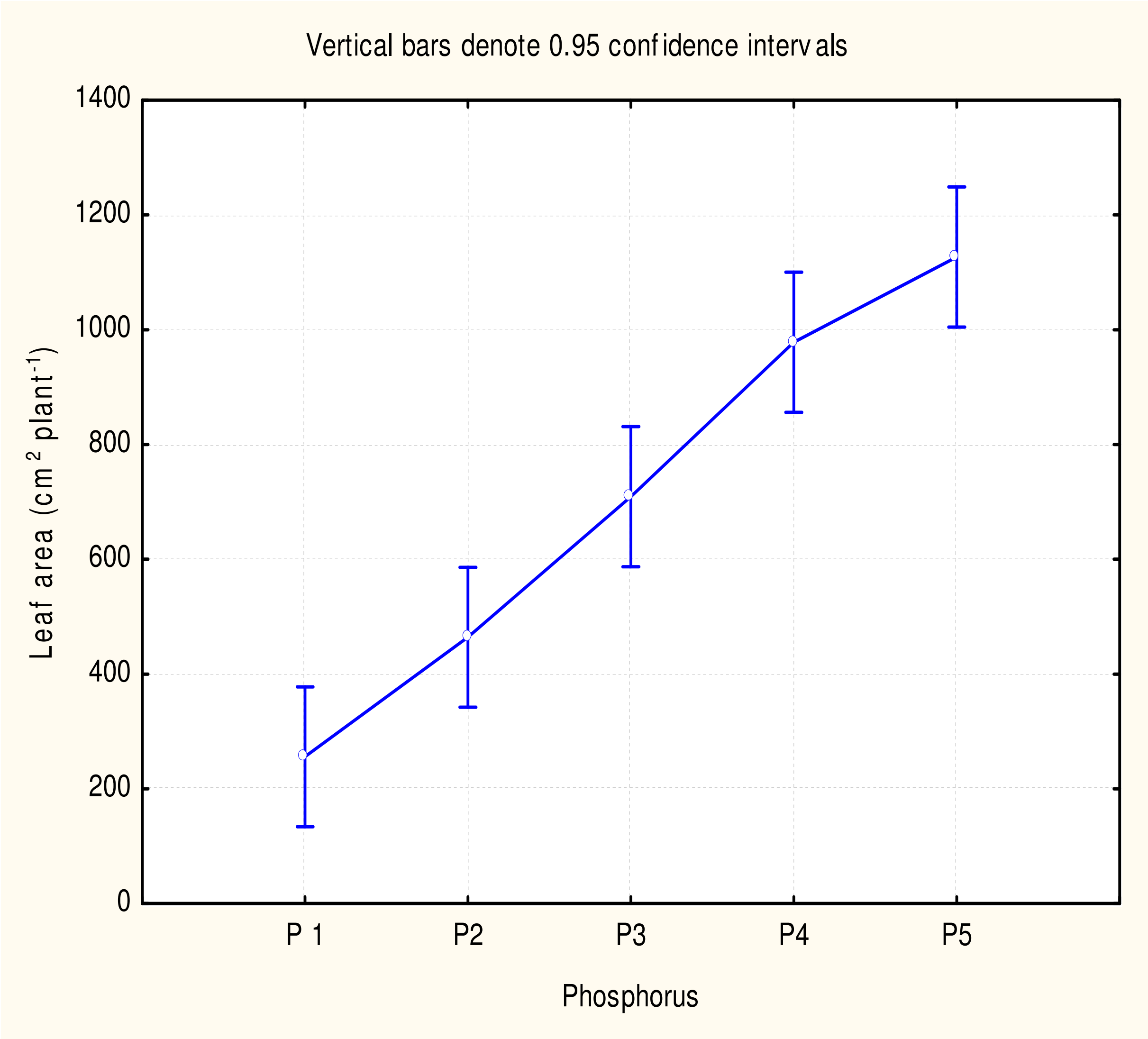

Figure 4.50 Effect of different P levels on leaf area of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

In Figure 4.51, the effect of P application levels on stem diameter of maize plants at 60 days after planting is shown. The highest plant stem diameter of 15.31 mm was recorded in plants receiving 40 kg P ha^{-1} (P_5). This value was however not significantly different from the stem diameter of P_4 plants (13.81 mm). Plants which received no P (P_1) had the lowest stem diameter of 9.00 mm.

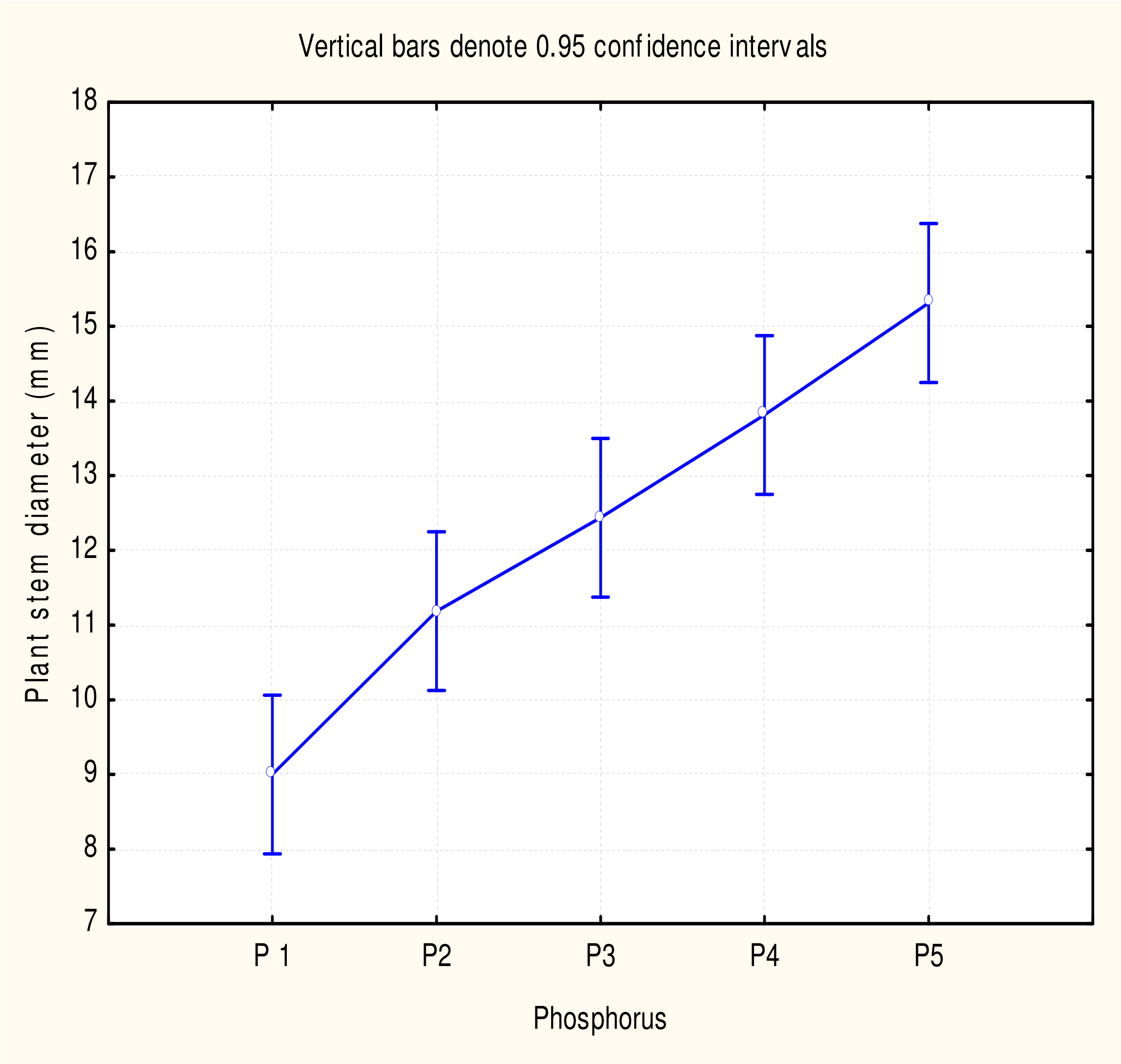

Figure 4.51 Effect of different P levels on stem diameter of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Maximum plant height of 1217.30 mm plant^{-1} was produced at P_4 (30 kg P ha^{-1}), which was significantly higher than the height of plants at P_1 (646.29 mm), P_2 (842.81 mm) or P_3 (979.88 mm), but was not statistically different from P_5 (1128.40 mm) (Figure 4.52).

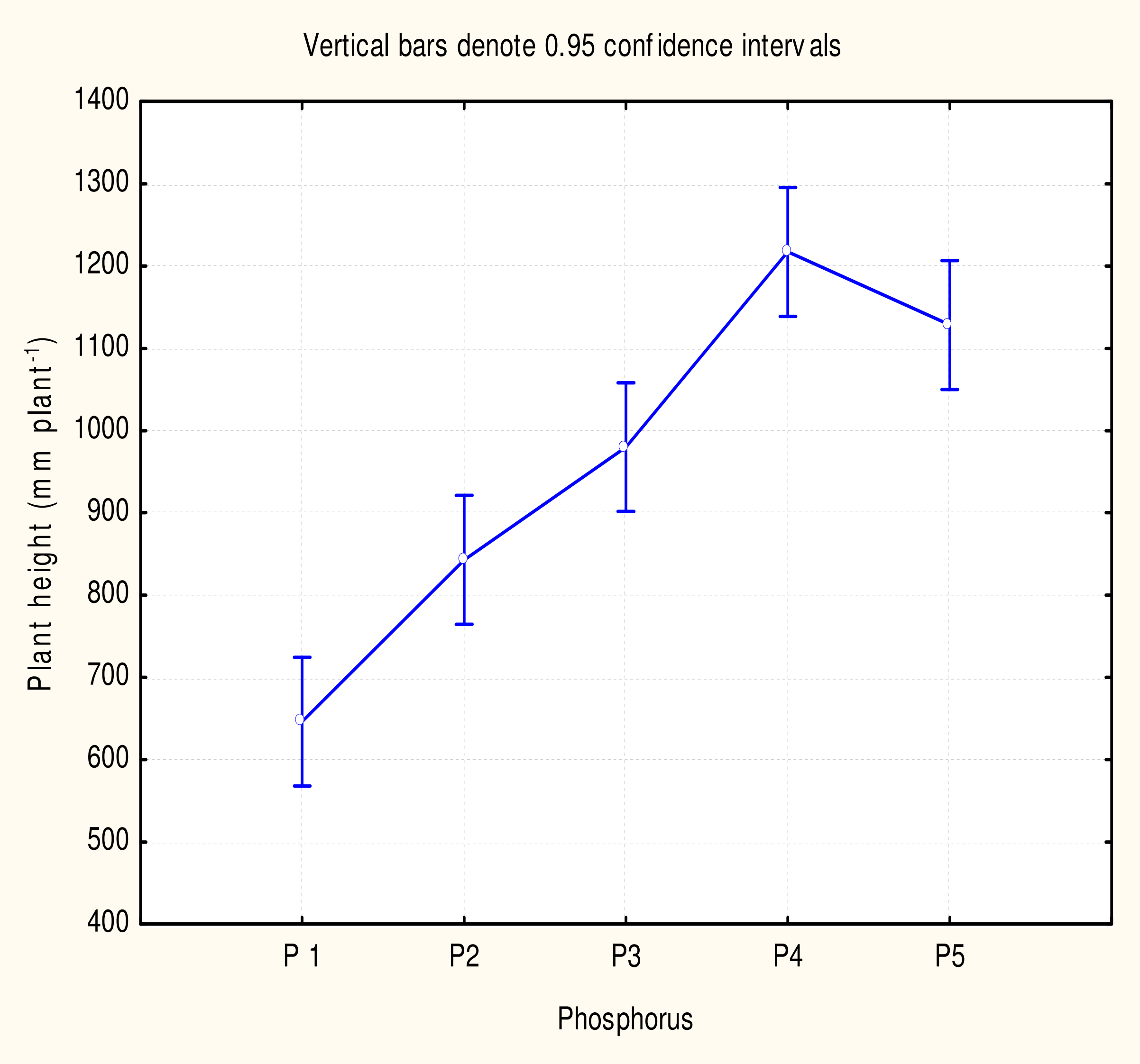

Figure 4.52 Effect of different P levels on height of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The results (Figure 4.53) showed an increase in root fresh mass with increasing P application levels from 7.669 g plant^{-1} at P_1 (0 kg P ha^{-1}) to 43.719 g plant^{-1} at P_5 (40 kg P ha^{-1}) without reaching a maximum. The root fresh mass at P_5 was statistically different from the root fresh mass produced with all the other P application levels.

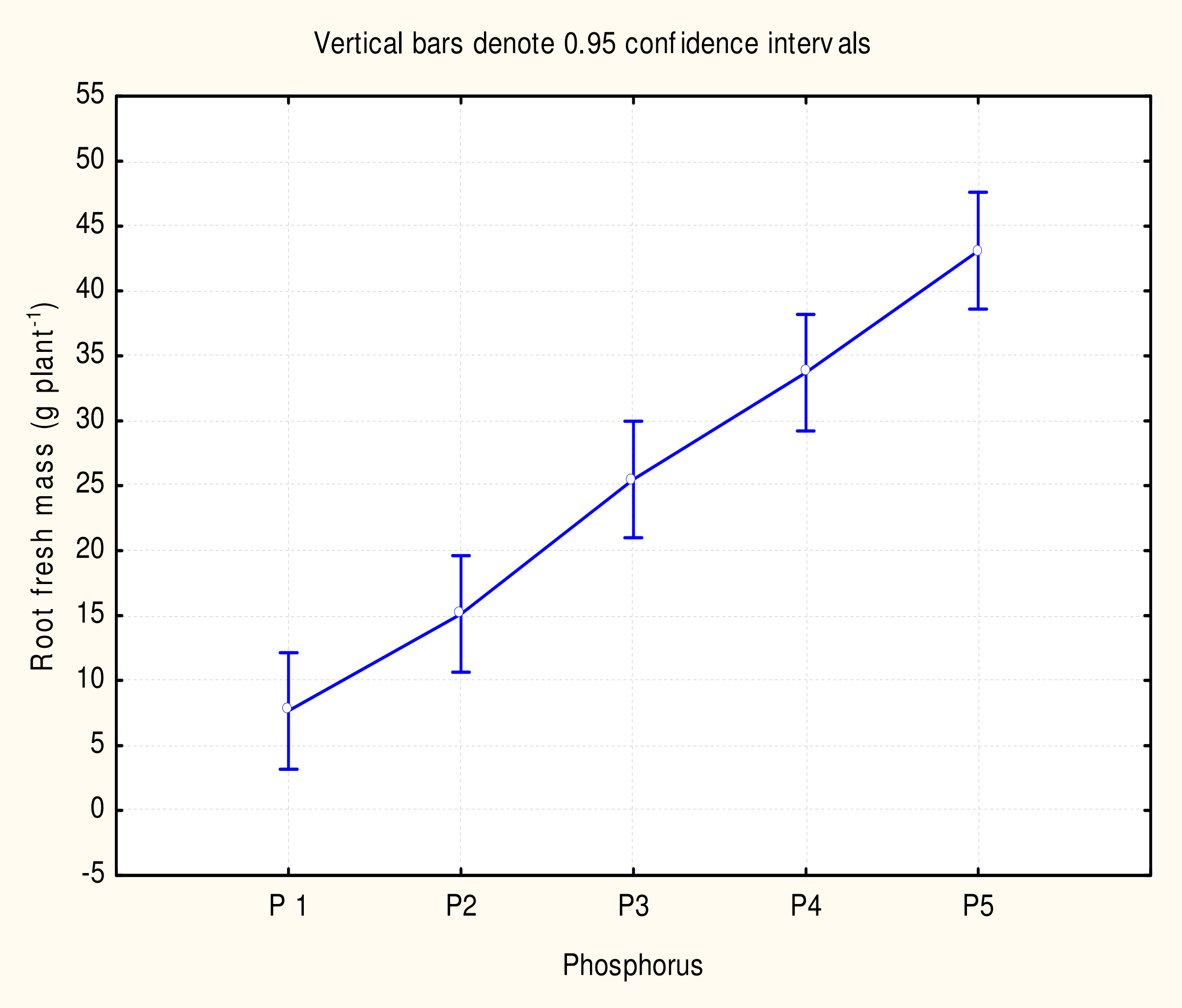

Figure 4.53 Effect of different P levels on root fresh mass of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The data on root dry mass (Figure 4.54) showed trends similar to root fresh mass (Figure 4.53). Root dry mass also increased with increasing P application levels from 1.351 g plant^{-1} with P_1, but reached a value of 8.150 g plant^{-1} at a P application level of 40 kg P ha^{-1}. This root dry mass was statistically different from root dry mass of all the other P application levels. The increase in root mass could be attributed to the stimulation of root development due to P fertilization (Griffith, 2004) and showed that for most parameters tested, a higher P application level (>40 kg P ha^{-1}) will be needed to obtain maximum production with maize in a sandy soil with low P content (<20 mg $P_{citric\ acid}$ kg^{-1} soil).

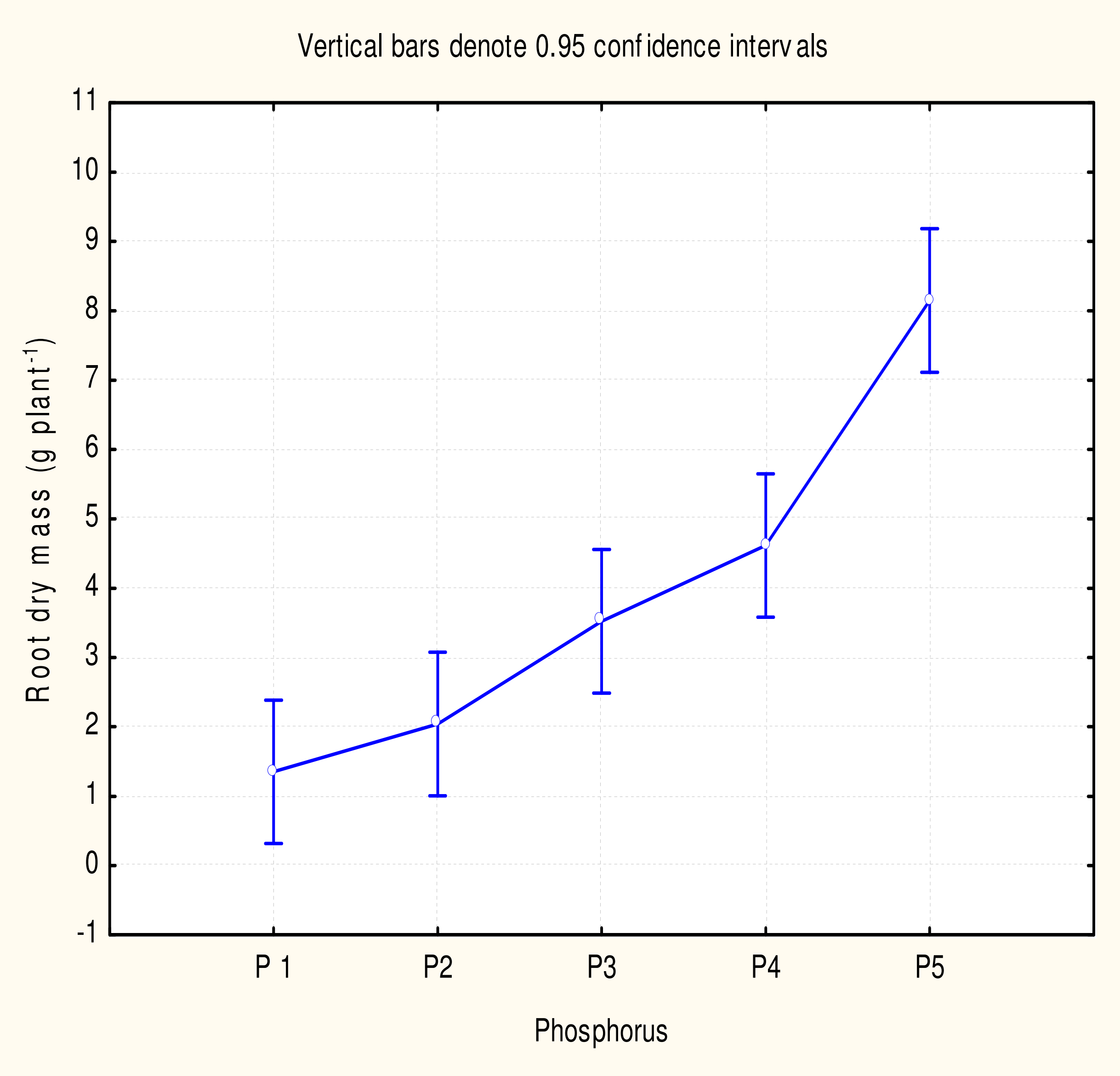

Figure 4.54 Effect of different P levels on root dry mass of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The highest moisture content of 86.74% was measured in plants receiving 20 kg P ha^{-1} (Figure 4.55). This value was however not significantly different from the moisture content of either the P_2 plants (86.36%) or the P_4 plants (86.41%). Plants which received 40 kg P ha^{-1} (P_5) had the lowest moisture content of 81.21%.

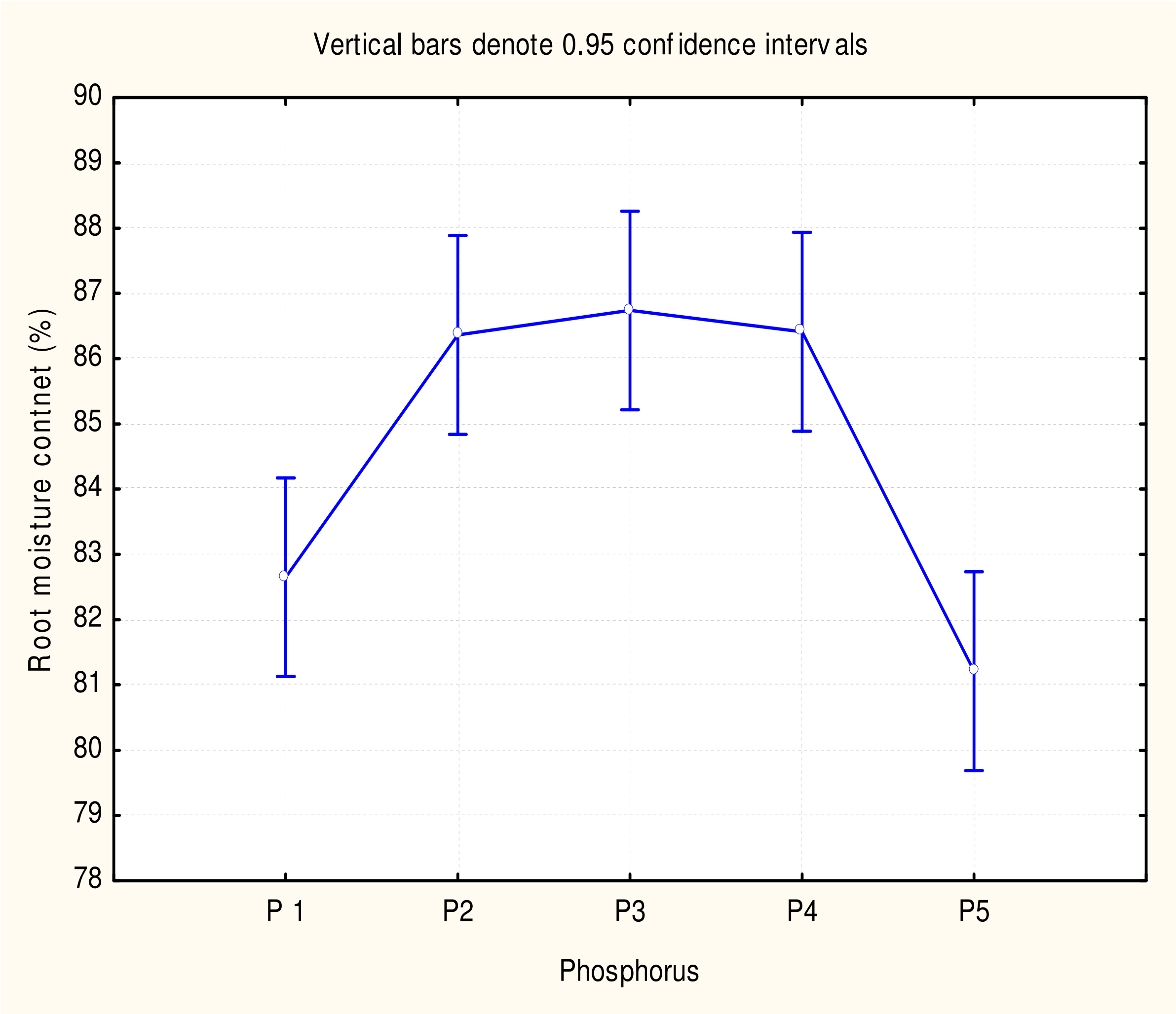

Figure 4.55 Effect of different P levels on root moisture content of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

The results (Table 4.16 and Figure 4.56) showed that mycorrhizal seed inoculation resulted in a significant increase in leaf area (from 633.77 cm^2 plant^{-1} to 779.95 cm^2 plant^{-1}), plant height (from 878.33 mm to 1047.50 mm), root fresh mass (from 21.80 g plant^{-1} to 28.25 g plant^{-1}), root dry mass (from 3.27 g plant^{-1} to 4.61 g plant^{-1}), plant fresh mass (41.84 g to 60.92 g) and plant dry mass (5.36 g to 7.89 g), but decreased root moisture content from 85.73% to 83.62%. A significant interaction between P and mycorrhizal treatments with regard to plant fresh mass and plant dry mass however indicated that the response to seed inoculation with AM fungi was affected by P application levels.

Table 4.16 Effect of mycorrhizal inoculation on growth of maize plants at 60 days after planting expressed as mean values per plant

	Measured parameters								
Treatments	Leaf area (cm^2)	Stem diameter (mm)	Plant height (mm)	Root fresh mass (g)	Root dry mass (g)	Root moisture (%)	Plant fresh mass (g)	Plant dry mass (g)	Plant moisture (%)
M_0	633.77b	12.05a	878.33b	21.80b	3.27b	85.73a	41.84b	5.36b	86.96a
M_1	779.95a	12.65a	1047.50a	28.25a	4.61a	83.62b	60.92a	7.89a	86.78a

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

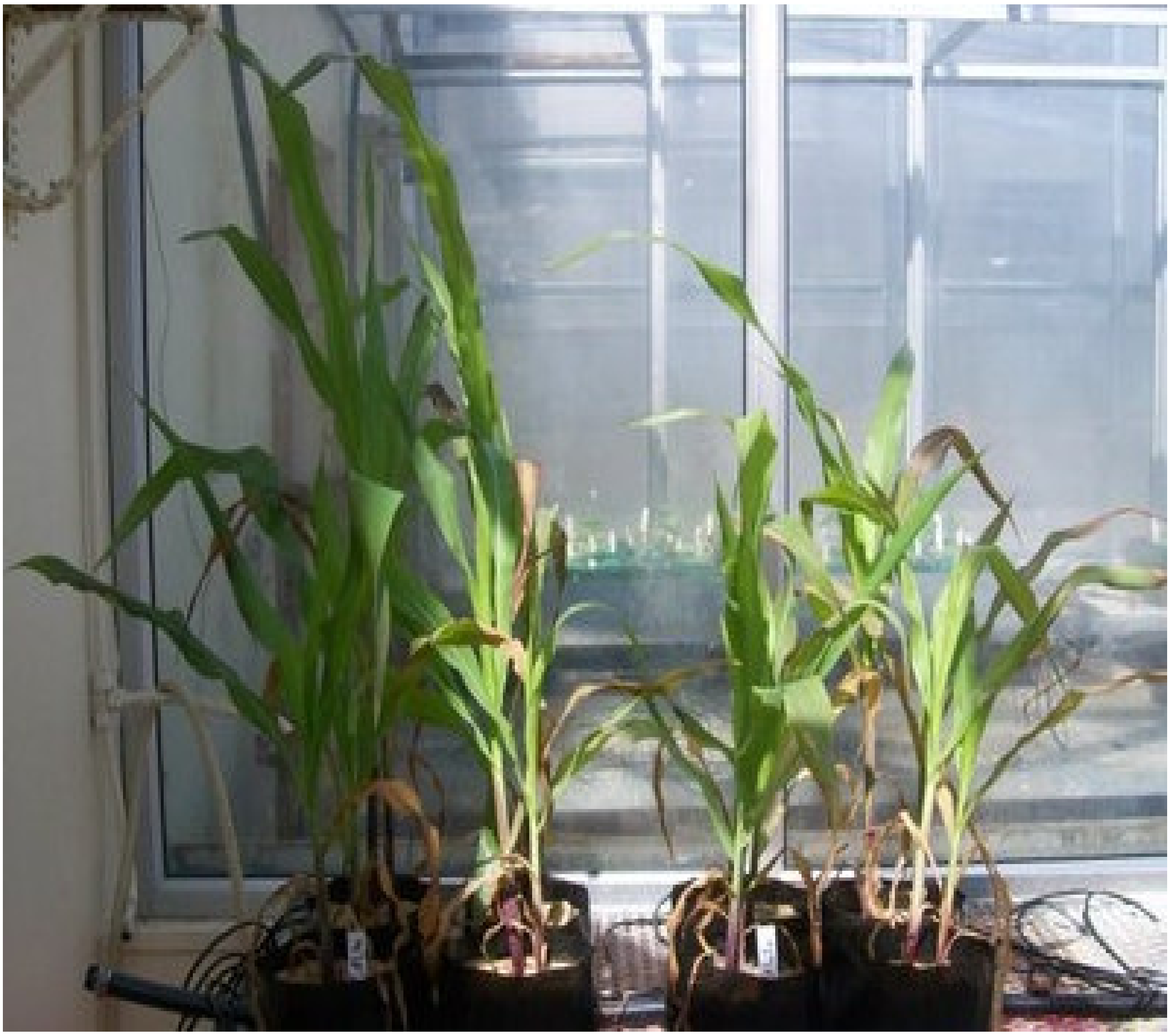

Figure 4.56 Mycorrhizal inoculated (M_1; left) and non-inoculated (M_0; right) maize plants at 60 days after planting at the P_1 (0 kg P ha^{-1}) level

Interaction between mycorrhizal inoculation and P application level

The fresh mass and dry mass per plant showed a significant interaction between P fertilization levels and mycorrhizal seed inoculation (Figures 57-59). In general both fresh and dry mass per plant showed an increase with an increase in P application level and tend to reach their maximum values at P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}) treatments, but responses to P treatment tend to be affected by mycorrhizal inoculation at these levels of P (30 kg P ha^{-1} and 40 kg P ha^{-1}). At these P application levels, mycorrhizal inoculated plants produced significantly higher fresh and dry mass plant^{-1} compared to non-mycorrhizal plants.

In this experiment seed inoculation with AM fungi did not have any beneficial effect on the fresh and dry mass of maize plants at P application levels of 0 (P_1), 10 (P_2) and 20 kg P ha^{-1} (P_3). Although somewhat unsuspected, this absence of a mycorrhizal effect at low P application levels in a P deficient soil, may suggest that at these low levels the P remain deficient even with the help of the mycorrhiza. This conclusion is supported by the results of fresh and dry mass which showed that the optimum P application level for maize in the sandy soil with P content of 18 mg kg^{-1} (citric acid) was not reached at the highest application levels of 40 kg P ha^{-1} due to the fact that maize has a high requirement for P.

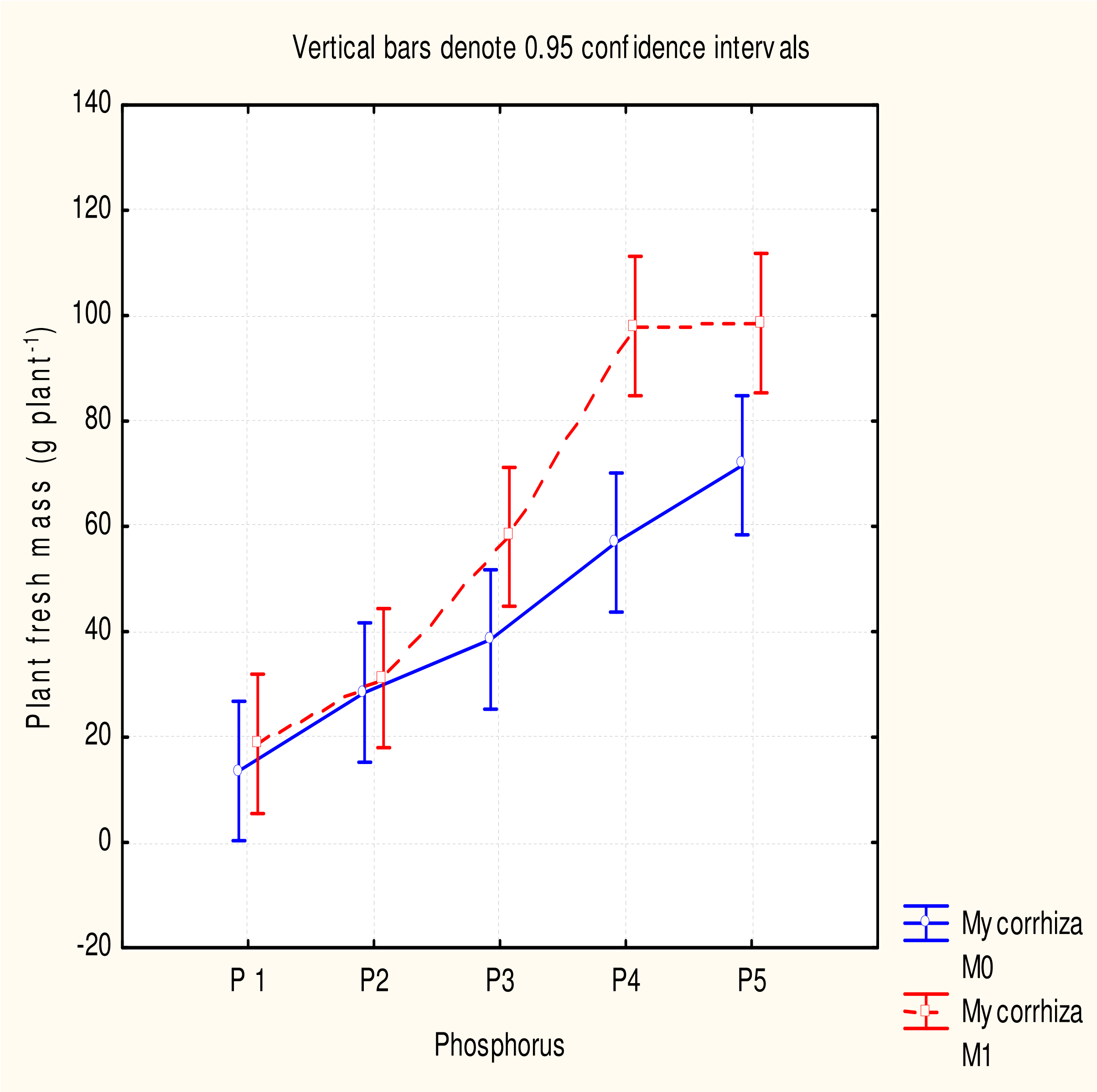

Figure 4.57 Effect of different P levels on the response of fresh mass of maize plants at 60 days after planting to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

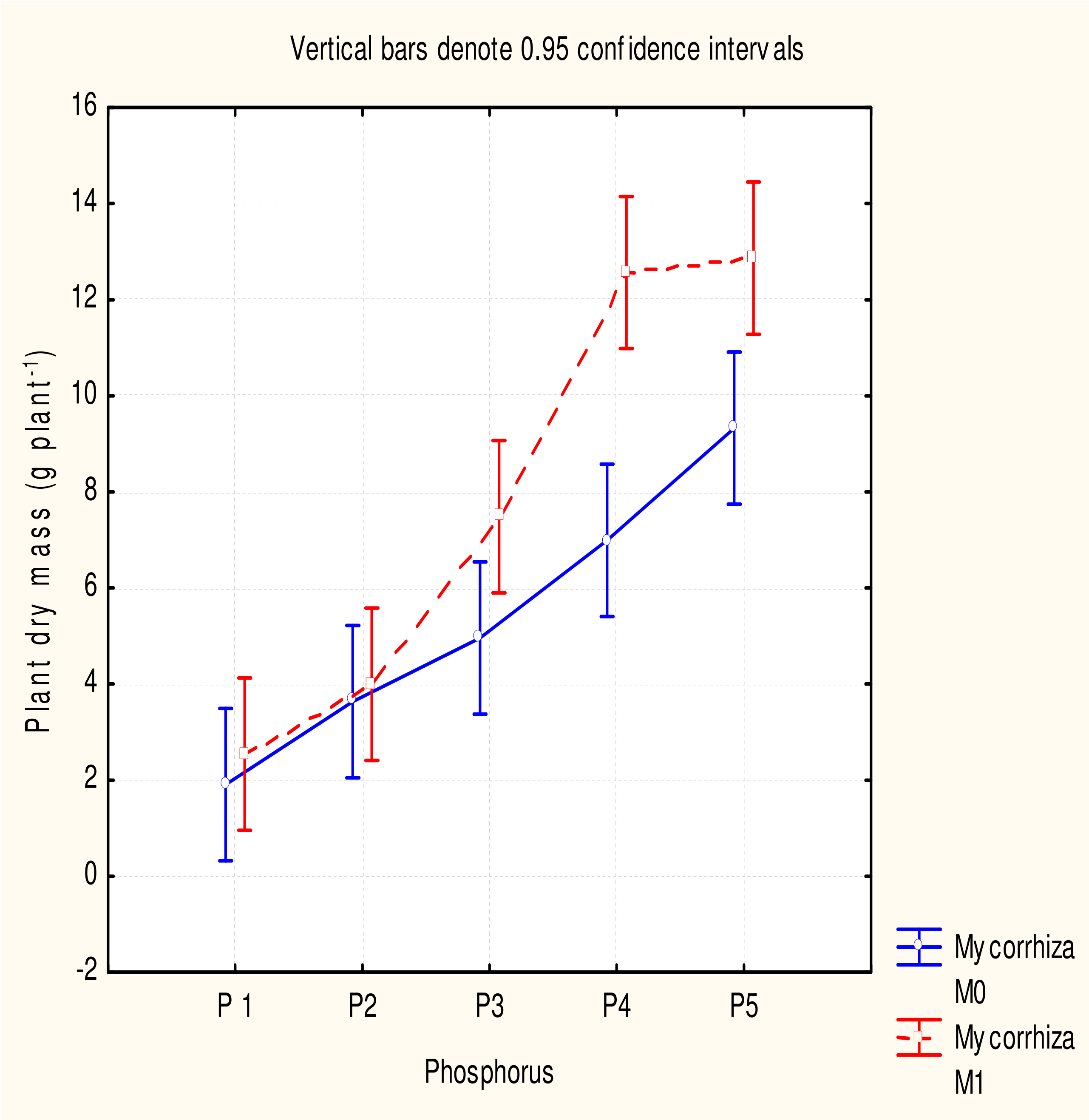

Figure 4.58 Effect of different P levels on the response of dry mass of maize plants at 60 days after planting to mycorrhizal seed inoculation.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Figure 4.59 Mycorrhizal inoculated (M_1; left) and non-inoculated (M_0; right) maize plants at 60 days after planting at the P_5 (40 kg P ha^{-1}) levels

Mineral Concentration of maize stover

The effect of P application levels, seed inoculation with AM fungi and their interaction on nutrient content of maize was determined at 60 days after planting. Results from analysis of variance are summarized in Table 4.17.

Phosphorus application levels had a significant effect on NH_4-N, K, Mg, Fe, Cu, Zn, Mn and B content. Mycorrhizal seed inoculation had a significant effect on NH_4-N, P, Fe, Cu, as well as on OC content. A significant interaction between P application levels and mycorrhizal seed inoculation with regard to OC content was found at 60 days after planting.

Only significant (P≤0.05) results will be discussed as follows.

Table 4.17 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on nutrient content of maize plants at 60 days after planting

Treatments	NH4 N (%)	P (%)	K (%)	Mg (%)	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	S (%)	OC (%)
P	*	NS	*	*	NS	*	*	*	*	*	NS	NS	NS
M	*	*	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*
M*P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

- NS: not significant at P≤0.05
- *: significant at P≤0.05

Effect of phosphorus

Increasing P application levels had a significant effect on NH_4-N, K, Mg, Fe, Cu, Zn, Mn, and B content (Table 4.18). All the measured elements tend to decrease with an increase in P application level. Increasing the P fertilizer levels from 0 to 40 kg P ha^{-1} resulted in a decrease in NH_4-N content from 2.62 to 2.07%, K from 3.05 to 2.34%, Mg from 0.15 to 0.13%, Fe from 54.53 to 38.51 mg kg^{-1}, Cu from 3.60 to 2.45 mg kg^{-1}, Zn from 36.82 to 23.36 mg kg^{-1}, Mn from 29.57 to 19.30 mg kg^{-1}, and B from 12.19 to 9.81 mg kg^{-1} respectively.

The results of this study indicate that P application may interfere with the availability and uptake by maize of these nutrient elements. The results are supported by Mulders' Chart of plant nutrient interactions which shows that higher P application reduces availability of Fe, K, Cu and Zn (Anonymous, 2009). Hussaini *et al.* (2008) reported a significant decline in Mg concentrations of maize with P applications up to 20 and 40 kg P ha^{-1} in a field experiment conducted in a dry season at Kadwa, Nigeria. Alloush & Clark (2001) observed that concentrations of K, Mg, Fe and Mn were greater in shoots of control plants and decreased slightly with application of phosphate rock in an acid soil.

Table 4.18 Effect of different P levels on nutrient content of maize plants at 60 days after planting

Treatments	NH4 N (%)	K (%)	Mg (%)	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})
P_1	2.62a	3.05a	0.15a	54.53a	3.60a	36.82a	29.57a	12.19a
P_2	2.41ab	2.73ab	0.14ab	46.71abc	3.12ab	32.31ab	23.95b	11.42ab
P_3	2.20bc	2.57ab	0.13b	43.98bc	3.14ab	30.70abc	22.86bc	10.61ab
P_4	2.13bc	2.56ab	0.13b	47.53ab	2.66bc	26.44bc	21.05bc	10.35ab
P_5	2.07c	2.34b	0.13b	38.51c	2.45c	23.36c	19.30c	9.81b

* Means with different letters within the same column are significantly different (P≤0.05).

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

Mycorrhizal seed inoculation resulted in a significant decrease in the content of several elements in maize plants at 60 DAP. Nitrogen (NH_4-N) content decreased from 2.41 to 2.17%, P decreased from 0.06 to 0.05%, Fe decreased from 48.30 to 44.21 mg kg^{-1}, Cu decreased from 3.14 to 2.85 mg kg^{-1} and OC decreased from 52.63 to 51.64% (Table 4.19). Kothari *et al.* (1991) reported an enhanced Cu concentration in roots (135%) but not in shoots of maize grown in a calcareous soil by mycorrhizal inoculation. Alloush & Clark (2001) also reported greater decrease in Fe concentrations when AM fungi were present in an acid soil.

Table 4.19 Effect of mycorrhizal inoculation on nutrient content of maize plants at 60 days after planting expressed as mean values per plant

	Measured elements				
Treatments	N (%)	P (%)	Fe (mg kg^{-1})	Cu (mg kg^{-1})	OC (%)
M_0	2.41a	0.06a	48.30a	3.14a	52.63a
M_1	2.17b	0.05b	44.21b	2.85b	51.64b

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application levels

The application of P fertilizer decreased OC content of maize in non-mycorrhizal plants, but not in the mycorrhizal inoculated plants (Figure 4.60). This resulted in a significantly higher (54.8%) OC content in non-mycorrhizal plants compared to mycorrhizal plants (50.7%) where no P was applied.

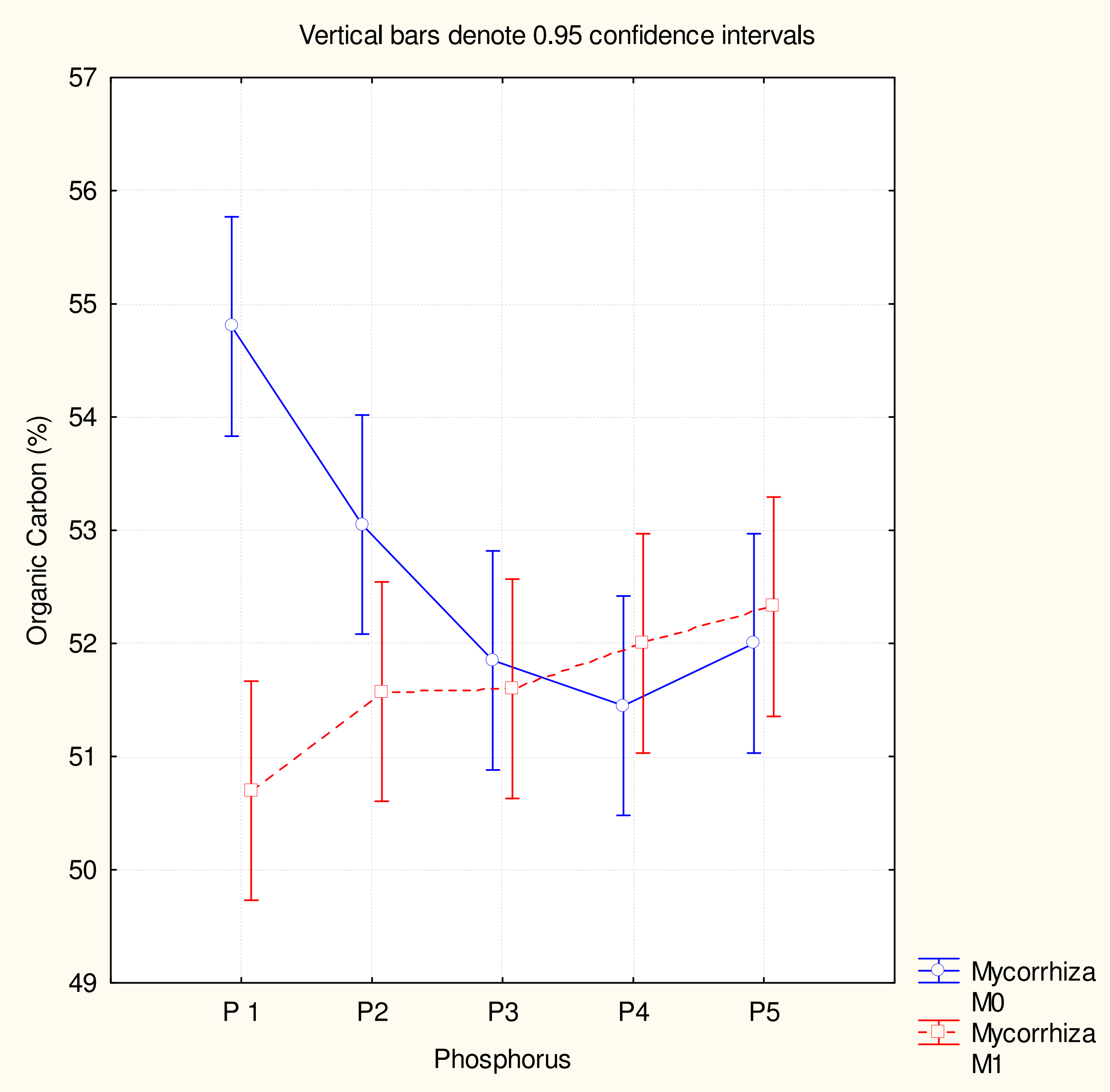

Figure 4.60 Effect of mycorrhizal seed inoculation and different P levels on organic carbon content of maize plants at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Nutrient uptake by maize plant

The effect of P application levels, seed inoculation with AM fungi and their interaction on nutrient uptake of maize was determined at 60 days after planting. Results from analysis of variance are summarized in Table 4.20.

Phosphorus application levels had a significant effect on all the nutrient elements. Mycorrhizal seed inoculation had a significant effect on all the nutrient elements except for aluminium (Al). A significant interaction between P application level and mycorrhizal seed inoculation with regard to NH_4-N, P, Zn, Mn, S and OC uptake was found at 60 days after planting.

Only significant (P≤0.05) results will be discussed as follows.

Table 4.20 Analyses of variance showing the effect of different P levels and mycorrhizal seed inoculation on nutrient uptake of maize plants at 60 days after planting

Treatments	NH4 N (g)	P (g)	K (g)	Mg (g)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Mn (mg)	B (mg)	Al (mg)	S (g)	OC (g)
P	*	*	*	*	*	*	*	*	*	*	*	*	*
M	*	*	*	*	*	*	*	*	*	*	NS	*	*
M*P	*	*	NS	NS	NS	NS	NS	*	*	NS	NS	*	*

- NS: not significant at P≤0.05
- *: significant at P≤0.05

Effect of phosphorus

Increasing P application levels had a significant effect on of NH_4-N, P, K, Mg, Na, Fe, Cu, Zn, Mn, B, Al, S and OC uptake of maize plants at 60 DAP (Table 4.21). All the measured elements tend to increase with an increase in P application levels, but tended to reach their maximum or at least level off at P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}). The application of P fertilizer increased NH_4-N from 0.06 to 0.22 g $plant^{-1}$, P from 0.001 to 0.006 g $plant^{-1}$, K from 0.07 to 0.26 g $plant^{-1}$, Mg from 0.0003 to 0.0013 g $plant^{-1}$, Na from 0.12 to 0.42 mg $plant^{-1}$, Fe from 0.12 to 0.41 mg $plant^{-1}$, Cu from 0.008 to 0.026 mg $plant^{-1}$, Zn from 0.08 to 0.25 mg $plant^{-1}$, Mn from 0.07 to 0.21 mg $plant^{-1}$, B from 0.03 to 0.11 mg $plant^{-1}$, Al from 0.003 to

0.10 mg plant^{-1}, S from 0.003 to 0.013 g plant^{-1} and OC from 1.16 to 5.80 g plant^{-1}where no P was applied (P_1) compared to a P rate of 40 kg P ha^{-1} (P_5) respectively.

The results of this study indicate that P application increased the uptake of these nutrient elements by maize plants. Alloush & Clark (2001) reported that application of phosphate rock increased the acquisition of P, K, Mg, Na, Cu, Zn, Mn, S and B in an acid soil. Hussaini *et al.* (2008) reported a total maize plant N uptake increase by 23.3% with P application of 40 kg P ha^{-1} in a field experiment conducted in a dry season.

Table 4.21 Effect of different P levels on nutrient uptake per plant of maize plants at 60 days after planting

Treatments	NH4 N (g)	P (g)	K (g)	Mg (g)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Mn (mg)	B (mg)	Al (mg)	S (g)	OC (g)
P_1	0.06c	0.001c	0.07c	0.0003c	0.12b	0.12c	0.008c	0.08c	0.07c	0.03c	0.03b	0.003c	1.16c
P_2	0.09c	0.002bc	0.10bc	0.0005bc	0.17b	0.17c	0.012c	0.12c	0.09c	0.04bc	0.04b	0.005bc	1.99bc
P_3	0.14b	0.003b	0.16b	0.0008b	0.33a	0.27b	0.019b	0.19b	0.14b	0.07b	0.07ba	0.007b	3.22b
P_4	0.20a	0.005a	0.25a	0.0012a	0.41a	0.45a	0.024a	0.26a	0.21a	0.10a	0.11a	0.011a	5.07a
P_5	0.22a	0.006a	0.26a	0.0014a	0.42a	0.41a	0.026a	0.25a	0.21a	0.11a	0.10ba	0.013a	5.80a

* Means with different letters within the same column are significantly different ($P \leq 0.05$).

[P1 = 0 kg P ha^{-1}; P2 = 10 kg P ha^{-1}; P3 = 20 kg P ha^{-1}; P4 = 30 kg P ha^{-1}; P5 = 40 kg P ha^{-1}]

Effect of mycorrhizal inoculation

In this pot trial with sandy P deficient soil, mycorrhizal seed inoculation resulted in a significant increase in the uptake of NH_4-N from 0.12 to 0.16 g plant^{-1}, P from 0.03 to 0.04 g plant^{-1} , K from 0.14 to 0.20 g plant^{-1}, Mg from 0.0007 to 0.0010 g plant^{-1}, Na from 0.25 to 0.33 mg plant^{-1}, Fe from 0.25 to 0.32 mg plant^{-1}, Cu from 0.016 to 0.020 mg plant^{-1}, Zn from 0.14 to 0.22 mg plant^{-1}, Mn from 0.12 to 0.17 mg plant^{-1}, B from 0.06 to 0.08 mg plant^{-1}, S from 0.006 to 0.009 g plant^{-1} and OC from 2.80 to 4.10 g plant^{-1} by maize plants (Table 4.22). Khaliq & Sanders (1997) also reported higher P contents in mycorrhizal inoculated maize plants grown in pots placed under field environment at 57 days after planting. Subramanian

& Charest (1997) reported significantly higher contents of N, Mn and Cu of drought-stressed mycorrhizal plants than non-mycorrhizal plants in a greenhouse experiment.

Table 4.22 Effect of mycorrhizal inoculation on nutrient uptake per plant of maize plants at 60 days after planting

	Measured elements											
Treatments	NH4 N (g)	P (g)	K (g)	Mg (g)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Mn (mg)	B (mg)	S (g)	OC (g)
M_0	0.12b	0.03b	0.14b	0.0007b	0.25b	0.25b	0.016b	0.14b	0.12b	0.06b	0.006b	2.80b
M_1	0.16a	0.04a	0.20a	0.0010a	0.33a	0.32a	0.020a	0.22a	0.17a	0.08a	0.009a	4.10a

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1 = seed inoculated with AM fungi

Interaction between mycorrhizal inoculation and P application levels

The NH_4-N, P, Zn, Mn, S and OC uptake showed a significant interaction between P fertilization and mycorrhizal seed inoculation (Figures 4.59-4.64). In general all measured elements showed an increase with an increase in P application level and tend to reach their maximum at P_4 and P_5 treatments, but responses to P treatment tend to be affected by mycorrhizal inoculation at high levels of P (30 kg P ha^{-1} and 40 kg P ha^{-1}).

Increase in P levels increased the N uptake in both non-mycorrhizal and mycorrhizal inoculated plants (Figure 4.61). However, at P_4 (30 kg P ha^{-1}) the mycorrhizal inoculated plants contained 0.24 g of N which was significantly higher than the 0.16 g plant^{-1} of the non-mycorrhizal plants.

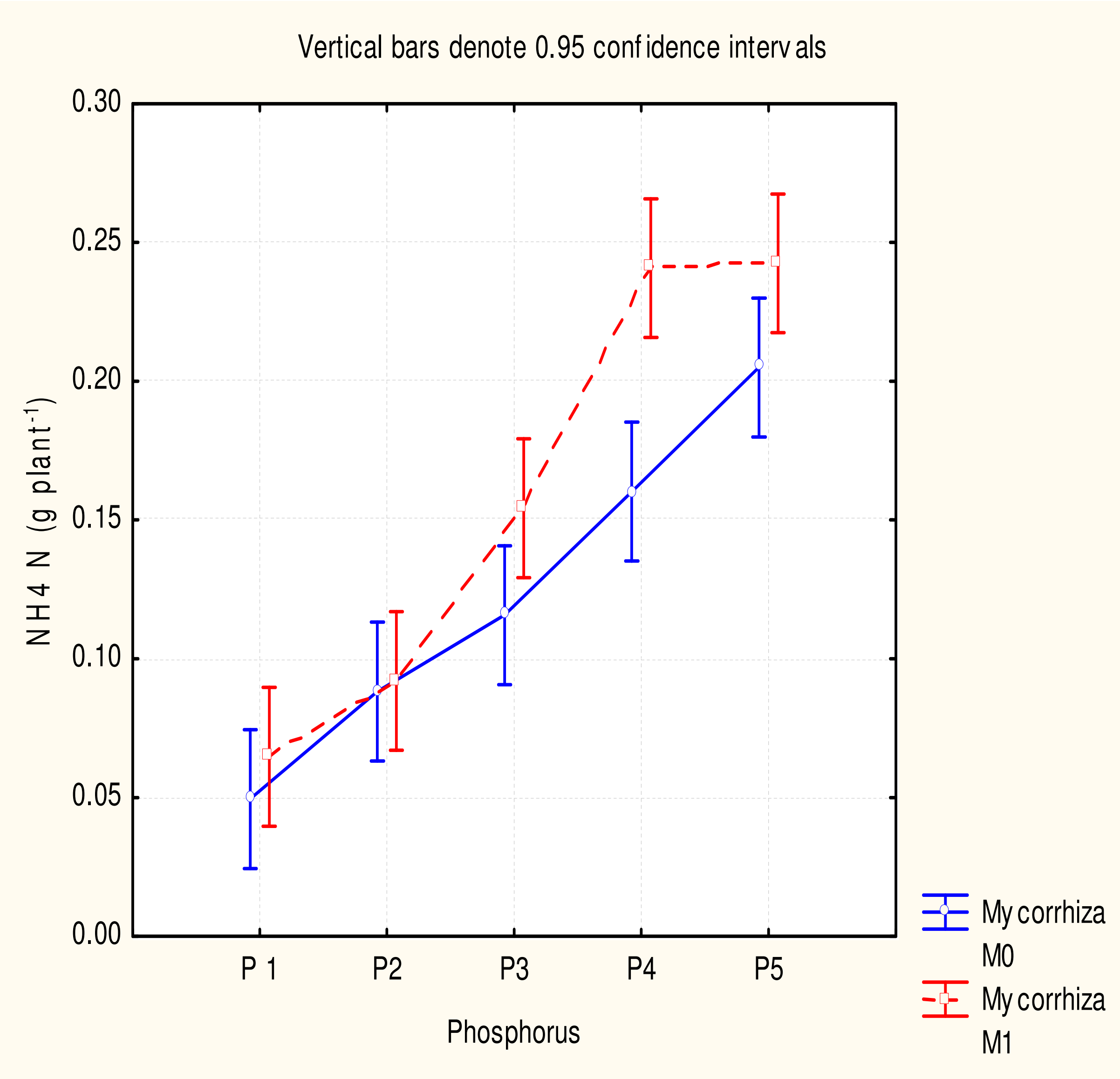

Figure 4.61 Effect of mycorrhizal seed inoculation on the N content of maize plants in response to different P application levels at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Increase in P levels increased P, Mn and S content (Figure 4.62-4.64) in non-inoculated and mycorrhizal inoculated plants. As also found with N content, significant differences were observed at P_4 (30 kg P ha^{-1}) with mycorrhizal inoculated plants containing significantly higher nutrient contents compared to non-mycorrhizal plants.

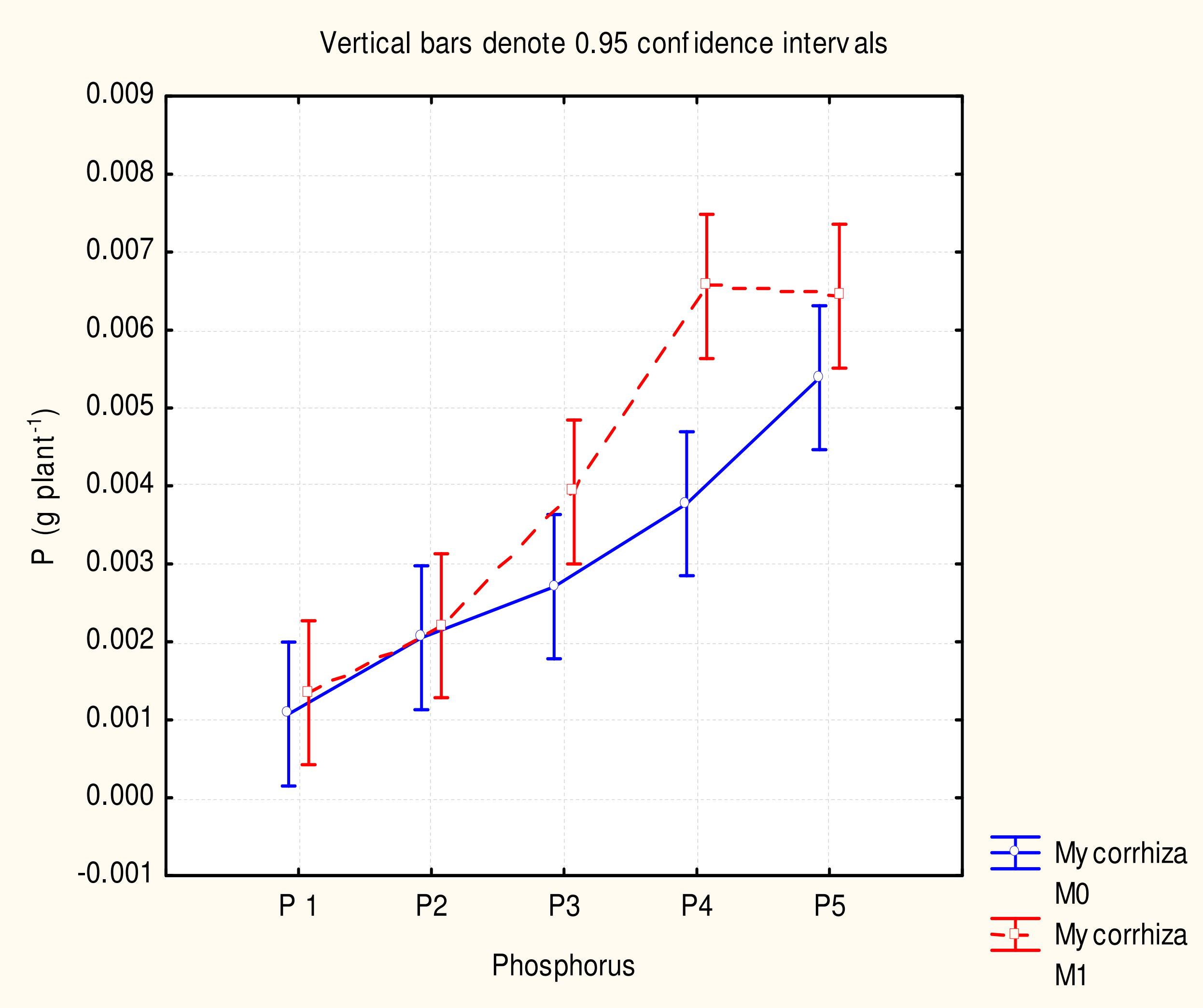

Figure 4.62 Effect of mycorrhizal seed inoculation on the P content of maize plants in response to different P application levels at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

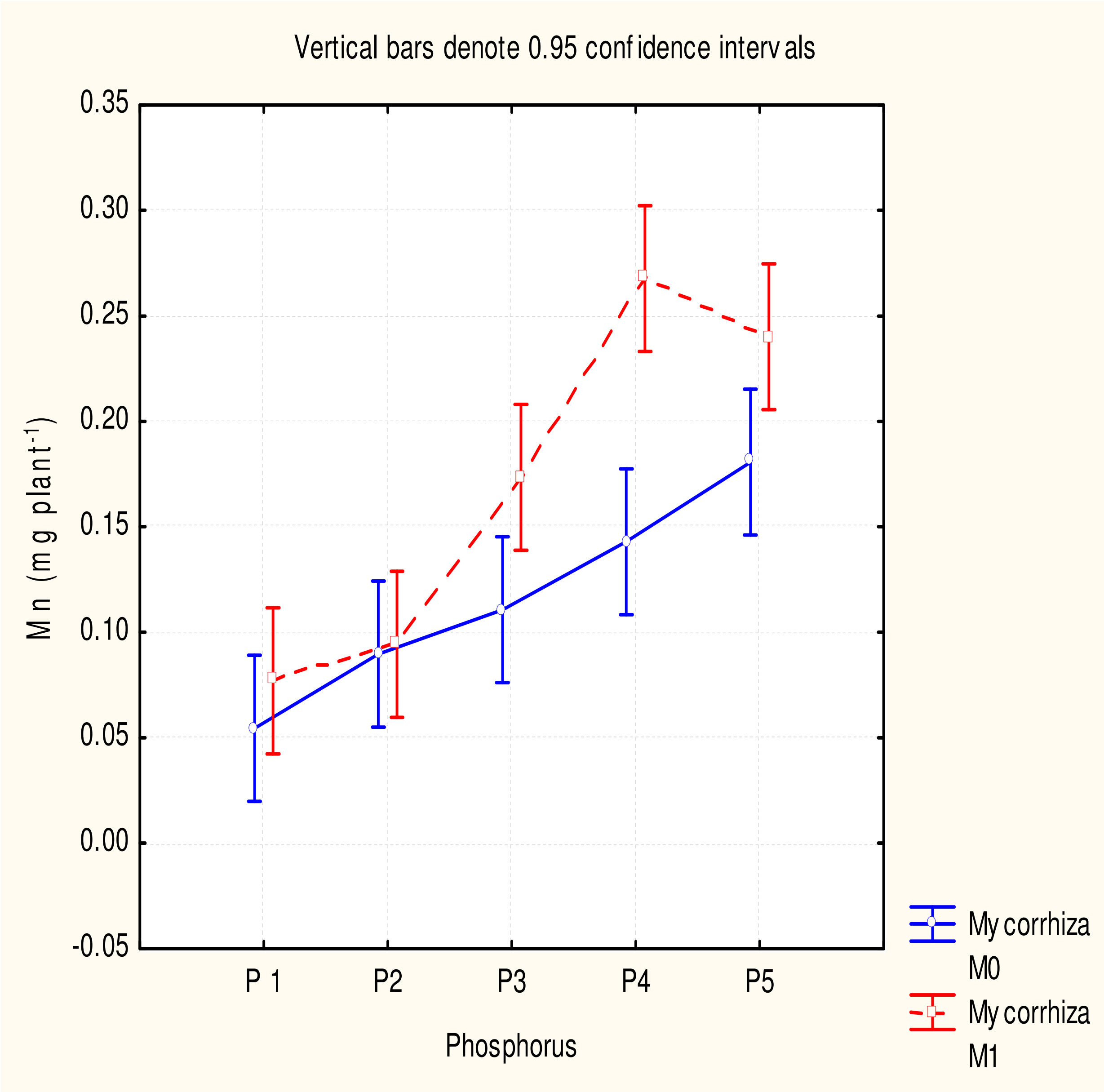

Figure 4.63 Effect of mycorrhizal seed inoculation on the Mn content of maize plants in response to different P application levels at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

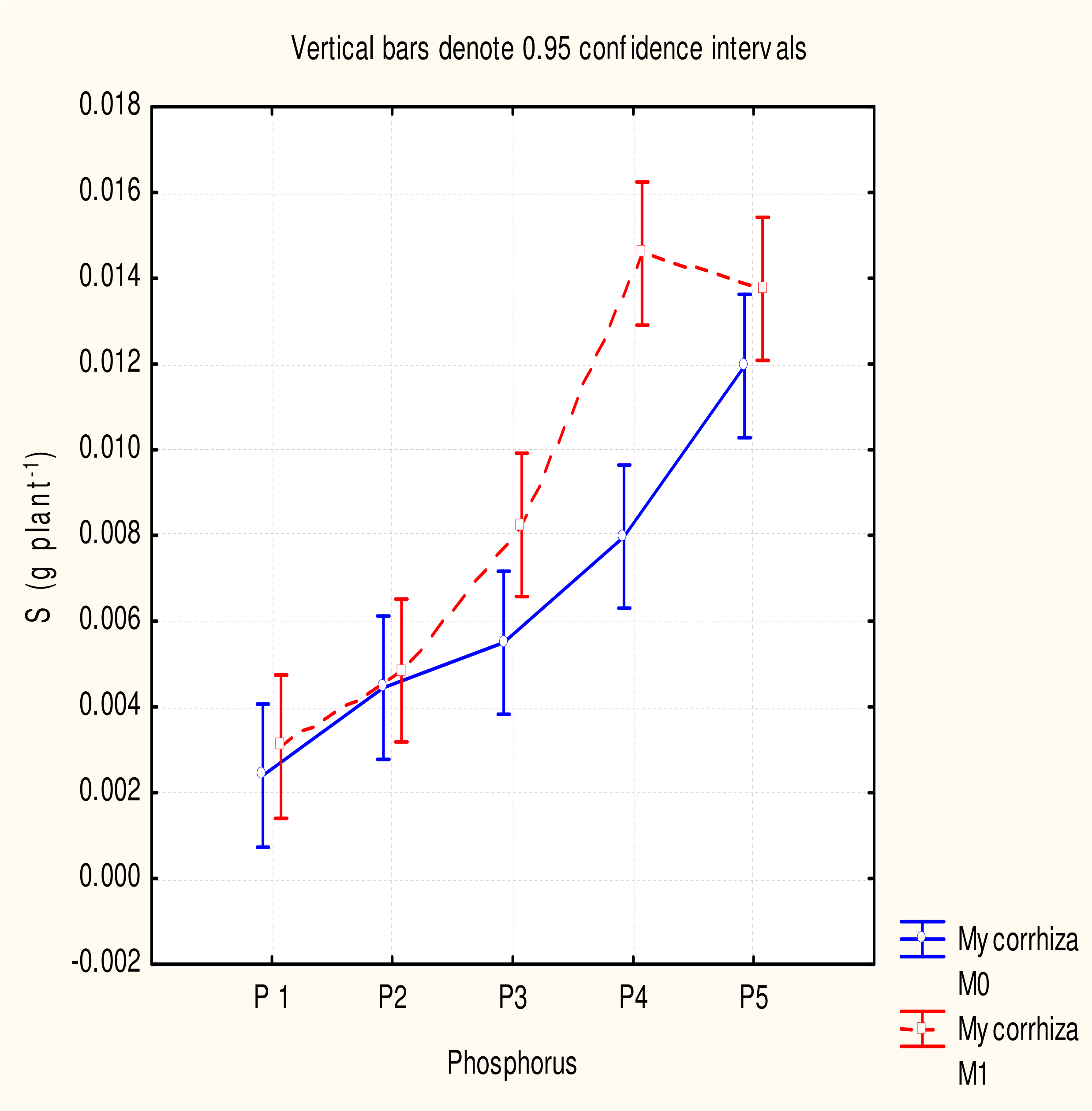

Figure 4.64 Effect of mycorrhizal seed inoculation on the S content of maize plants in response to different P application rates at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

In general the Zn and OC content also showed an increase with an increase in P application levels in both inoculated and non-inoculated plants, but in contrast to the other elements mycorrhizal inoculation had a significant effect at both the P_4 (30 kg P ha^{-1}) and P_5 (40 kg P ha^{-1}) rates. At these P application levels, mycorrhizal inoculated plants contained significantly higher Zn and OC content compared to non-mycorrhizal plants.

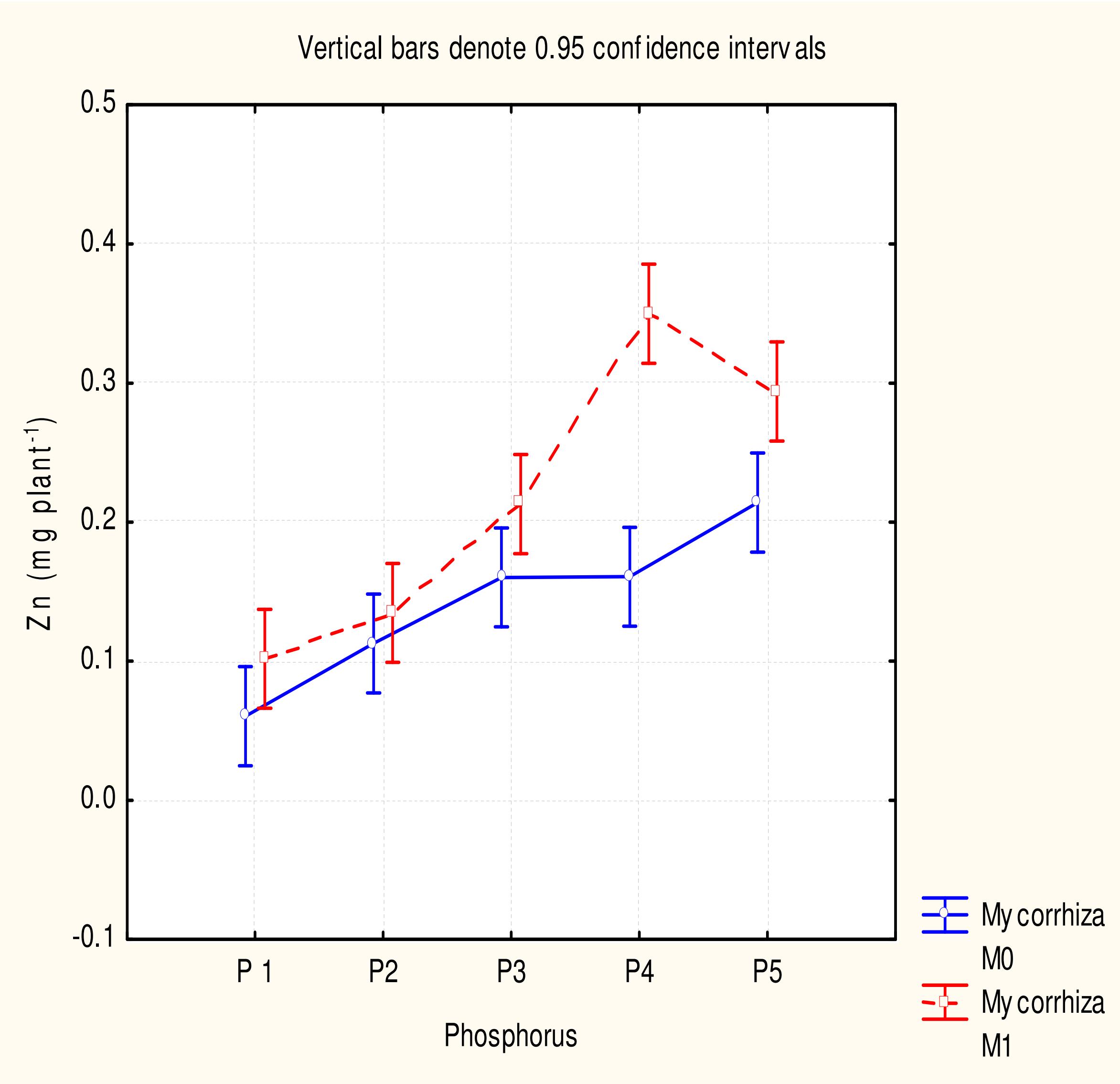

Figure 4.65 Effect of mycorrhizal seed inoculation on the Zn content of maize plants to P application at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

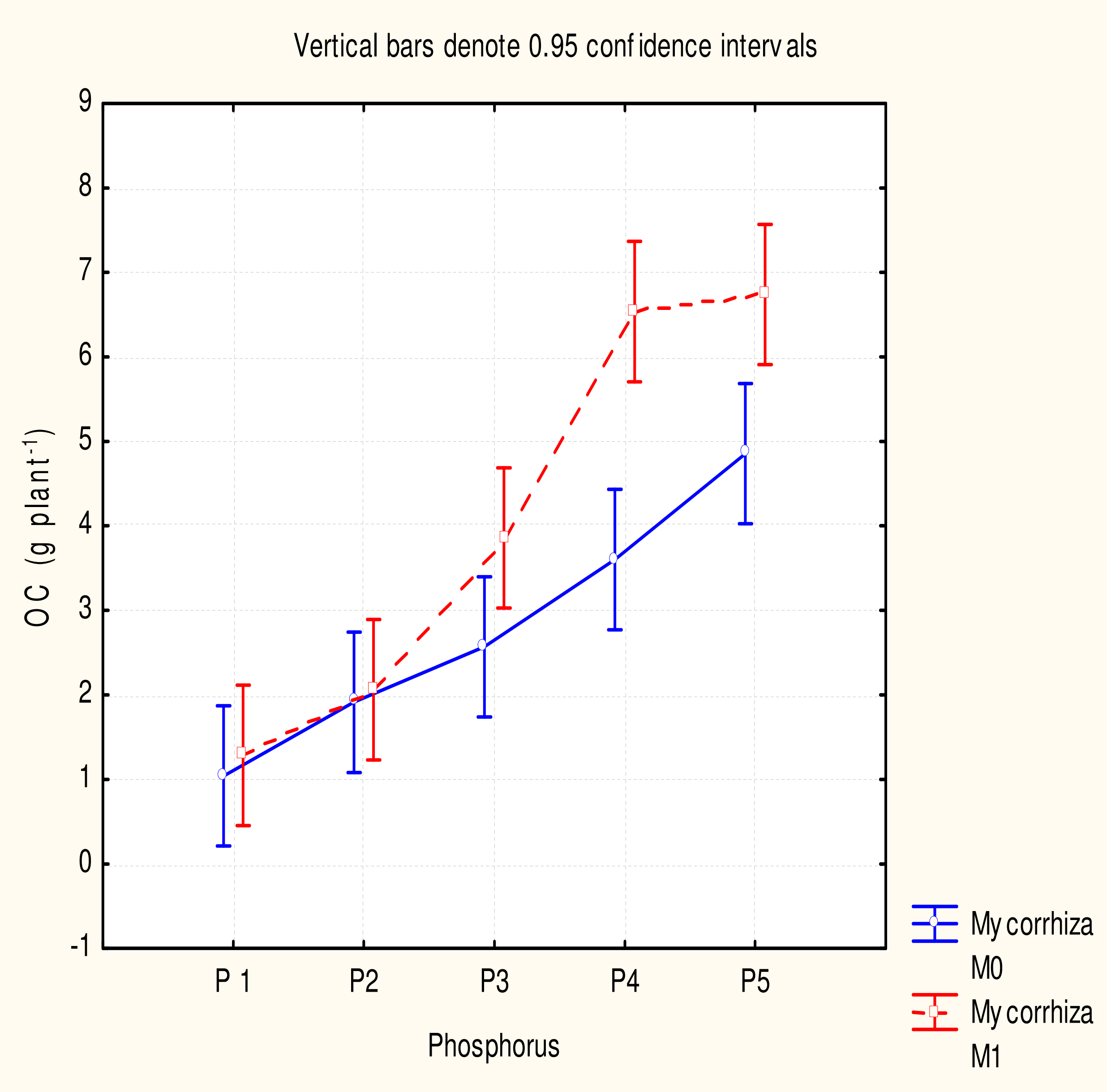

Figure 4.66 Effect of mycorrhizal seed inoculation on OC content of maize plants in response to different P application rates at 60 days after planting.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

[M_0 = uninoculated seed; M_1 = seed inoculated with AM fungi]

Mycorrhiza usually increase the growth of plants by enhancing nutrient uptake especially P. In this experiment seed inoculation with AM fungi did not have any beneficial effect on the nutrient uptake of maize plants at P application levels of 0 (P_1), 10 (P_2) and 20 kg P ha^{-1} (P_3). Although somewhat unsuspected, this absence of a mycorrhizal effect at low P application levels in a P deficient soil, may suggest that at these low levels the P remain deficient even with the help of the mycorrhiza. The results of this experiment showed that nutrient contents were higher in mycorrhizal plants than in non-mycorrhizal plants. The total amount of nutrients in maize above ground parts of mycorrhizal plants may be greater than that of non-mycorrhizal plants due to increased above ground growth. Thus, increased amounts of nutrients in above ground parts of mycorrhizal plants can often be explained by higher nutrient demand due to enhanced plant P uptake.

These results are supported by the result of P concentration which showed that the P concentration in plants above ground material never reached the critical levels (0.25%; FSSA, 2007). This conclusion is also supported by the results of fresh and dry mass which showed that the optimum P application level for maize in the sandy soil with P content of 18 mg kg^{-1} (citric acid) was not reached at the highest application levels of 40 kg P ha^{-1} due to the fact that maize has a high requirement for P. Gerdemann (1964) reported that in a pot experiment with a 3 to 1 steam sterilized soil-sand mixture, because of much greater weight of mycorrhizal plants, they contained larger quantities of all nutrients.

Conclusion

One of the main soil constraints in crop production is the phosphate availability. The results of this study indicate that wheat growth and yield and maize growth were significantly enhanced by the application of different P levels and mycorrhizal seed inoculation in P deficient sandy soil. The results suggested that the optimum P application level for wheat and maize in the sandy soil with P content of 18 mg kg^{-1} (citric acid) was between 30 and 40 kg P ha^{-1}, but some parameters tested showed no significant increase when P application levels were higher than 20 kg P ha^{-1}. Compared with wheat, maize benefited from mycorrhiza because it is considered to be highly dependent on mycorrhiza as compared to wheat which has a lower mycorrhizal dependency (Seymour, 2009). Although mycorrhizal inoculation did not have a significant effect on wheat and maize performance during the early growth stages, the effect became more significant as the crop matured. Because mycorrhiza improves nutrient and water use efficiency, it can improve crop performance and yield particularly under semi arid conditions.

This study showed that P application levels and mycorrhizal inoculation decreased the mineral concentration of maize stover. However, P application levels significantly enhanced the content of P, K, Mg and S in wheat plants while mycorrhizal seed inoculation resulted in a significant decrease in the content of Mn. The results of this experiment showed that nutrient contents were higher in mycorrhizal plants than in non-mycorrhizal plants. The total amount of nutrients in wheat and maize above ground parts of mycorrhizal plants may be greater than that of non-mycorrhizal plants due to increased above ground growth. Thus, increased amounts of nutrients in above ground parts of mycorrhizal plants can often be explained by much greater weight of mycorrhizal plants.

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CHAPTER 5

THE EFFECT OF MYCORRHIZAL INOCULATION AND PHOSPHORUS LEVELS ON GROWTH AND YIELD OF A MAIZE CROP GROWN ON A PHOSPHORUS DEFICIENT SANDY SOIL IN BOTSWANA

Abstract

Despite numerous reports on the positive effects of AM fungi on plant growth and yield in P deficient soils, surprisingly no data exist on the importance of AM fungi for crop growth and yield on P deficient sandy soils of Botswana. A field experiment was conducted in the 2009/2010 summer growing seasons to investigate the effects of mycorrhizal inoculation and P fertilizer levels on percent mycorrhizal colonization, growth and yield of maize (*Zea mays* L.) grown on a P deficient sandy soil of Botswana. At 60 days after planting, P fertilizer levels had a significant increasing effect ($P \leq 0.05$) on percent mycorrhizal colonization, fresh weight and dry weight. However, mycorrhizal inoculation had no significant effect at this growth stage and there was no significant interaction between the mycorrhizal seed inoculation treatment and P fertilizer levels. Substantial percent mycorrhizal colonization occurred at low P levels. At high P levels, percent mycorrhizal colonization was not suppressed as expected and thereby illustrating the P deficiency in the soil used because the mycorrhizal associations tend to decrease with increasing background levels of soil P. At 125 days after planting, mycorrhizal inoculation had a significant increasing effect ($P \leq 0.05$) on grain yield per cob and 100 seed weight while P fertilizer levels had a significant increasing effect ($P \leq 0.05$) on grain yield per cob and number of grains per cob. However, there was no significant interaction between mycorrhizal seed treatment and P fertilizer levels on all the measured parameters. The results of this study showed that on severely P deficient sandy soils of Botswana, P application and mycorrhizal inoculation can lead to enhanced maize growth and yield.

Introduction

Maize (*Zea mays* L.) is next to sorghum (*Sorghum bicolor* (L) Moench), the most important cereal crop grown under rainfed conditions during the summer rainy season in Botswana. Maize grain is used for both human consumption and poultry feed (Ibrahim & Kandil, 2007). It provides a high percentage of the daily calories in most of the diets of the people of Botswana (Lekgari & Setimela, 2001). As in many other semi-arid regions, rainfall and soil fertility (especially N and P) are the major environmental constraints affecting maize production in Botswana. Soil moisture content seems to be the most limiting factor for crop production in Botswana with the mean annual rainfall varying from a maximum of about 650 mm in the extreme northeast area of the Chobe District to a minimum of less than 250 mm in the extreme southwest part of the Kgalagadi District. Limited soil moisture availability can inhibit plant growth both directly due to water stress, and indirectly as a result of limited uptake of nutrients such as P (Ramolemana *et al.,* 2002).

However, P fertilizer application is also of special importance for crop production in Botswana because most soils have below optimal levels of this nutrient (Jones, 1984). The FAO Soil Mapping Project found that the majority (69%) of soils tested in Botswana, excluding the Western Region have soil P less than 5 mg kg^{-1} (Beynon, 1991).

Optimal use of fertilizers, especially P, could therefore help to withstand drought stress and improve water use efficiency due to larger root systems thus enabling crops to produce higher yields (DAR, 1995). Maize require about 4.5 kg of P to produce a grain yield of 1000 kg ha^{-1}, and it requires soil P contents (Bray 1) of 21-27 mg P kg^{-1} of soil for optimum growth (FSSA, 2007). The economically optimal level of P recommended for Botswana soils is 48 kg P ha^{-1} (457 kg ha^{-1} single super phosphate) (Beynon, 1991). In Botswana, traditional farmers rarely apply fertilizer to their crops, because chemical fertilizers are expensive and often beyond affordability by small scale farmers. More effective methods of fertilization and more efficient water use techniques are required to improve productivity of arable soils.

The symbiotic relationship between some soil fungi and plant roots, which is known as mycorrhiza, can improve the uptake of nutrients such as P and Zn as well as water from the soil (Mullen & Gammie, 2003; Meyer, 2007). Mycorrhizal fungi live in a symbiotic relationship with plants. They grow in close association with the roots and play an important role in the uptake and transfer of nutrients and water from the soil to the plant in exchange of sugars from the plant (Meyer, 2007; Farahani *et al.,* 2008). Mycorrhizal roots due to their extrametrical hyphae can explore more soil volume than the non-mycorrhizal roots and thus increase the supply of water and the slowly diffusing ions such as P to the plant (Khaliq & Sanders, 2000; Meyer, 2007). In addition, mycorrhiza competes much better than plant roots with other soil micro-organisms for the nutrients that are available to the plants, mostly because they are effectively distributed (Joubert & Archer, 2000). Phosphorus travels to the roots via diffusion and mycorrhizal hyphae reduce the distance required for diffusion thus increasing uptake (Anonymous, 2010).

Most crop plants are colonized by AM fungi (Babana & Antoun, 2006). Besides improving the potential for uptake of poorly mobile nutrients, mycorrhizal symbioses benefit plant growth by other mechanisms of action such as improving drought tolerance, protecting plants against pathogens or channelling carbon to the soil, thus improving soil aggregation (Babana & Antoun, 2006). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky, 1986; Manjunath & Habte, 1988).

The beneficial effects of mycorrhiza on P uptake and crop growth may however differ between crops and may be affected by growth conditions. Some crops rely strongly on mycorrhiza for P uptake (Baylis, 1975; Janos, 1980) and mycorrhizal formation and activity may differ between seasons of the year (Fitter, 1989). Maize have a very high mycorrhizal dependency while the other major summer crops such as sorghum and sunflower have a high mycorrhizal dependency (Seymour, 2009). Little is however known on the effect of AM fungi on P uptake and P responses of this crop grown in P deficient soils of Botswana.

The objective of this study was

- To evaluate the growth and yield of maize in response to different phosphorus fertilizer application levels in combination with seed inoculation with AM fungi in a field trial in a phosphorus deficient sandy soil.

Materials and Methods

Locality

The study was conducted in the 2009/2010 summer planting season at Pandamatenga Agricultural Research Station Farm (18^o 33′ S; 25^o 38′ E and 945 m above sea level) in the North-West District of Botswana, to evaluate the effect of different levels of single superphosphate as a P source on growth and yield of maize and investigate the possibility of improving its value by seed inoculation with AM fungi. A basal fertilizer application consisting of agricultural lime, N, K and micronutrients was applied to all the plots according to the soil analysis results (Table 5.1). The soil at the experimental site is Ferralic Arenosols. These Arenosols have a very low physical and chemical fertility and are not suitable for low input agriculture because this limited fertility means that it can be relatively easily pushed irreversibly beyond their capacity to sustainable use (Pardo *et al.* 2003). The average rainfall varies from 394 mm to 1050 mm per annum. The total rainfall received during the maize growing season of 2009/2010 was 494.8 mm while the average maximum day temperature recorded during the growing season was 30.3^oC.

Treatments and agronomical techniques

The test crop, maize (*Zea mays* L.: KEP), was planted in rows of 1.0 m apart with plant spacing of 0.50 m within the rows. The mycorrhiza inoculation plots (main plots) sizes were 500 m^2 (10 m x 50 m) while the total experimental area was 2450 m^2 (50 m x 49 m). The main plots were divided into 2 subplots of 24 m x 10 m spaced 2 m apart. The sub-plots were divided into 5 mini plots of 10 m x 4 m. Four maize seeds were planted in each row every 50 cm and thinned to two plants per plant hill during the seedling stage. Half of the plots (20 plots) were sown with inoculated seeds while the other half was sown with uninoculated seeds designated mycorrhiza inoculated (M_1) and uninoculated (M_0) respectively.

Table 5.1 Soil characteristics of the experimental site at Pandamatenga Agricultural Research Farm (Botswana) and optimum soil nutrient levels for cereal crops (sampled before planting 2009)

Soil characteristics	Measured values	Optimum levels	Reference
Texture	Sand		
pH (KCl)	4.6	>6.0	DAR, 2010
Calcium	0.82	>1.00 cmol+/kg	DAR, 2010
Magnesium	0.53	>0.30 cmol+/kg	DAR, 2010
Potassium	36	>0.10 cmol+/kg	DAR, 2010
Sodium	4	<1.00 cmol+/kg	DAR, 2010
Phosphorus (citric acid)	7	>30 mg/kg	FSSA, 2007
Total cations	1.95	>2.50 cmol+/kg	DAR, 2010
Copper	0.44	0.5 – 3 mg/kg	Aubert & Pinta, 1977
Zinc	0.51	1.5 - 2 mg/kg	FSSA, 2007
Manganese	34.03	1.0 mg/kg	Aubert & Pinta, 1977
Boron	0.01	0.1 – 2.0 mg/kg	Aubert & Pinta, 1977
Carbon	0.51%	>0.2%	DAR, 2010
Sulphur	2.10	5 – 10 mg/kg	Jez, 2008
Total Nitrogen	0.02%	-	

Mycorrhizal seed inoculation was done at sowing by applying 7.35 g of inoculum (*Glomus intraradices*) obtained from Biocult (Pty) Ltd Somerset West, RSA to 1225 g of maize seed. Phosphorus was applied as single superphosphate at five different levels at planting. The experimental treatments comprising of control P_1 = 0 kg P ha^{-1}, P_2 = 10 kg P ha^{-1}, P_3 = 20 kg P ha^{-1}, P_4 = 30 kg P ha^{-1} and P_5 = 40 kg P ha^{-1}, were arranged as a 2 x 5 factorial design with each treatment combination replicated four times and making provision for two sampling times.

Crop care and management measures included the use of glyphosate as a pre-planting herbicide followed by manual weed control during the crop cycle. Pesticides (Alpha Cypermethrin) were applied on maize plots during the cropping season to control pests (stalk borer, aphids and termites). Casual labourers were employed to scare wild animals during the day (monkeys) and at night (spring hare, buffalo and kudu).

Data collected

Plant components and yield

Plant samplings were taken at 60 days after planting (silking stage) to determine plant height, stem diameter, number of plants per m^2, shoot mass, nutrient content of above ground parts as well as percent mycorrhizal colonization. Number of seed per cob, 100 seed weight and grain yield per cob were also determined at 125 days after planting (maturity stage).

Plant height was measured from the ground level to the tip of the tallest leaf (Fageria, *et al*., 2006) using a meter ruler. Roots were dug out of the soil and then thoroughly washed to get rid of all the sand granules and thin layers of soil still coating the roots. The stem diameter of maize plants was measured after the maize plants were cut at the ground level. After that, above ground parts were weighed to determine the fresh mass before oven dried at $80^{o}C$ for 24 hours to determine dry weights (Jones, 1984). To measure the grain yield components, plants were randomly selected from each plot in a 1 m^2 area, and all the yield components were measured on them. Because of damage done by wild animals the total plot area could not be harvested.

Mycorrhizal colonization (%)

Mycorrhizal colonization was determined during the sampling at silking stage (60 days after planting). Roots were stained according to the modified method by Phillips & Hayman (1970). The roots were first cleared in 10% potassium hydroxide (KOH) for 20 minutes by autoclaving at $120^{0}C$. The KOH was then washed off three times with tap water and 5%

hydrochloric acid (HCL) was added and allowed to sit for 1 minute. Root samples were then stained overnight using mycorrhizal stain consisting of glycerol, lactic acid and trypan blue. After staining, the root pieces were transferred into containers containing 50% glycerol and kept overnight to remove stain.

About 5 randomly cut 2 cm roots from each stained sample were mounted on a microscopic glass slide and the root segment observed under the light microscope (10X magnification) for the presence of mycorrhizal structures (hyphae, vesicles, spores and arbuscules). The length of the root cortex in each root segment colonized with mycorrhiza was assessed using a stage micrometer. Three slides of each sample were analyzed for mycorrhizal colonization and the length of colonization was averaged for each slide. Root colonization (%) by the fungi was estimated by the grid-line intersect method (Giovanetti & Mosse, 1980). These were then expressed in percentage colonization:

- % colonization = [Σ colonized root length ÷ total root length x 100] ± Standard deviation.

Data analysis

The data recorded at the different sampling stages was subjected to analysis of variance by Statistica Version 9.0 System (StatSoft Inc., 1993) procedure to determine the least significant differences between the treatment means. Tukey HSD test was used to separate the means of the measured parameters at $P \leq 0.05$.

Results and discussion

The effect of P application levels, seed inoculation with AM fungi and their interaction on the growth and yield of maize were measured at 60 and 125 days after planting respectively. Results from the analysis of variance are summarized in Table 5.2.

At 60 days after planting, P application levels had a significant effect on plant fresh and dry mass as well as on mycorrhizal percent colonization (Table 5.2). At 125 days after planting, P application level had a significant effect on all parameters except for 100 seed weight. Although results tend to be variable due to uneven plant growth within the plots (due to damage by termites and other wild animals), these results clearly illustrated the P deficiency in the soil used in this study.

Table 5.2 Analyses of variance showing the effect of mycorrhizal seed inoculation and different P levels on growth and yield of maize at different growth stages in a field trial on P deficient sandy soil in Botswana

<table>
<tr><td>Sampling stage</td><td>Treatments</td><td>Plant stem diameter (mm plant^{-1})</td><td>Plant height (cm plant^{-1})</td><td>Plant fresh mass (g plant^{-1})</td><td>Plant dry mass (g plant^{-1})</td><td>Mycorrhizal colonization (%)</td></tr>
<tr><td rowspan="3">60 days after planting</td><td>P</td><td>NS</td><td>NS</td><td>*</td><td>*</td><td>*</td></tr>
<tr><td>M</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>M*P</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td rowspan="4">125 days after planting</td><td>Treatments</td><td>Grain yield/cob (g cob^{-1})</td><td colspan="2">100 seed weight (g)</td><td colspan="2">No. of grains cob^{-1}</td></tr>
<tr><td>P</td><td>*</td><td colspan="2">NS</td><td colspan="2">*</td></tr>
<tr><td>M</td><td>*</td><td colspan="2">*</td><td colspan="2">NS</td></tr>
<tr><td>M*P</td><td>NS</td><td colspan="2">NS</td><td colspan="2">NS</td></tr>
</table>

- NS: not significant at P≤0.05
- *: significant at P≤0.05

The effect of seed inoculation with AM fungi varied between different maize growth stages (Table 5.2). At 60 days after planting, mycorrhizal seed inoculation had no significant effect on the measured parameters. However, at 125 days after planting, mycorrhizal seed inoculation had a significant increasing effect on grain yield per cob and 100 seed weight. These results indicated that the mycorrhizal effect became more pronounced as the plant became more mature.

Analysis of variance showed no significant interaction between the P application levels and mycorrhizal seed inoculation at 60 days after planting or at 125 days after planting respectively (Table 5.2).

Only significant ($P \leq 0.05$) results will be discussed as follows.

60 days after planting

Effect of phosphorus

Phosphorus application levels significantly increased plant fresh and dry mass as well as % mycorrhizal colonization (Figures 5.1-5.3). The best response to P application levels on plant mass occurred at 40 kg P ha^{-1} (P_5) treatment and although results were variable, plant mass tended to increase with increasing P rates (Figure 5.1).

Plant dry mass showed similar results with a highest value of 116.15 g $plant^{-1}$ at 40 kg P ha^{-1} compared to 66.81 g $plant^{-1}$ where 10 kg P ha^{-1} was applied (Figure 5.2). Khan *et al.* (2005) also reported a significant effect of P levels on maize stalk yield in salt affected soils, while Khaliq & Sanders (1997) and Njul & Musandu (1999) also found increased dry matter production with P application in various pot and field trials.

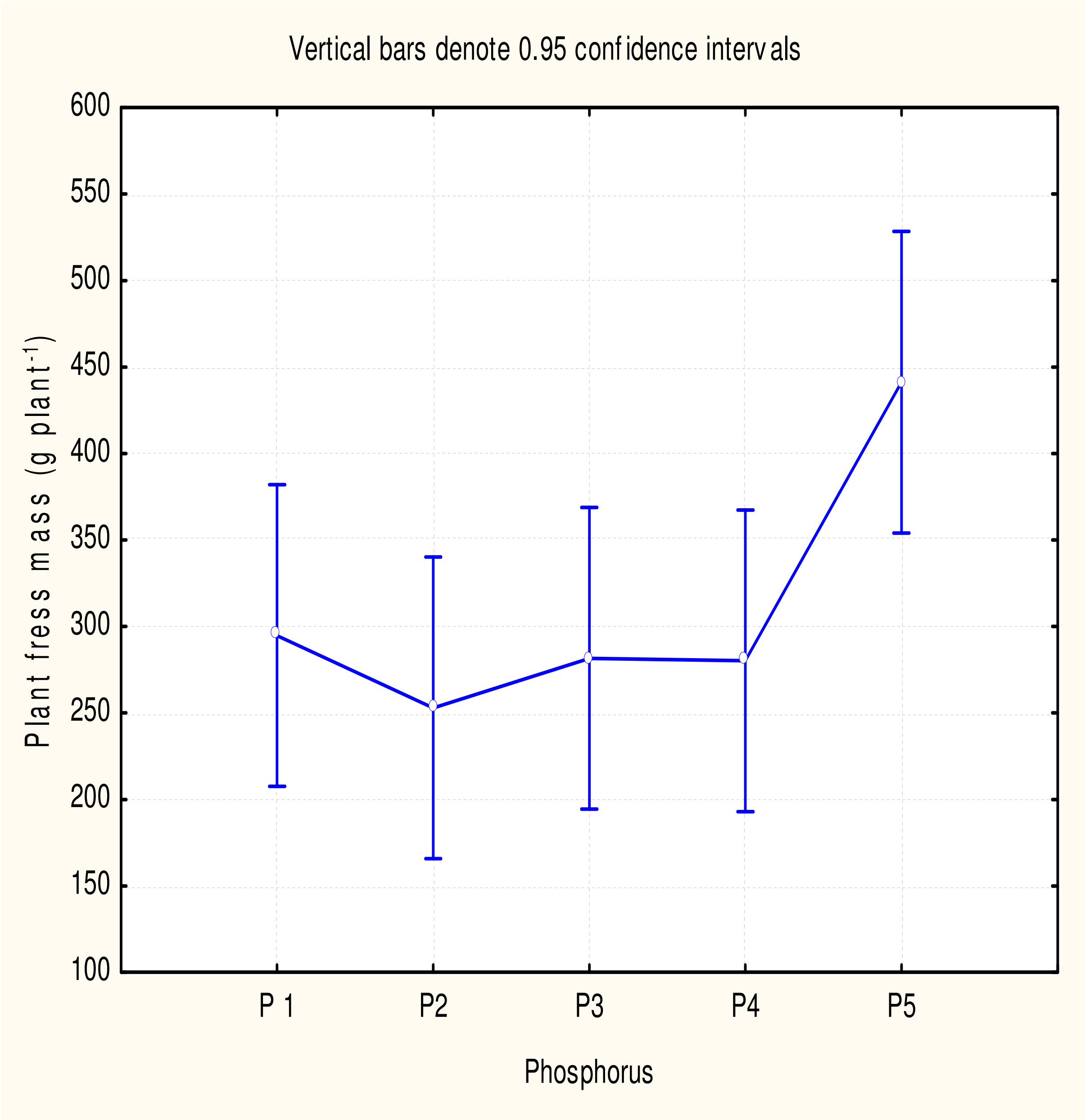

Figure 5.1 Effect of different P levels on fresh mass of maize plants at 60 days after planting in a field trial in Botswana.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

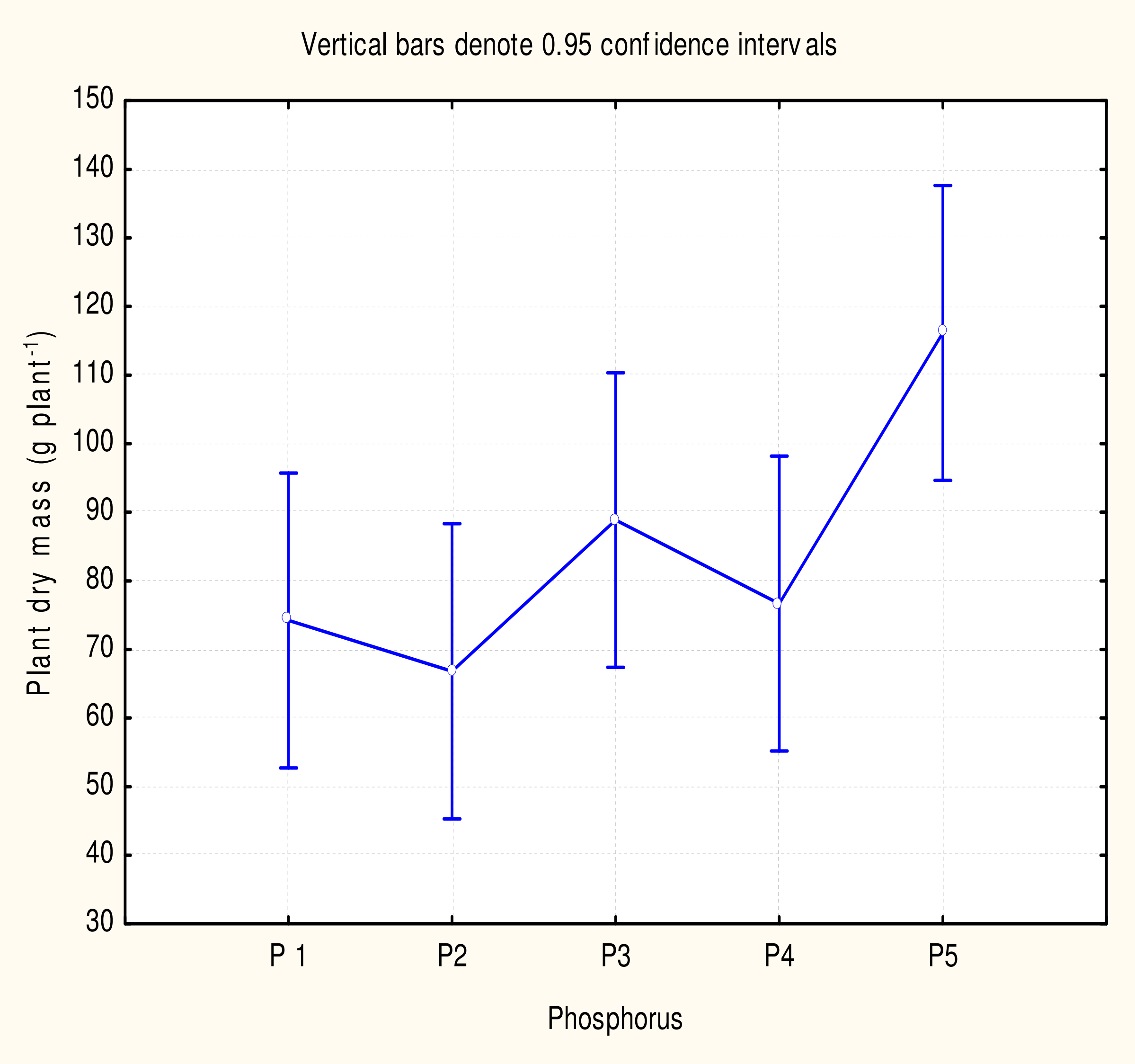

Figure 5.2 Effect of different P levels on dry mass of maize plants at 60 days after planting in a field trial in Botswana.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

As also found with plant mass, results of % colonization also tend to be variable, but showed an increasing tendency with increasing P application levels. The highest colonization of 3.27% was thus observed in plants receiving 40 kg P ha^{-1} (Figure 5.3). This value were however not significantly different from the colonization with the 0 kg P ha^{-1} (2.30%), 10 kg P ha^{-1} (3.19%) or 30 kg P ha^{-1} (2.39%) levels. These results clearly illustrated that the mycorrhizal associations tend to decrease with increasing background levels of soil P as also shown by Grant *et al.* (2005).

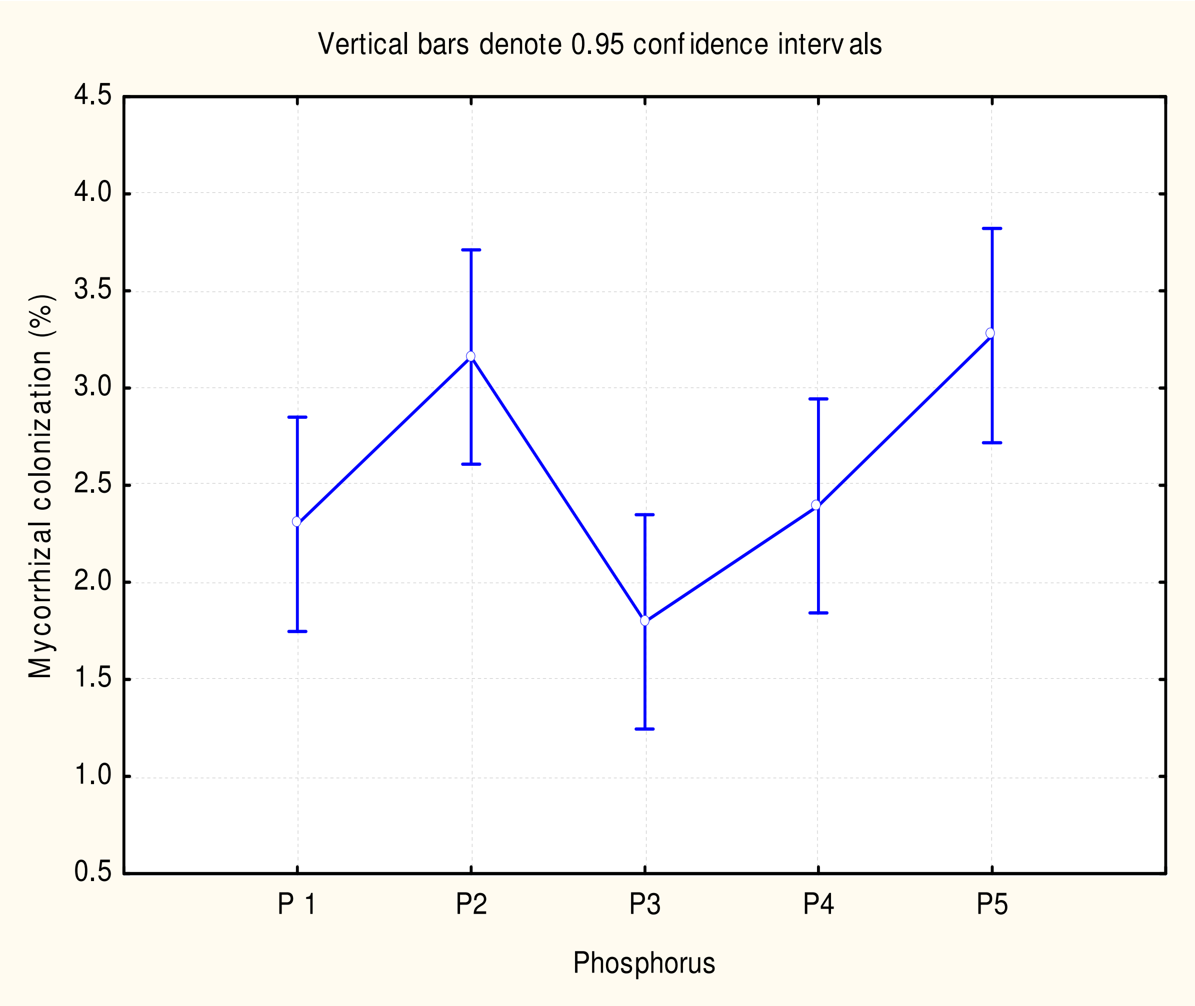

Figure 5.3 Effect of different P levels on mycorrhizal colonization (%) of maize plants at 60 days after planting in a field trail in Botswana

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

125 days after planting

Effect of phosphorus

Phosphorus application significantly increased maize grain weight cob^{-1} as well as number of grains cob^{-1} (Figure 5.4-5.5). Grain yield of maize plants increased from 23.01 g cob^{-1} where no P was applied to 46.47 g cob^{-1} at a P level of 40 kg P ha^{-1} (Figure 5.4). No significant differences were however found between 20 kg P ha^{-1}, 30 kg P ha^{-1} and 40 kg P ha^{-1} levels. Khan *et al.* (2005) reported a significant increase in grain weight per cob of maize plants under saline conditions.

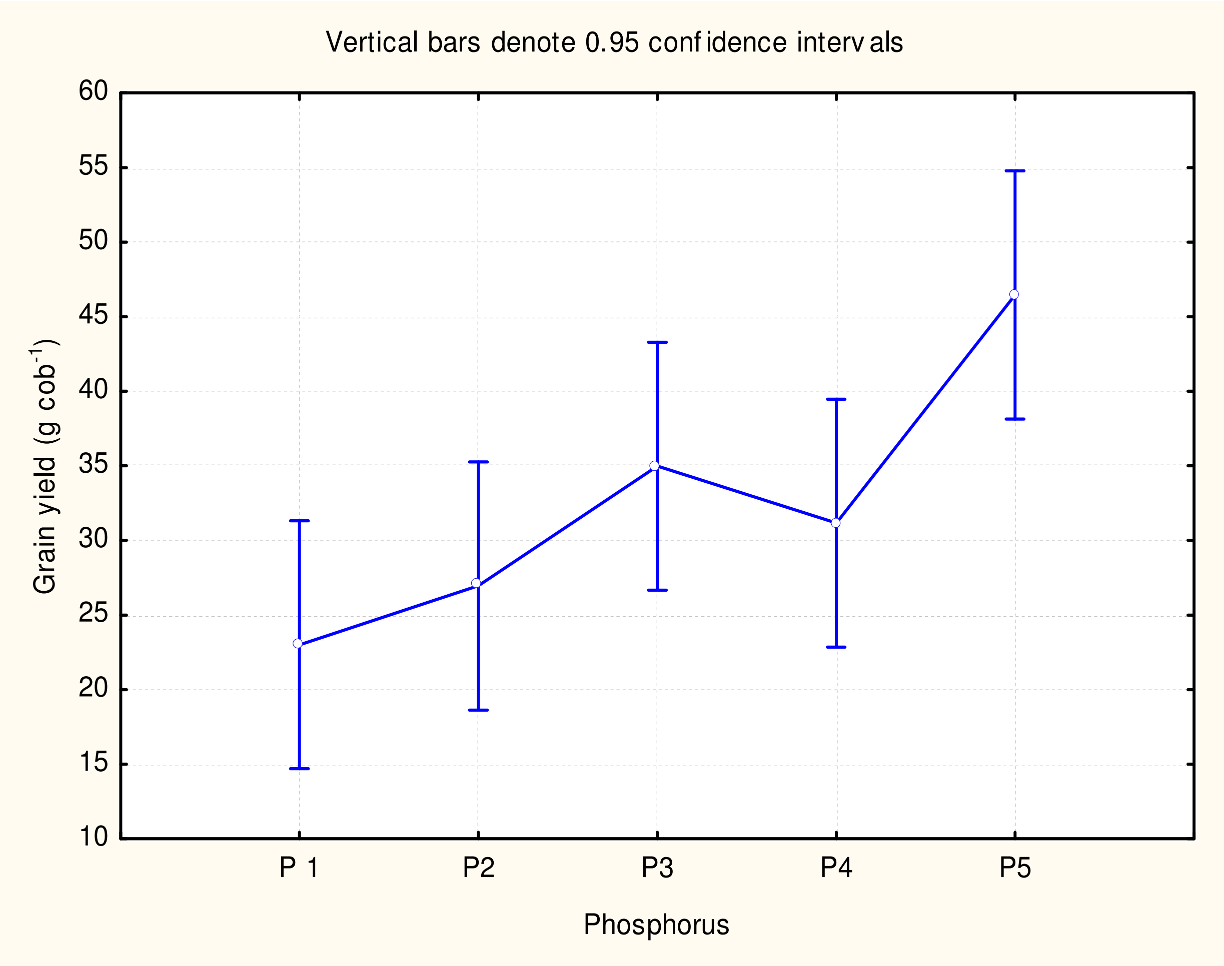

Figure 5.4 Effect of different P levels on grain yield per cob of maize plants at 125 days after planting in a field trail in Botswana.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

The number of grains per cob increased with an increase in P application level (Figure 5.5). A P application level of 40 kg P ha^{-1} produced 169 grains cob^{-1} which was significantly more than 85 grains cob^{-1} produced by 0 kg P ha^{-1}. Grains produced per cob when 40 kg P ha^{-1} was applied, were however not significantly higher than that of the 10 kg P ha^{-1} (107 grains cob^{-1}), 20 kg P ha^{-1} (151 grains cob^{-1}) or 30 kg P ha^{-1} (129 grains cob^{-1}) treatments. The results obtained in this trial in sandy soil with P content of 7 mg kg^{-1}, supported earlier studies (Khan *et al.,* 2005) under saline conditions, which showed a significant increase in grains per cob of maize with an increase in P application level. These results are also in accordance with those of Sharma & Sharma (1989) who reported that P fertilizer applications significantly affected the grains per cob.

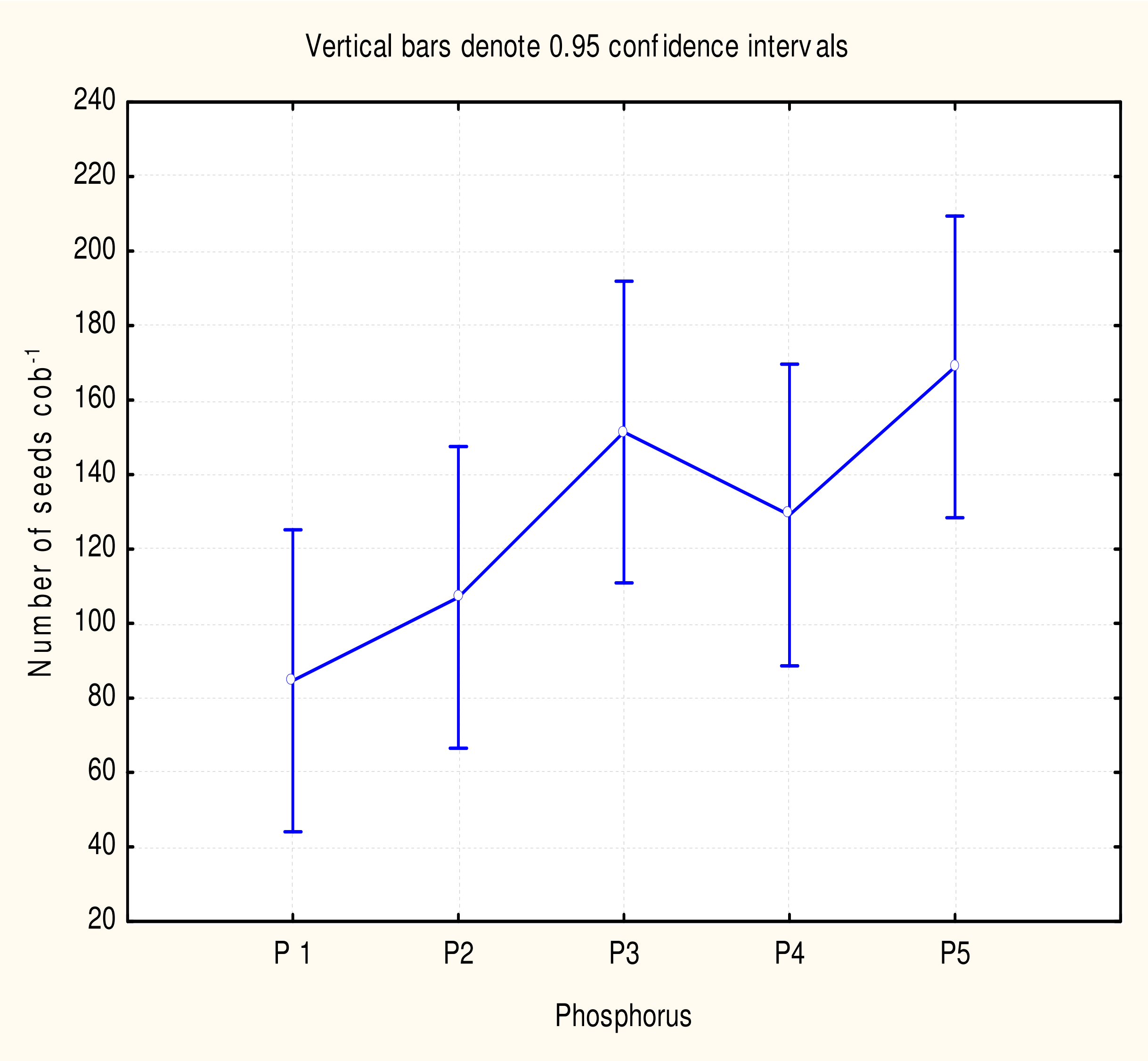

Figure 5.5 Effect of different P levels on number of seeds per cob of maize plants at 125 days after planting in a field trail in Botswana.

[P_1 = 0 kg P ha^{-1}; P_2 = 10 kg P ha^{-1}; P_3 = 20 kg P ha^{-1}; P_4 = 30 kg P ha^{-1}; P_5 = 40 kg P ha^{-1}]

Effect of mycorrhizal seed inoculation

The results showed that mycorrhizal seed inoculation of maize had a significant effect on grain yield per cob and 100 grain weight measured at maturity (Table 5.3). Seed inoculation with AM fungi significantly increased grain yield from 27.51 to 37.51 g cob^{-1} and 100 grain weight from 23.57 to 26.67 g. Khan (1972) reported that mycorrhiza increased the number of maize grains per ear and grain weight almost twelve times in steam-sterilized sand.

Table 5.3 Effect of mycorrhizal inoculation on yield of maize plants at maturity (125 DAP)

Treatments	Measured parameters	
	Grain yield cob^{-1} (g)	100 seed weight (g)
M_0	27.51b	23.57b
M_1	37.51a	26.67a

* Means with different letters within the same column are significantly different (P≤0.05).

M_0 = uninoculated seed M_1= seed inoculated with AM fungi

Conclusion

The results of this experiment showed that the application of different P levels and mycorrhizal seed inoculation had significant positive effects on the growth and yield of maize grown under field conditions in a P deficient sandy soil in Botswana. The results also showed that the effects of seed inoculation with AM fungi became more pronounced as the maize crop matured. However, this experiment did not show interactions between P fertilizer and mycorrhizal seed inoculation. In general, mycorrhiza promote plant growth and nutrient uptake of soils low in fertility and can help increase the effectiveness of P fertilizer added to soils that are P deficient or have a high P-fixing capacity. The absence of any significant interaction between the P level and the seed inoculation with AM fungi may be due to the fact that even with the highest application level of 40 kg P ha^{-1}, the growth and yield response of maize to P did not cease (reach a plateau) in a sandy soil with P content of 7 mg kg^{-1} (citric acid). This indicated that even with the highest application level, the maize still benefit from the seed inoculation with AM fungi.

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CHAPTER 6

GENERAL CONCLUSIONS

Arbuscular mycorrhizal (AM) fungi have shown beneficial effects on plant growth and health due to increased nutrient and water uptake and the synergistic interactions with other beneficial soil microorganisms such as N-fixers and P-solubilizer. The AM fungi help the plant take up water and immobile soil nutrients such as P, Cu and Zn by extending from the plant roots and expanding the volume of soil that the root system cannot explore by itself. The amount of P fertilizer application in crop production systems can be reduced when effective arbuscular mycorrhizal associations have been formed. The application of P fertilizers and mycorrhizal seed inoculation can have significant effects on the growth and yield of crops grown under field conditions in P deficient soils such as those in Botswana. Wheat and maize are important crops in semi-arid areas that have economic value as a source of food and animal feed crops. However, little is known on the effect of AM fungi on P uptake and P responses of these crops grown in P deficient soils of semi-arid areas in Southern Africa.

One of the main soil constraints in crop production is the phosphate availability. Low soil fertility and poor efficiency of nutrient and water use may lead to low crop yields, particularly under traditional farming systems. Glasshouse and field experiments were conducted to evaluate the growth and yield of wheat and maize in response to different P fertilizer application levels in combination with seed inoculation with AM fungi in P deficient sandy soils. To achieve this, pot trials were conducted to determine the effect of mycorrhizal seed inoculation on early growth of wheat and maize and also to confirm the role of mycorrhizal inoculation and P fertilizers in increasing wheat growth and yield and maize growth in a P deficient sandy soil at the University of Stellenbosch, in the Western Cape Province of South Africa. A field study was also conducted to determine the effect of different P levels and mycorrhizal seed inoculation on the growth and yield of maize grown under field conditions in a P deficient sandy soil in Botswana.

The first pot experiments to determine the effect of mycorrhizal seed inoculation on early growth of wheat and maize showed that during the early stages of growth of wheat (10 – 30 days after planting) and maize (14 – 21 days after planting) both wheat and maize were negatively affected by mycorrhiza. However, it was found that mycorrhizal seed inoculation resulted in a significant increase in wheat root dry mass at 10 DAP while the mycorrhiza significantly increased maize root fresh and dry mass at 28 DAP. At these early growth stages of the plant when the mycorrhiza is still in the process of colonizing the plant roots, it may not be able to enhance the efficiency of nutrient uptake but still obtained carbohydrates from the plants to ensure its survival and thereby suppresses crop growth. Mycorrhiza therefore seemed to have a suppressing effect on wheat and maize growth during the seedling and early growth stages. Contrarily, maize growth was improved by mycorrhiza at a later growth stage (28 DAP) which indicates that mycorrhiza have formed an association with plant roots to survive and therefore enhanced water and nutrient uptake by plant roots. Maize benefited from mycorrhiza because it is considered to be highly dependent on mycorrhiza as compared to wheat which has a lower mycorrhizal dependency. Although wheat performance was adversely affected by addition of mycorrhiza during the early growth stages (10 – 30 DAP), its performance can be improved as the crop matures. Because mycorrhiza improves nutrient and water use efficiency, it can improve crop performance and yield particularly under semi arid conditions.

The follow-up pot experiments were conducted to evaluate the growth and yield response as well as nutrient content of wheat and maize to seed inoculation with AM fungi in combination with different P fertilizer application levels in a pot trial with P deficient sandy soil. The results of this study indicate that wheat growth and yield and maize growth as well as nutrient content were significantly enhanced by the application of different P levels and mycorrhizal seed inoculation in P deficient sandy soil. The effect of P application levels, seed inoculation with AM fungi and their interaction on the growth of wheat were measured at tillering (50 DAP) and anthesis (90 DAP), while the effect on yield components and nutrient content were measured at maturity (120 DAP). Phosphorus application levels had a significant enhancing effect on all the measured parameters except for root fresh mass, root moisture content and thousand kernel weight. The effect of seed inoculation with AM fungi varied between different wheat growth stages. Wheat percent root moisture was significantly increased at the tillering stage but was significantly decreased at anthesis, while percent plant moisture was significantly increased at both the tillering and anthesis stages of growth. At maturity, grain yield as well as plant fresh mass and plant dry mass were significantly increased by the seed treatment with mycorrhiza. Significant negative interaction between the P application level and mycorrhizal inoculation was found at anthesis with regard to percent root moisture while a significant positive interaction was observed at maturity stage with regard to number of grains per plant, thousand kernel weight as well as fresh and dry mass per plant.

Phosphorus application levels significantly increased the concentrations of P, K, Mg and S but significantly decreased concentrations of Fe, Cu, Zn, Mn, and OC in wheat plants. No significant effect due to mycorrhizal seed inoculation was found on any of the measured elements except for Mn concentration. A significant positive interaction between P application level and mycorrhizal seed inoculation with regard to Zn concentration was found at 120 DAP, however, significant negative interaction was found with regard to Mn concentration at this stage of growth. Phosphorus application levels enhanced the content of all the nutrient elements except for Mg content. A significant increasing effect due to mycorrhizal seed inoculation was found on NH_4-N, P, Mg, Fe, Cu, Zn, S and OC content. A significant positive interaction between P application level and mycorrhizal seed inoculation with regard to all nutrient elements except for Mn uptake was found at 120 DAP.

The effect of P application levels, seed inoculation with AM fungi and their interaction on the growth of maize were measured at 30, 45 and 60 DAP respectively while nutrient content of maize plants was determined at 60 DAP. Phosphorus application levels had a significant positive effect on all the measured parameters except for percentage plant moisture at 30 and 60 DAP respectively. However, P application levels significantly decreased percent root moisture at 30 DAP. The effect of seed inoculation with AM fungi varied between different maize growths stages. At 30 DAP, mycorrhizal seed inoculation had no significant effect on the measured parameters. At 45 DAP, mycorrhizal seed inoculation significantly increased stem diameter, root fresh mass and percentage root moisture, while at 60 DAP all measured parameters except stem diameter and plant moisture were significantly increased by mycorrhizal seed treatment. However, mycorrhizal seed inoculation significantly decreased percent root moisture. These results indicated that the mycorrhizal effect became stronger as the plant became more mature. A significant positive interaction between P application level and mycorrhizal seed inoculation with regard to plant height as well as fresh and dry mass per plant was found at 30 and 60 days after planting respectively.

Increasing P application levels had a significant decreasing effect on NH_4-N, K, Mg, Fe, Cu, Zn, Mn and B concentrations in maize plants. Mycorrhizal seed inoculation resulted in a significant decrease on NH_4-N, P, Fe, Cu, as well as on OC concentrations. A significant negative interaction between P application level and mycorrhizal seed inoculation with regard to OC content was found at 60 DAP. Phosphorus application levels significantly increased maize contents of all the nutrient elements. Mycorrhizal seed inoculation resulted in a significant increase on contents of all the nutrient elements except for Al content. A significant positive interaction between P application level and mycorrhizal seed inoculation with regard to NH_4-N, P, Zn, Mn, S and OC uptake was found at 60 DAP.

The results of a field experiment to evaluate the growth and yield of maize in response to different P fertilizer application levels in combination with seed inoculation with AM fungi in a field trial on P deficient sandy soil in Botswana showed that the application of different P levels and mycorrhizal seed inoculation had significant positive effects on the growth and yield of maize grown under field conditions in a P deficient sandy soil in Botswana. The results have also shown that the effects of seed inoculation with AM fungi became more pronounced as the maize crop matured. However, this experiment did not show interactions between P fertilizer and mycorrhizal seed inoculation. In general, AM fungi promote plant growth and nutrient uptake of soils low in fertility and can help increase the effectiveness of P fertilizer added to soils that are P deficient or have a high P-fixing capacity. The absence of any significant interaction between the P level and the seed inoculation with AM fungi may be due to the fact that even with the highest application level of 40 kg P ha^{-1}, the growth and yield response of maize to P did not cease (reach a plateau) in a sandy soil with P content of 7 mg kg^{-1} (citric acid). This indicated that even with the highest application level, maize still benefit from the seed inoculation.

This study further confirms the role of mycorrhizal inoculation and P fertilizers in increasing growth and grain yield in wheat and maize production. From the result of the experiments, application level of between 30 and 40 kg P ha^{-1} may be recommended for increasing wheat and maize yield particularly in the semi arid regions, but some parameters tested showed no significant increase when P application levels were higher than 20 kg P ha^{-1}. This will greatly benefit farmers in areas where supply of P fertilizer is low or in cases where farmers cannot afford the cost of high fertilizer input.