# EFFECT OF MYCORRHIZAE ON PHOSPHORUS UPTAKE AND GROWTH OF ONIONS (*Allim cepa* L.) IN KENYAN SOILS



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A thesis submitted in partial fulfilment for the degree of Master of Science in the University of Nairobi, Department of Soil Science.

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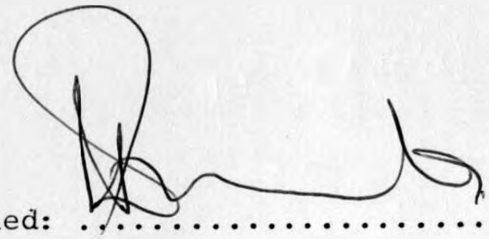
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.



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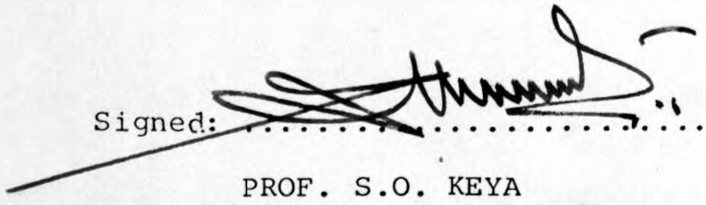

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Date: 16TH JUNE 1989.....

# DEDICATION

To my Parents I dedicate this work

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ABSTRACT

This study aimed at determining the presence of mycorrhizal fungi in Kenyan soils. Using onion as a test crop, the interaction between mycorrhizal fungi and phosphorus was investigated in five different phosphorus deficient Kenyan soils. Spore density of mycorrhizal fungi determined by the Wet-sieving method was used as an indicator of infection. There was a significant variation in the spore population and this ranged from 5 spores/g dry soil in Ferralsol to 11 spores/g dry soil in Luvisol.

Onions were planted in pots in a glass house using the above soils. Some plants were raised in steam sterilised soils while others were grown in unsterilised soils. Roots of plants grown in unsterilised soils were heavily infected while the roots of plants in the sterilised soils remained uninfected. Plant growth parameters namely fresh weight, dry weight, height, bulb diameter and bulb fresh weight were larger for infected plants than non-infected plants. These growth differences were significant after eight weeks of growth. Increase in shoot dry weight expressed as a percentage of the non-mycorrhizal plants ranged from 990 - 6983% after 21 weeks of growth, while the increase in percentage phosphorus varied from 214% to 410%. The root:shoot ratio was higher for non-mycorrhizal plants. It was concluded that mycorrhizal fungi enhances phosphorus uptake and consequently promotes growth.

In another experiment, onions were grown in both sterilised and unsterilised soils in which either no phosphorus was added or phosphorus was added at varying rates. It was observed that at low to moderate levels of soil phosphorus, the mycorrhizal onions performed better than non-mycorrhizal ones Infected plants also contained higher percentage phosphorus in their tissue. However, at very high phosphorus levels (100 ppm) both mycorrhizal and non-mycorrhizal onions had comparable growth and tissue phosphorus content. Mycorrhizal plants at 50 ppm of phosphorus grew as well as non-mycorrhizal plants at 100 ppm of phosphorus. Thus mycorrhizal fungi contributed approximately 50 ppm of phosphorus. These results demonstrated that mycorrhizal infection may have practical significance in low phosphate soils but not at high soil phosphorus concentration.

It was concluded that onions are dependent on vescular arbuscular mycorrhizal fungi for normal growth and development. It was recommended that similar experiments be extended to other Kenyan soils and other crops to ascertain the presence and importance of mycorrhizal fungi.

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# 1. INTRODUCTION

Mycorrhizae are symbiotic associations between fungal hyphae and roots of vascular plants. The association is beneficial to both the plant and fungus. These associations are divided into two broad classes based primarily on spore morphology. The first, ectomycorrhizae, contain a fungal mantle surrounding the outermost root cells as well as intercellucar fungal growth in the first few cell layers of the root cortex. The intercellular growth is referred to as the hartig net. The second, endomycorrhizae, contain a loose fungal network in the soil and the fungus grows both intra- and intercellularly in the root cortex. The endomycorrhizae are further sub-divided into two groups. The first group is produced by septate fungi. The second group is produced by non-septate fungi and is referred to as vescular arbscular mycorrhizae (VA mycorrhizae).

Though mycorrhizal associations were observed as early as 1885 (Frank 1885), research on fungal symbionts began much later with the development of techniques for maintaining these fungi in pot cultures (Mosse, 1953). More advances were made when it was found that plants inoculated with vescular arbuscular fungi grew better due to increased phosphorus uptake (Gerdemann, 1964). The possible importance of mycorrhizae can be seen from the fact that almost all plant species of

economic importance in the tropics are able to be infected by vescular arbuscular fungi. Most form vescular arbuscular mycorrhizae but some tree species form ectomycorrhizae (Gerdemann, 1975). The only notable exception is rice grown under paddy conditions where infection is rare. But when grown in non-flooded soils, it becomes infected (Asai, 1934).

In Kenya, intensive agricultural practices is characteristic of high potential areas. However, soil fertility in these areas is diminishing with increased cultivation and hence the need for application of fertilizers. Similarly, in the low potential and highly eroded areas, there is need for high amounts of fertilizers, especially phosphorus. Phosphorus is deficient in East African Soils (Robinson, 1953) and is one of the important nutrients limiting productivity of the major soils in Kenya (Hinga, 1973). The cost of chemical fertilizers is astronomical and is rapidly escalating. Moreover, phosphate fertilizers, unlike nitrogen fertilizers cannot be produced by chemical or biological means. Thus, we heavily rely on the already scarce sources which are being depleted at an alarming rate.

In Africa, there are only twenty known phosphate deposits, and of these, only two are being exploited. Phosphate fertilizers have an added disadvantage of being 'fixed' by tropical soils. Hinga(1973) observed that phosphorus fixation in Kenya may be very high and

can vary greatly for different soils. Therefore, there is need for search of alternative ways and means of increasing nutrient absorption and sustaining productivity with low fertilizer input if possible. Given that mycorrhizae enhances phosphorus uptake, research on mycorrhizal inoculation could partly offer an answer to this problem. Soils which are deficient in/and/or fix phosphorus as those described by Keter (1983) and/or are fumigated to remove plant pathogens should receive more attention to mycorrhizae (Hayman, 1930).

Although numerous investigators have demonstrated that mycorrhizae can enhance plant growth, information on the presence and importance of mycorrhizal fungi in Kenyan soils is limited or totally lacking. The objective of this study was therefore to:

1. Establish prevalence of mycorrhizal fungi in some Kenyan soils.
2. Study the effect of mycorrhizae on growth of onion plants.
3. Assess the effect of mycorrhizae on phosphorus uptake using onions as a test crop.
4. Determine the effect of phosphate fertilizers on mycorrhizal infection.

# 2. LITERATURE REVIEW

## 2.1. Mycorrhizal Fungi

Vescular arbuscular mycorrhizal (VAM) fungi are non-septate phycomycetes in the order Mucorales and the family Endogonaceae (Schenk and Perez, 1987). Presence of spores of members of Endogonaceae in association with vescular arbuscular mycorrhizal roots was established long ago by Peyronell (1923) but were not accepted by many as the true mycorrhizal fungi until the work of Mosse (1953 and 1956). Fungal species causing vescular arbuscular mycorrhizae are the same in the tropical countries as in other parts of the world (Redhead 1977) but other types of mycorrhizal fungi may be found when more research is conducted in the tropics.

Attempts to grow vescular arbuscular fungi in pure culture have been unsuccessful. Thus, isolation of these fungi from the soil is by wet sieving through a series of sieves with decreasing pore diameter (Gerdemann, 1955; Gerdemann and Nicolson, 1963). Other methods include, Adhesions Floatation (Sutton and Barron, 1972), "Floatation bubbling", (Furlan and Fortin, 1975), a sucrose centrifugation method originally developed to extract nematodes from soil (Smith and Skipper, 1979) and a method which involves direct plating of soil suspension on filter paper. Skipper and Smith (1979) compared the effectiveness of the above

methods in recovering total spore population and found that the plate method constantly yielded higher spore counts.

Recently researchers have demonstrated that vescular arbuscular fungal spores can germinate on water agar media (Daniels and Trappe, 1980, Hepper, 1981; Koske, 1981), but the levels of germination will differ for each species. Germination on water agar can be improved for certain fungi by amendment with non-sterile soil (Mosse, 1959) implying that the presence of a microbially produced soil constituent stimulated germination. Mosse (1962) found that high level of germination were achieved in the absence of soil if a Pseudomonas spp were present. The importance of microorganisms in germination of vesculur arbuscular mycorrhizae fungi was further demonstrated by Daniels and Menge (1981) who found that germination was reduced if spores were surface sterilized. Though spore germination on agar media is a tremendous step towards commercial production of vescular arbuscular mycorrhizae fungi, the conditions to initiate spore production and maturation are still unknown.

Studies on the number of spores per gram of soil indicate that spores of many types are widespread in the soil (Hayman, 1970; Hayman and Stovold 1979; Powell 1977). The number of spores in the rhizosphere often does not relate to the intensity of mycorrhizal infection in the roots of associated plants (Porter,

1979). The discrepancy could be due to: (i) extraction technique which may not remove all the spores, (ii) spores are not the only source of inoculum. Moreover, mycorrhizal development in a given soil depends not only on the inoculum present, but also on the rate of root colonization (Abbott and Robson, 1982). However, there are some reports of a correlation between the number of spores and mycorrhizal infection (Daft and Nicolson 1969a; Hayman 1970). Realistic estimates of the number of effective propagules have been obtained using the most probable number methods (Porter 1979; Powell, 1980).

Higher spore populations are found in agricultural soils than in either grassland or undisturbed bushland and (Hayman and Stovold, 1979). Similar findings have been reported by Mosse and Bowen (1968b).

## 2.2. Root Infection

Plants infected with vescular arbuscular fungi often take up more phosphorus from P-deficient soils than uninfected plants (Mosse, 1973; Gerdemann, 1975). Thus the assessment of infection is an essential part of the study of vescular arbuscular mycorrhiza.

The rate and extent of initial infection of a root not only depends on the amount of propagules present, but also at the rate at which the propagules germinate in the soil and initiate root colonization

(Abbot and Robson, 1982). The time at which growth response is apparent is earlier the higher the number of spores used (Daft and Nicolson, 1969a). Thus, low number of propagules in the field soils may result in low levels of infection (Hall and Armstrong, 1979; Moorman, et al 1979; Powell, 1980).

Sanders et al (1977) found one fungus, probably Glomus macrocarpus, was slow to colonize roots, hence achieved less infection of the root length and did not result in increased phosphorus uptake and growth of onions. However, three other fungi, Glomus mosseae, Glomus macrocarpus var. geospora and Gigaspora calospora which colonized faster, resulted in increased phosphorus uptake and growth of onions.

Infection intensity is not always proportional to mycorrhizal effects. Fungal efficiency in phosphate uptake not only depends on the fungal ability to colonize the root and extensively develop in the soil, but also the capacity of external mycellium to absorb phosphorus from the soil and transport to the plants. The little data available indicate that external mycellium increases during the active phase of internal mycellium development and it is related to phosphorus uptake by mycorrhizal plants (Sanders et al 1977; Bethlenfalvay, 1982).

Mycorrhizal infection is influenced by many environmental factors and this includes light supply, temperature, soil pH and nutrients in the soil. The fungi involved in mycorrhizal association derive their carbon energy source from host plant and these rely on the photosynthetic ability of the host plant and the transportation of the photosynthates to the root. Therefore, light is obviously a limiting factor. Daft and El-Ghiami (1978) showed that plants exposed to greater radiant energy had greater mycorrhizal infection than plants grown under low radiant energy. Infact, daylength may play a vital role in vescular arbuscular mycorrhizal development. Longer photoperiods stimulate more mycorrhizal infection (Hayman, 1974).

Air temperature affects photosynthesis, hence it is possible that it influences mycorrhizal development. Furlan and Fortin (1973) showed that infection levels in onion roots increased with ambient temperature up to 26$^{o}$C, and this was sometimes associated with increased plant growth responses. However, in these studies, there is need to consider the high soil temperature in the tropics. Smith and Bowen (1979) showed that infection by fungi from a temperate soil increased with increasing temperature and reached maximum at 16-20$^{o}$C whilst in Senegal, peanuts sown during hot season showed a root infection of 53% after

sixteen days of growth. In view of the beneficial effect of light and temperature, it is expected that under the high temperatures and light intensity in the tropics, mycorrhizal infection could be high and growth responses marked.

Vescular arbuscular fungi are adapted to certain pH ranges and can be profoundly affected by pH changes (Daniels and Trappe 1980; Mosse, 1972). This fact is demonstrated by Hayman and Mosse (1971) who obtained infection and growth stimulation of Coprosoma robusta with Glomus mosseae in two soils of pH 5.6 and 7.0, but not in acid soils of pH 3.3 to 4.4. However after liming to pH 6.5, infection and growth stimulation occured in most of the soils.

Most work reported on nutrients concerns the effects of soil phosphorus. Daft and Nicolson (1966) reported an inverse relationship between percentage infection of the tomato plants and the relative level of phosphate applied as bone meal. In another experiment, Daft and Nicolson (1969b) investigated the relationship between percentage infection of maize by Endogone, Glomus macrocarpus var geospora, the amount and duration of application of fertilizer. The relationship between amount of soluble $KH_2PO_4$ supplied and the infection of the root still held regardless of whether $KH_2PO_4$ was applied once or over a period of time.

Similar findings have been reported by Mosse (1973). Bolgiano et al (1983) grew onions at four levels of phosphorus and found that mycorrhizal infection was high only when phosphorus concentration were below 15-20 $\mu g/cm^3$.

Control of vescular arbuscular mycorrhizal infection of roots by phosphorus was stressed by Sanders and Tinker (1973) who found that there was a correlation between the final percentage of infection and quantity of phosphorus added to the soil. Later work by Sanders (1975) in which onion plants were given phosphorus as a "foliar feed" showed that the phosphorus concentration within the plant plays a more important role than soil phosphorus levels in determining the degree of mycorrhizal infection. Menge et al (1978a) using a "split-root" technique found that phosphorus fertilization of half of the root system of Sudan grass could significantly reduce the number of chlamydosphores of the VAM fungus Glomus fascicutus in the unfertilized half of root system. In a further experiment, they were able to show that it is the concentration of phosphorus within the plant and not soil phosphorus which leads to reduction in colonization, infection and spore production of Glomus fasciculutus.

Most measurements of infection are based on assessment of the proportion of host tissue occupied by fungus. In this case, observations are made on stained material. The commonly used method is that of Phillips and Hayman (1970).

There are many methods of recording the percentage infection of the stained roots. Giovanetti and Mosse (1980) compared the accuracy of the four commonly used methods, viz, Gridline intersect, visual, slide length and slide (±). The Gridline intersect and visual methods were more accurate. The visual is more appropriate since it is less laborious. Another method is based on the yellow pigment formed in the infected roots of some host species (onion and maize, Becker and Gerdemann, 1977). However, Abbott and Robson, (1978) found that not all species form the pigment. Moreover, the pigment is light sensitive, hence requires immediate determination.

2.3. Growth of Host Plant

Many experiments demonstrate that infection with vescular arbuscular mycorrhizae causes increased growth of both tropical and temperate plants when the amount of available phosphorus is a limiting factor (Gerdemann, 1964, 1968; Mosse, 1973; Sanders et al 1977). Asai (1943) as quoted by Kleinschmidt (1972) grew a large number of plants in sterilized and unsterilized soils

to which he had added a small amount of non-sterile garden soil. Many species which grew poorly in sterilized soil grew normally in the inoculated soils if they became mycorrhizal. Similar findings have been reported by Khan (1972).

Kleinschmidt and Gerdemann, (1972) found that citrus seedlings grown in fumigated nurseries were non-mycorrhizal, stunted and Endogone spores were absent. On the contrary, seedlings grown in normally scattered areas of those nurseries were mycorrhizal. They noted that all mycorrhizal plants had greater dry weight and higher percentage phosphorus than did non-mycorrhizal plants. They suggested that increased growth was due to increased phosphorus uptake. In similar experiments, Gray and Gerdemann (1969) and Ross (1971) found that mycorrhizal plants always have higher phosphorus than non-mycorrhizal plants. Further to these, Gerdemann (1964) found that phosphorus deficiency symptoms on Zea mays L. grown in steamed soil can be prevented by inoculation of plants with endomycorrhizal fungi.

Recently, Yost and Fox (1979, and 1982) using a sterilized and a non-sterilized oxisol with moderate phosphorus levels demonstrated that cowpea and soybean plants grown in sterilized soil were slow in their development and had lower phosphorus content when compared to those

plants in non-sterile soil. These findings agree with those of Nelsen et al (1981).

Improved growth has been demonstrated for a very wide range of host plants, including crop plants and trees and is manifested as increased root and shoot growth, increased vascular tissue, flower production and yield in some host plants (Daft and Nicolson, 1969a; 1966, 1972; Daft and Okusanya, 1973a). Reduction in the root: shoot ratio has also been observed in a few plant species (Sanders, 1975; Hayman and Mosse 1971; Mosse and Hayman, 1971). All these effects on growth can be attributed directly or indirectly to improved mineral nutrition in many cases. Similar changes have been shown to take place in response to fertilizer application in the absence of mycorrhizal infection. (Murdoch et al 1967; Ross 1971; Mosse 1973).

Reduction in plant dry weight following mycorrhizal infection may occur (Baylis 1967). This growth depression is probably due to carbohydrate utilisation by fungi as suggested by Furlan and Fortin (1973; Smith 1980). This view has been questioned on the basis that there is insufficient fungal tissue associated with the root to provide a vigorous sink of photosynthates (Cooper, 1975). However, Hepper (1977) found that the fungus could constitute 4-17% of the dry weight of the root and could therefore exert pressure on carbon

compounds especially during the early stages of growth (Bethlenfalvay et al 1982a and 1982b).

Though most natural soils contain indigenous vescular arbuscular fungi (Khan 1971a) it has been observed that addition of a more effective vescular arbuscular fungi generally improved the uptake of nutrient and growth of onions (Ojala et al 1983; Wamocho et al 1984). It is also known that diferent species/strains differ in their ability to increase plant growth (Mosse, 1972a,b, Mosse and Hayman 1971; Abbott and Robson, 1978). Therefore if a soil contains insufficient inoculum or the efficiency of the strain is low, then inoculum with a more effective mycorrhizal strain can offset phosphorus deficiency and increase plant growth (Howeller and Sieverding, 1983).

Elimination of native mycorrhizal fungi through soil sterilization causes significant response to inoculation with vescular arbuscular fungi under field condition as shown for maize and cowpea (Islam and Ayanaba 1981), and onions (Nelsen et al 1981). However, the benefit of added mycorrhiza has been limited so far to perennial nurseries where fumigation is practical. Trials in non-sterile natural field soil sometimes gives variable and non-significant responses (Bagyraj et al; 1979, Powell et al; 1980). However, there have been reports of significant yield increases (Khan, 1972; Hayman and Mosse, 1979; Wamocho et al 1984; Mosse, 1977).

## 2.4. Mycorrhizal Infection and Growth of Onions in Relation to Phosphorus

Increased growth of mycorrhizal plants is usually accompanied by high concentration of nutrients (Mosse 1975). The most notable nutrient and on which most research has been carried out is phosphorus (Gerdemann 1964; Daft and Nicolson, 1966). Much emphasis is laid on phosphorus for it is required in large amounts by plants and is commonly deficient in soils. Robinson (1953) reported that phosphorus is deficient in East African soils, particularly in acid and leached soils of Western Kenya (Doughty, 1953). Phosphorus has been shown to limit productivity of Kenyan soils. Increased supply of phosphorus and other nutrients is due to increased uptake of nutrients through hyphal network of the VAM fungi which ramify within and beyond the rhizophere (Allen, 1980; Sanders and Tinker 1973; Hayman and Modde, 1971; Mosse 1973).

It has been estimated that for every cm of infected root, there are 80 cm of hyphae (Sanders and Tinker 1973) making about 46 to 107 connections with the root (Mosse 1959). Some hyphae have been reported to grow 1 cm to 7 cm from host root (Hattigh *et al*;1973) hence exploit a large volume of soil. Increased rate of nitrogen and phosphorus absorption from soil by onions

as a result of colonization by VAM fungi has been reported (Smith et al 1986). Other researchers have also demonstrated increased dry matter accumulation in plants colonized by VAM fungi under low to moderate phosphorus conditions (Manjunath and Bagyraj, 1986, Skipper and Strubble, 1984; Wamocho et al 1984).

Diffusion of phosphate ions in soil solution is slow compared to the rapid absorption of phosphate by the root resulting in a depletion zone around them (Nye and Tinker 1977). Thus mycorrhizal plants are advantaged for hyphae extend beyond the depletion zone into unexploited soil. Increased inflow of phosphate into both onion and clover roots infected by VA fungi has been demonstrated (Sanders, and Tinker, 1971; Sanders et al 1977; Smith, 1982).

There have been suggestions that mycorrhizal roots can exploit insoluble phosphorus sources. Murdoch, et al (1967) showed that growth of mycorrhizal maize responded to the application of rock phosphate, whereas the non-mycorrhizal plants did not respond to the fertilizers. Similar suggestions have been reported by Hall (1975), Mosse, Powell and Hayman (1976). Excretion of hydroacids by hyphae which would increase the availability of rock phosphate has been suggested as a mechanism underlying the increased uptake (Johnston, 1956; Johnston and Muller, 1959; Smith 1980). However,

$^{32}$phosphorus labelling experiments have repeatedly shown that in unamended soils, mycorrhizal roots, absorb from the labile pool of the native phosphorus as non-mycorrhizal roots do. There is no difference in specific activity of $^{32}$phosphorus in the two groups of plants (Sanders and Tinker, 1971; Hayman and Mosse, 1972; Mosse, Hayman and Arnold 1973; Powell 1975a).

## 2.5. Effects of High Phosphorus Levels on Growth of Host Plant.

Growth differences between mycorrhizal and non-mycorrhizal plants decreases with increasing amount of phosphate fertilizer (Baylis 1967; Mosse 1973, Khan, 1972; 1975). At extreme quantities of phosphorus in the soil, the growth of non-mycorrhizal plants can exceed that of infected ones (Smith 1982; Bethlenfalvay *et al* 1983; Kierman *et al* 1983). Infact some researchers have found that for the same concentration of phosphorus in the tissue, the mycorrhizal plants can have lower yields than non-mycorrhizal plants (Pairunan *et al*, 1980). These yield reductions in mycorrhizal plants could be due to high utilization of photosynthates by fungi as suggested by Smith (1980), Bulwalda and Goh (1982).

Nelsen *et al* (1981) studied the effects of high treatments of phosphorus on root infection and growth of onions grown on two muck soils, which were low and

high in phosphorus. To these soils, 4 levels of phosphorus were added (0,30, 97 and 193 kg P $ha^{-1}$). In the soil that was low in phosphorus, bulb weight increased with added phosphorus. Root infection by mycorrhizal fungi were negatively correlated with added phosphorus. But in soil with high available phosphorus, the bulb weight and root infection were not influenced by added phosphorus. Root data from both soils suggested a threshold level of soil phosphorus below which mycorrhizal infection was high and above which mycorrhizal infection was low. Similar findings have been reported by other researchers (Bolgiano et al; 1983; Sanders and Tinker, 1973; Hayman et al; 1975). Recently, Ssali and Keya (1986) studied interaction of native mycorrhizae with nitrogen and phosphate fertilizers. They grew three bean cultivars at 2 levels of phosphorus, (0 and 150 kg P $ha^{-1}$) and 2 levels of N (10 and 100 kg N $ha^{-1}$). They observed that where phosphorus was not applied, the percentage root infection was 94 - 100%, but where phosphorus was applied, the infection ranged between 0 - 40%.

Effects of high phosphorus on root colonization varies with host plant and it has been proposed that it is the phosphorus concentration within the plant tissue that affects mycorrhizal activity (Sanders, 1975; Menge et al 1978a). Reduction of infection at high phosphorus levels has been attributed to the

decreased membrane mediated root exudates necessary for colonization to occur. (Ratnayake et al, 1978). While this may be true, recent studies have shown increase in phosphate may directly disturb the physiology of the fungi before significantly affecting their ability to colonize the roots (Bethlanfalvay et al 1983; Pearson and Read, 1975). There is evidence that some mycorrhizal fungi tolerate higher levels of soluble phosphates than others (Giltrap and Lewis; 1981).

## 2.6. Benefits of Mycorrhizae to Plants

Apart from the enhanced growth and phosphorus absorption, studies on uptake of other nutrients by mycorrhizal plants has been reported for K. (Powell, 1975), Cu (Menge et al 1978)and Zn(Benson and Covey, Jr. 1976). Other documented advantages of mycorrhizae include increased plant tolerance to moisture stress (Bolgiano et al, 1983; Lavy and Kirkum, 1980; Safir et al 1972). A reduction of plant diseases has been noted (Schenk and Kellan, 1978). Improvement on nodulation and nitrogen fixation in some leguminous plants has also been observed (Abbot and Robson, 1982; Mikola 1986; Mosse, 1973 and 1976).

Recently researchers have demonstrated nutrient transfer between roots of same plant or roots of different plants of the same species and of different species (Francis and Read, 1984, Heap and Newman,

1980a,b; Kessel et al 1985; Francis et al 1986). This benefit is significant in the tropics in maximising productivity in the mixed plant communities and maintaining nutrients cycling.

# 3. MATERIALS AND METHODS

## 3.1. Soils

Five Kenya soils classfied as (i) Mollic Andosol, (ii) Humic Nitosol, (iii) Chromic Luvisol, (iv) Orthic Ferralsoland (v) Plinthic Acrisol in the FAO-UNESCO Soil Classfication Legend (1974) were chosen for this study. These are agricultural soils and are intensively cultivated. They occur in different agro-climatic zones in the country. The soils sampled were from fields in Agricultural Research Stations.

The following considerations were observed in each of the chosen sites before any decision to sample was reached; the FAO-UNESCO classfication unit of the soil in the area, the soil available phosphorus had to be less than 15 ppm (Double-acid method), phosphate fertilizer had not been applied to the field for at least ten years at the time of sampling, and soil pH was slightly acidic to neutral. Background information required for this selection was obtained from Kenya Soil Survey report (Siderius and Muchena, 1977). The characteristics of the sites are described in greater detail below.

### 3.1.1. Humic Nitosol

The soil was sampled from the National Agricutural Laboratories, Nairobi, behind the Pathology Laboratory. This station is 6 km west of Nairobi City Centre and

its Centre Co-ordinates are $1^{o}15'S-36^{o}46'E$. It is at an altitude of 1740 m. The mean annual rainfall is 973 mm and falls mainly in the month of mid March to May and mid October to December. The research station is on a volcanic ridge and had an experimental field of maize, sunflower, irish potatoes, tomatoes and flowers. These are the crops grown by the surrounding community. The station originally was a woodland with Marthimia, Albizzia and Ficus as the dominant trees and Digitaria scalarum as the dominant grass.

The soil had pH 5.12 in 1:2.5 soil/water suspension and pH 4.17 in 1:2.5 soil/1 N KCl mixture. The amount of organic matter was 2.5%, nitrogen 0.19% and phosphorus 1.5 ppm (Double acid method). The soil cation exchange capacity was 21.2 me/100 g soil. The exchangeable bases measured in terms of me/100 g soil were: Ca, 5.7; Mg, 2.4; K, 9.8 and Na, 1.2. The mechanical analysis showed that the soil had high clay content (47.88%) with moderate amount of Silt (28.72%)and Sand (18.4%).

3.1.2. Chromic Luvisol

The soil was sampled from a field behind the staff houses of the National Dryland Research Station, Katumani. The research station is about 10 km south of Machakos town, Machakos district, Eastern Province.

Its centre co-ordinates are $01^{o}35'S$ - $37^{o}14'E$. It is situated in Ecological Zone IV, $P/E_o$ = 40%. The mean annual rainfall is 718 mm and falls mainly in the month of March to June and October to December. The remaining months are usually dry.

The soil is classfied as Chromic Luvisol (FAO - UNESCO 1974) and is usually deep to very deep. The station is situated on gently undulating slopes at an altitude of 1575 m. Surface sealing has been noticed (Mbuvi and Van de Weg, 1975) to be predominant hence the soils are vulnerable to erosion. Most farms are terraced for control of soil erosion.

The soil had pH 5.92 in 1:2.5 soil/water suspension and pH 5.17 in 1:2.5 soil/1 N KCl mixture. The soil contained 1.97% organic carbon, 0.14% nitrogen and was low (22 ppm) in phosphorus (Double acid method). The bases expressed as me/100 g soil were: Ca, 9.1; mg, 1.8; K, 24 and Na, 1.4. The cataion exchange capacity of the soil was 14.4 me/100 g soil. The amount of sand present was 42.4%; clay, 48.88% and silt, 8.72%.

The area was originally under acacia bushland which was cleared for cultivation and research on various cereals, maize in particular. Some research was being carried out also on sorghum, beans and

pasture. The crops grown in the locality include maize, bean, and pigeonpeas.

3.1.3. Mollic Andosol

The soil was sampled from the demonstration farm at the Pyrethrum Research Station, Molo. The Research Station is 5 km North West of Molo town, Nakuru district, Rift Valley Province. The centre co-ordinates are $0^{o}14'S$ - $35^{o}44'E$. The station is on an undulating volcanic upland at an altitude of 2550 mm. It is in Ecological Zone II. $P/E_{o}$=75%. The mean annual rainfall is 1117 mm and is well distributed throughout the year, with the highest amount in April and August. The rainfall is reliable.

The soil is classfied as Mollic Andosol (FAO-UNESCO, 1974) and is very deep and well drained. The soil had pH 6.32 in 1:2.5 soil/water mixture and pH 4.91 in 1:2.5 soil/1 N KCl suspension. The amount of organic carbon present was 3.74%, nitrogen 0.27% and phosphorus 1.75 ppm (Double acid method). The bases expressed as me/100 g soil were: Ca, 13.0; Mg, 1.2; K, 22 and Na, 1.7. The cation exchange capacity of the soil was 23.9 me/100 g soil, while the particle size distribution showed that sand constitutes 27.78%, clay, 43.52% and silt, 28.72%.

There were experimental fields for pyrethrum as well as high altitude crops and many fruit trees.

Maize and pyrethrum are the major crops grown in this area.

3.1.4. Orthic Ferralsol

The soil was sampled from a pasture field from the National Agricultural Research Station, Kitale. The research station is 3 km south west of Kitale town, Trans-Nzoia district, Rift Valley Province. The centre co-ordinates are $1^{o}01'N - 34^{o}39'E$. The station is located on a slightly undulating upland at an altitude of 1860 m and the ecological zone is III, $P/E_o$ = 66%. The mean annual rainfall is 1193 mm and falls mainly in March to September.

The soil is classified as Orthic Ferralsol (FAO-UNESCO, 1974). The soil had pH 5.49 in 1:2.5 soil/water mixture and pH 4.26 in 1:2.5 soil/1 N KCl suspension. The magnitude of organic matter was 2.57%, nitrogen 0.15% and phosphorus, 4.15 ppm (Double acid method). The bases expressed as me/100 g soil were Ca, 4.8; mg, 1.8; K, 5.8 and Na, 0.8. The cation exchange capacity of the soil was 12.7 me/100 g soil while the mechanical analysis showed that amount of sand present was 22.76%, clay, 22.5% and silt, 54.76%. Originally, the area was under moist combretum woodland to bushland which was cleared to pave way for maize and research. The local people grow mainly maize and beans.

3.1.5. Plinthic Acrisol

The soil was sampled from the Agricultural Research Station, Alupe near the Metereological Station. The research station is about 5 km North East of Busia town, Busia District, Western Province. The centre co-ordinates are $0^{o}28'N - 34^{o}07'E$. The station is situated on a gently undulating upland, at an altitude of 1178 m and ecological zone II, $P/E_{o}$ = 85%. The mean annual rainfall is 1775 mm. The area receives rain throughout the year with the highest amount in April. The rainfall is reliable and there is no real dry season.

The soil is classfied as Plinthic - Acrisol (FAO UNESCO, 1974). The soil is shallow to very shallow and is well drained. The soil had pH 5.68 in 1:2.5 soil/water suspension and pH 4.43 in 1:2.5 soil/1 N KCl mixture. The soil contained 2.77% organic carbon, 0.18% nitrogen and had low phosphorus, 8.67 ppm (Double acid method). The exchangeable bases expressed as me/100 g soil were: Ca, 6.4; mg, 1.8; K, 4.4 and Na, 1.2. The cation exchange capacity was 17.1 me/100 g soil, while the texture determination revealed that the soil had high clay (50.52%) content and moderate amounts of sand (32.76%) and silt (16.72%).

There was research being carried out on, cotton, sorghum, and animal husbandry. The local community

grows cotton, maize and sorghum.

3.2. Soil Sampling and Preparation for Analysis

The soils were sampled from uncultivated land where phosphate fertilizer had not been applied for at least the last ten years. Five spots (about 2 m apart) in the same area were selected and the grass and surface debris removed with the aid of a jembe and panga. The jembe and panga were cleared of all the soil and thoroughly washed prior to sampling at the next research station.

Surface soil samples (0 - 15 cm) were dug out using a jembe. Each spot measured 1 m x 2 m. Using a spade the soil was put into large jute bags. The jute bags were new and had been thoroughly washed and dried to avoid contamination. A card labelled in pencil with the name of the research station was put in a small polythene bag, sealed and placed in each jute bag. This way, the card could not be torn and was protected from the soil damp.

The jute bags were sealed and the soil transported back to the glass-house in a Land Rover. From each research station we carried four or five bags of soil. At the time of sampling all the soils were moist except in Katumani . From the freshly sampled soil at each site, five subsamples were put in small

polythene bags and kept in afridge at $4^{o}C$ for one month awaiting spore density determination and spore identification.

Soil samples from a given site were poured on to large polythene papers and mixed thoroughly. This ensured that a representative sample was obtained. Large clods were broken into smaller aggregates with the aid of a stick. A representative sample from each site (about 15 kg) was separated for laboratory analysis. The bulk of the soil was put in the glass house for filling in pots. Half of the soil from each site was steam sterilized at 65°C and 2.5 bars of pressure for two and half hours to kill the native mycorrhizal fungi. This process was repeated after a fortnight to kill the resistant spores. The soil was stored for one month to allow it to stabilize. Soil sterilization at high temperatures changes the soil chemically, structurally and biologically. It has been argued that high temperatures kill the microorganisms which could improve phosphate availability or the bacteria which convert manganese from solution to insoluble form. Hence manganese toxicity could occur. However, this has been discounted since there is good correlation between plant weight and percentage mycorrhizal infection suggesting that mycorrhiza function in some way to make phosphorus available and increase its uptake. The phosphorus content in all the soils was adjusted to 13 ppm using $KH_2PO_4$, except

Katumain soils whose phosphorus concentration was already high (22 ppm).

For the laboratory analysis, the soils were air dried by spreading on polythene sheets on the laboratory benches for two weeks. The dry soils were ground into fine powder using a pestle and mortar, then sieved to pass through a 2.0 mm sieve.

The soil organic carbon (%C) was determined by the Walkley and Black wet oxidation method (Black, 1965) using 0.4 g to 0.50 g of soil depending on the organic matter level. The soil pH was measured with a glass electrode pH meter and using 1:2.5 soil/water (1N KCl solution) suspension.

The total soil nitrogen content (%N) was determined by a modified kjeldahl digest method (Fleige et al. 1971).

In order to determine the exchangeable cataions, the soil was leached with neutral ammonium acetate (pH 7). Sodium and potassium in the leachate were determined by flame emission spectrophotometry.

Calcium and magnesium in the leachate were determined by titration. The cation exchange capacity (C.E.C.) of the soil was determined by distilling $NH_4^+$ in the $2^{nd}$ leachate (Black, 1965).

The particle size distribution was determined by the hydrometer 3 hour method (Day, 1965).

In soil phosphorus determination the Double-Acid Method (Black, 1965) was used.

3.3. Determination of Spore Density in the Soil

Spore population of native mycorrhizal fungi was determined using the wet sieving and decanting method (Daniels and Skipper, 1982) with slight modification (IVAM, 1985). Each soil had four replicates. Freshly sampled soil weighing 50 g was thoroughly mixed with tap water in a 500 ml beaker and stirred for five minutes with a mechanical stirrer. The suspension was left to settle for 10-15 seconds and then decanted into sieves with 355 µm sieve at the top to hold large debris and vegetative matter. Below there was a 45 µm sieve to hold the spores. This process was repeated five times in order to extract almost all the spores.

All the decanted material was directed into one end of the sieve under gently running tap water. The material was put into centrifuge tubes, suspended in water and spun for five minutes at 2700 r.p.m. The supernatant was poured into the drain and 40% sucrose solution added to the tubes till half full. The tubes were centrifuged for one minute and the spining broken. The supernatant was poured through

a clean 45 µm sieve and the spores immediately washed with distilled water. The spore material was collected in a plastic petridish with grids at the base. The spores were observed under a microscope at a magnification of x 50 and counted.

3.4. Handling of Plants in the Glass House

The experiment was conducted in a glass house at the University of Nairobi, Faculty of Agriculture, Field Station, Kabete, Kenya. One hundred and sixty pots were used. Each pot was filled with 3 kg of soil. The soil was further mixed with 1 kg of thoroughly washed sand (2-4 mm). The sand improves soil structure, aeration and drainage. Sixteen pots contained sterilised soil and sixteen others had unsterilised soil from each site. The onion was used as the test crop. The onion seeds used were acquired from East Africa Seed Company (Onions are important economically and have been found to be heavily mycorrhizal.).

Eight certified onion seeds var Red creole were sown in each pot. Thinning was done one week after germination so as to leave four plants per pot. Tap water was used to maintain soil moisture at field capacity. The 160 pots were placed in a 5 x 32 arrangement along the length of the glass house. The pots were placed on wire-mesh stands one metre above the ground level to avoid the draft of air through

the open lower part of the glass house. The bottom of the pots had perforations large enough to allow aeration and drainage. A completely randomised design was used. Randomization was done at planting and fortnightly thereafter.

The plants were harvested at 4, 8, 15 and 21 weeks after germination. At each sampling time, plants were removed from four pots containing sterilised soil and four pots containing unsterilised soil. The soil in the pots was loosened by wetting and pressing the sides of the pots gently in a rotational manner. By tilting the pot upside while holding the plants leaves, in between the V finger shape, the pot was pulled off the roots.

Plant tops were cut at the soil level and the height of the longest leaf measured using a ruler and recorded. The tops were weighed to determine the fresh weight. They were then put in labelled paper bags, dried in an oven for 72 hours at 72$^{o}$C and then weighed again.

The roots were removed from the soil. Most of the terminal feeder roots were clipped off, put in a refrigerator and kept for assessment of mycorrhizal infection. The bulk of the roots were washed to remove the soil particles and bloated to remove the adhering water, then weighed. The dry weight was determined as for the tops.

3.5. Observation of Mycorrhizal Infection

Measurement of mycorrhizal infection was based on the root slide technique (Nicolson, 1960). The terminal feeder roots were washed thoroughly and cut into 1-2 cm segements. The segments were put into 10% KOH and left to clear overnight. The KOH was drained off and the roots rinsed in tap water 3-5 times before being allowed to sit in water for 1-24 hours. The excess water was drained off and the roots placed in 0.05% trypan blue stain for 12-36 hours. The segments were rinsed in tap water 3-5 times to completely remove the excess stain.

A sample of 10-18 segments was randomly selected, put on a slide squashed under a large coverslip and examined for mycorrhizal infection under a microscope at a magnification of x 100. At early harvest, the roots were so small that fewer segments were observed. The percentage root infection was calculated according to the formula below:

$$\% \text{ root infection} = \frac{\text{Number of mycorrhizal sections}}{\text{Total number of sections examined}} \times 100$$

This method does not take into account the size of the infected area nor the stage of mycorrhizal development.

3.6. Determination of Phosphorus in Plant Tissue

The dry roots and shoots collected at each harvest were pooled, finely ground and put in labelled paper bags. Four replicates of each ground sample weighing up to 0.50 g were put in 25 cm porcelain crucibles and ignited at 500$^{o}$C for 6-8 hours in a Muffle furnace. The furnace could accomodate 30 crucibles. The ashed material was allowed to cool, and 5 ml of 1N $HNO_3$ was added to each crucible, then evaporated to dryness on a sandbath. The material was returned to the furnace and heated at 400$^{o}$C for 15-20 min. The hot crucibles were removed from the furnace with help of tongs and then allowed to cool to room temperature. To each crucible, 10 ml of 1N HCl was added. The samples were filtered through Whatman No 42 filter paper into a 50 ml volumetric flask. The crucibles and filter paper were washed 3 times with 10 ml of 0.1N HCl and the volume topped to the mark with 0.1N HCl.

For colour development, the necessary reagents were first prepared. Reagent "A" comprised of 12 g of ammonium molybdate, 0.2908 g antimony potasium tatrate and 148 ml of conc. $H_2SO_4$ (SP.g 1.98) all dissolved in 1000 ml of distilled water and made to 2000 ml. Reagent "B" was prepared by dissolving 1.054 g of ascorbic acid in 200 ml of Reagent A.

Aliqouts of 0.1 ml to 1.0 ml of the filtrate were taken in a 50 ml volumetric flask to which 8 ml of reagent B was added for colour development. The volume was topped to the mark by distilled water. A standard curve was prepared using a phosphorus solution containing 0.1 to 5 ppm. Measurements were taken on a Pye Unicam Spectrophotometer, SP-500 series at 882 µm.

3.7. Assessment of Root Infection

The rate of root infection by vescular arbuscular fungi not only depends on the amount of propagules present but also on the rate at which the propagules germinate in the soil and initiate root colonization (Abbot and Robson, 1982). Therefore the experiment aimed at studying the infection rate of onion roots grown in five Kenyan soils. Five unterilised soils, namely, Luvisol, Acrisol, Ferralsol, Nitosol and Andosol were used. Soil from each site was filled in 18 pots. Each pot contained 4-6 plants.

Harvesting of the plants was done after 14, 18, 25, 32, 39 and 53 days of growth. At each sampling time, a total of twelve plants were removed from three selected pots for each soil. After harvesting the plants, the roots were thoroughly washed, stained in trypan blue and examined for presence of mycorrhizal infection. The whole root system was used except at

day 39 and 53. The percentage root infection was then calculated.

### 3.8. Interaction of V.A.M. fungi and Phosphorus Fertilizer

Three Kenyan soils classfied as, Humic Nitosol, Mollic Andosol and Orthic Ferralsol were selected for the study. These soils were part of those used in the preceding experiments.

Soils from one site were put in 24 pots with 12 pots containing sterilised and 12 pots having unsterilized soil. This constituted 3 replicates for each phosphorus treatment. There were four levels of phosphorus used:-Initial phosphorus, adjusted phosphorus to give 20 ppm, 50 ppm and 100 ppm.

Eight certified seeds (East African Seed Company) of onions (Allium cepa L.) var Red-creolewere sown. One week after germination the plants were thinned to five plants per pot. The soil moisture content was maintained at field capacity by adding tap water. A completely randomised design was used.

The plants were allowed to grow for 12 weeks then harvested. Plant height, fresh and dry weight of shoot and root were determined. Also the percentage root infection and the phosphorus content in the tissues were analysed.

## 3.9. Analysis of Data

The data was analysed using the students' t-test and comparison was done between mycorrhizal and non-mycorrhizal plants at every sampling time for each parameter measured. For data on rate of root infection, interpretation was made using the graphs drawn. In the experiment in which phosphorus was added to the soil, comparison was made between mycorrhizal and non-mycorrhizal plants at each phosphorus treatment.

# 4. RESULTS

## 4.1. Number of Spores in the Soil

Most natural soils contain native vescular arbuscular fungi (Khan, 1971a) of varying magnitude. Low numbers of spores in the soil may result in low levels of infection (Powell, 1980). Therefore, the experiment was carried out to determine the mycorrhizal fungi spore density in some Kenyan soils.

The number of spores per gram of dry soil is shown in Table 1. It was observed that the number of spores varied amongst the soils, and ranged from as low as five to as high as eleven spores/g dry soil. The ferralsol had a significantly low number of spores while the Luvisol had a significantly higher spore count than the other three soils.

The spore population was influenced by the agricultural activities and climate at the site. The Luvisol was from a semi-arid area and the field had been cultivated. The high number of spores in this soil could be due to heavy reliance on mycorrhizae during periods of moisture stress (Lavy and Kirkum, 1980). The Acrisol, Ferralsol, Nitosol and Andosol were from virgin lands within the research stations and had lower spore counts. This results agree with those of Hayman, and Stovold (1979) who found that agricultural soils had higher spore populations than either grassland or undisturbed bushland.

Table 1: Magnitude of Vescular Arbuscular Fungal Spores

| Soil type | Spores |
|---|---|
| Plinthic Acrisol | 9[a] |
| Humic Nitosol | 7[a] |
| Mollic Andosol | 8[a] |
| Orthic Ferralsol | 5 [b] |
| Chromic Luvisol | 11 [c] |

Numbers refer to spore count per gram of dry soil. Means followed by the same letter are not significantly different. Means followed by different letters are significantly different at 5% and 1%.

4.2. Height of Plants

Height of what is a good measure of growth. Height of plants was measured to determined the time when growth differences between mycorrhizal and non-mycorrhizal plants occured.

Table 2 shows the height of plants grown in sterilised and unsterilised soil. Four weeks after germination, there was no significant difference between the height of mycorrhizal and non-mycorrhizal plants. However, the mycorrhizal plants were taller except for those in the Luvisol and Ferralsol. This could be a growth depression as suggested by Smith (1980). Eight weeks of growth showed a marked difference in height between the infected and uninfected plants. This significant difference persisted throughout up to the

end of the experiment.

The colonized plants were healthy and tall, while the uncolonized plants were stunted, unhealthy and grew shorter with time because the leaf tips withered. At this point it was observed that the first leaves were dying and falling off. This was more pronounced amongst the non-mycorrhizal plants. The colonized plants at 3rd harvest (15 weeks of growth) were much taller than those at 4th harvest (21 weeks of growth). This was due to the withering of the tops at the 4th harvest as a result of senescence.

Generally it was noted that while mycorrhizal plants were continously growing taller, the height of non-mycorrhizal plants was relatively constant except in the Luvisol. This is illustrated in plates 1 to 5. It was further observed that the mycorrhizal plants in the Luvisol and Andosol were superior to their counterparts in the Nitosol, Ferralsol and Acrisol. This can be attributed to differences in nutrient content amongst the soils. All the non-mycorrhizal plants except in Luvisol exhibited the same performance. Soil sterilization did not affect seed germination.

Table 2: Height of Onion Plants (cm)

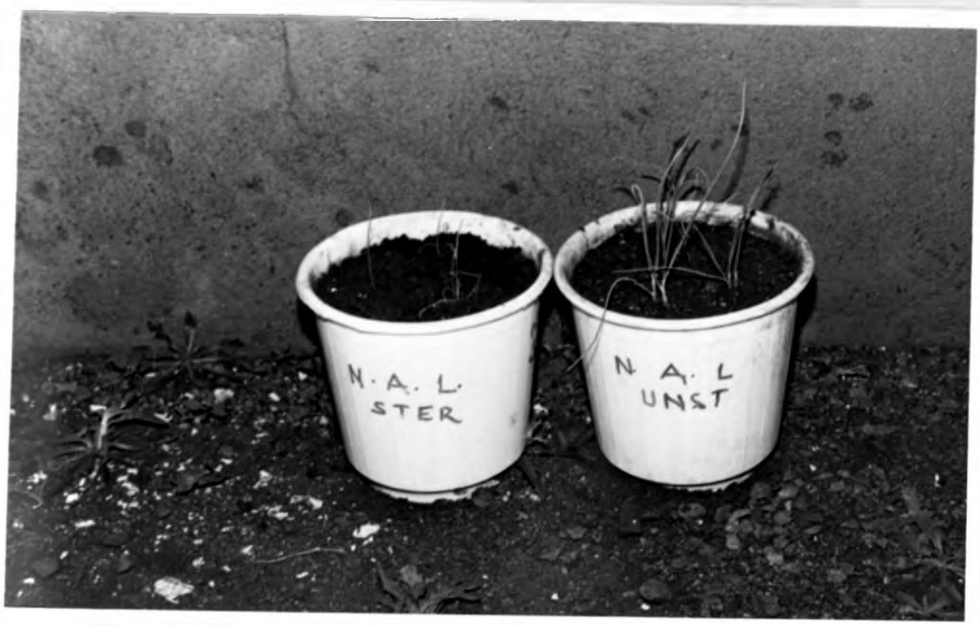
| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 14.75$^{NS}$ | 16.06$^{**}$ | 36.48$^{**}$ | 40.79$^{**}$ |
| Sterilised | 13.93 | 10.55 | 11.72 | 10.47 |
| Humic Nitosol | | | | |
| Unsterilised | 12.95$^{NS}$ | 15.33$^{*}$ | 30.23$^{**}$ | 40.70$^{**}$ |
| Sterilised | 12.83 | 10.56 | 9.80 | 9.74 |
| Mollic Andosol | | | | |
| Unsterilised | 17.48$^{NS}$ | 33.77$^{**}$ | 54.30$^{**}$ | 47.04$^{**}$ |
| Sterilised | 15.68 | 14.77 | 14.55 | 18.78 |
| Orthic Ferralsol | | | | |
| Unsterilised | 13.10$^{NS}$ | 14.49$^{*}$ | 25.06$^{**}$ | 42.71$^{**}$ |
| Sterilised | 14.13 | 11.88 | 11.63 | 10.46 |
| Chromic Luvisol | | | | |
| Unsterilised | 13.15$^{NS}$ | 30.00$^{**}$ | 62.63$^{**}$ | 56.10$^{**}$ |
| Sterilised | 14.15 | 14.89 | 20.28 | 26.51 |

Values are means of four replicates comprising of a total of twelve plants. NS, Not significant. **,* significantly different from non-mycorrhizal plants at 1% and 5% respectively.


a 8 weeks b

c 21 weeks d

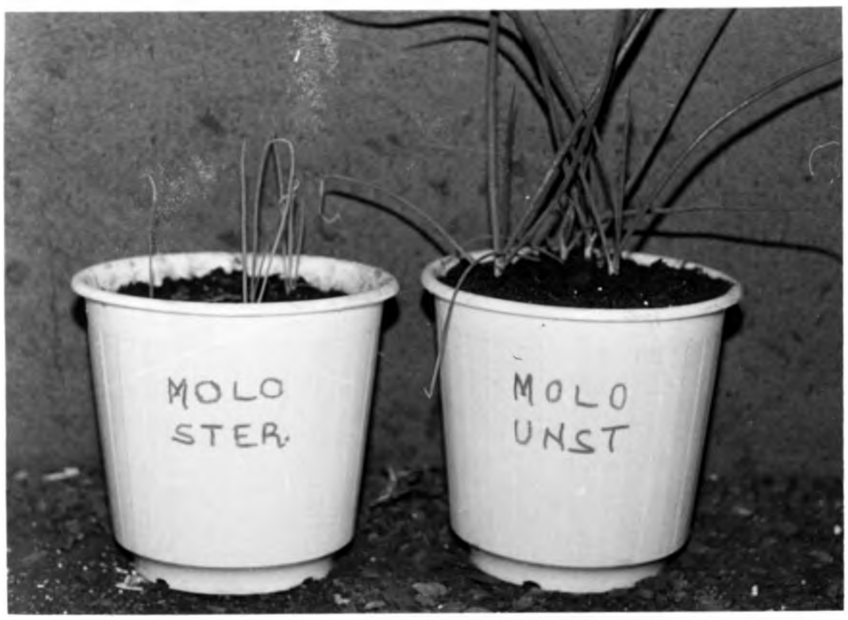
Plate 1: Plate 1 a/d shows the growth of onions in sterilised Acrisol after 8 and 21 weeks of growth respectively. Plate 1 b/c shows growth of onions in unsterilised Acrisol 8 and 21 weeks of growth respectively.




a 8 weeks b

c 21 weeks d

Plate 2: Plate 2a/d shows the growth of onions in sterilised Nitosol after 8 and 21 weeks of growth respectively. Plate 2b/c shows growth of onions in Unsterilised Nitosol after 8 and 21 weeks of growth




a 8 weeks b

c 21 weeks d

Plate 3: Plate 3a/c shows the growth of onions in sterilised Andosol after 8 and 21 weeks of growth. Plate 3b/d shows growth of onions in unsterilised Andosol after 8 and 21 weeks of growth


a 8 weeks b

c 21 weeks d

Plate 4: Plate 4a/d shows the growth of onions in sterilised Ferralsol after 8 and 21 weeks of growth. Plate 4b/c shows growth of onions in unsterilised Ferralsol after 8 and 21 weeks of growth


a 8 weeks b

c 21 weeks d

Plate 5: Plate 5b/c shows growth of onions in sterilised Luvisol after 8 and 21 weeks of growth. Plate 5a/d shows growth of onions in unsterilised Luvisol after 8 and 21 weeks

4.3. Fresh and Dry Weight of Shoot

Fresh and dry weight of shoot are good indicators of growth. These two parameters were measured to determine the difference between the growth rates of mycorrhizal and non-mycorrhizal plants.

Shoot fresh and dry weight of mycorrhizal and non-mycorrhizal plants is shown in Table 3a and 3b respectively. At first harvest, there were varying values for shoot fresh weight. In the nitosol and acrisol there was no significant difference between mycorrhizal and non-mycorrhizal plants. In the other three soils, there were differences with the mycorrhizal plants in the Andosol having superior weights to non-mycorrhizal plants. On the contrary, the uninfected plants in the Ferrolsol and Luvisol exhibited higher fresh weights than the infected ones.

At the second harvest all the mycorrhizal plants had higher fresh weights than the non-mycorrhizal ones. This difference became greater with time and was significant at $P = 0.05$ and even $P = 0.01$. There was tremendous increase in fresh weight from 8 to 15 weeks implying that this is a period of rapid growth. The mycorrhizal plants in the Luvisol and Andosol showed no significant increase in weight from 15 - 21 weeks indicating that maximum growth had been reached.

Table 3a: Shoot Fresh Weight (g) Per Onion Plant

| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0.11$^{NS}$ | 0.31$^{**}$ | 4.03$^{**}$ | 7.26$^{**}$ |
| Sterilised | 0.11 | 0.14 | 0.16 | 0.12 |
| Humic Nitosol | | | | |
| Unsterilised | 0.12$^{NS}$ | 0.44$^{*}$ | 4.13$^{**}$ | 9.02$^{**}$ |
| Sterilised | 0.08 | 0.12 | 0.10 | 0.09 |
| Mollic Andosol | | | | |
| Unsterilised | 0.20$^{*}$ | 2.70$^{**}$ | 20.84$^{**}$ | 17.13$^{**}$ |
| Sterilised | 0.15 | 0.24 | 0.40 | 0.68 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.10$^{*}$ | 0.37$^{**}$ | 2.24$^{**}$ | 7.86$^{**}$ |
| Sterilised | 0.14 | 0.16 | 0.18 | 0.12 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.12$^{*}$ | 2.01$^{**}$ | 25.25$^{**}$ | 26.35$^{**}$ |
| Sterilised | 0.16 | 0.31 | 0.97 | 2.11 |

Figures are means of four replicates comprising of a total of twelve plants. NS, Not Significant. **,* significantly different from non-mycorrhizal plants at 1% and 5% respectively.

Table 3b: Shoot Dry Weight (g) Per Onion Plant

| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0.01$^{NS}$ | 0.03$^{*}$ | 0.37$^{**}$ | 0.80$^{**}$ |
| Sterilised | 0.10 | 0.02 | 0.03 | 0.02 |
| Humic Nitosol | | | | |
| Unsterilised | 0.01$^{NS}$ | 0.04$^{*}$ | 0.37$^{**}$ | 0.85$^{**}$ |
| Sterilised | 0.01 | 0.01 | 0.02 | 0.01 |
| Mollic Andosol | | | | |
| Unsterilised | 0.02$^{NS}$ | 0.18$^{**}$ | 2.20$^{**}$ | 1.84$^{**}$ |
| Sterilised | 0.01 | 0.03 | 0.06 | 0.10 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.01$^{NS}$ | 0.03$^{*}$ | 0.23$^{**}$ | 0.69$^{**}$ |
| Sterilised | 0.01 | 0.02 | 0.03 | 0.02 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.01$^{NS}$ | 0.13$^{**}$ | 1.95$^{**}$ | 2.55$^{**}$ |
| Sterilised | 0.01 | 0.04 | 0.12 | 0.26 |

Figures are means of four replicates comprising of a total of twelve plants. NS, Not significant. **,* significantly different from non-mycorrhizal plants at 1% and 5% respectively.

On the contrary, the mycorrhizal plants in the Nitosol, Acrisol and Ferralsol still displayed rapid growth at the 21st week.

The fresh weight of the non-mycorrhizal plants was relatively constant throughout the experiment with an exception of the plants in the Luvisol and Andosol in which there was an increase with time.

The shoot of mycorrhizal plants was thicker. Shoot dry weight at first harvest (4 weeks of growth) reflected the results of the fresh weight though there is no significant difference between mycorrhizal and non-mycorrhizal plants in Andosol and Ferralsol. It would appear that the plants had an increased water uptake without a corresponding increase in dry matter. At second harvest (eight weeks of growth) there was significant difference between mycorrhizal and non-mycorrhizal shoot dry weight. This trend continued upto the end of the experiment. Increase in shoot dry weight expressed as a percentage of the non-mycorrhizal plants ranged from 990% to 6983%. These results agree with the findings of many researchers (Khan, 1972; Wamocho et al., 1984; Mosse, 1973; Sanders et al. 1977).

4.4. Root Fresh and Dry Weight

Mycorrhizal plants have been found to have highly branched roots and greater root weight (Daft and Nicolson, 1969a; Mosse, 1973; Hayman, and Mosse, 1971), hence the experiment aimed at comparing the root weight of mycorrhizal and non-mycorrhizal plants.

Root fresh and dry weight of mycorrhizal and non-mycorrhizal plants is shown in Table 4a and 4b respectively. Root fresh weight of mycorrhizal plants at first harvest (four weeks of growth) was not significantly different from that of non-mycorrhizal plants, though mycorrhizal plants had higher weights. The exception was in the Luvisol where non-mycorrhizal plants had higher fresh weight. At 2$^{nd}$ harvest (eight weeks of growth) the mycorrhizal plants had significantly higher root fresh weight. This difference was maintained upto the 21$^{st}$ week of growth.

It is worth pointing out that the values indicated in the Table (4a) are approximated for the roots were loosing water fairly fast. The situation was further complicated by the fact that the bloating of water from the roots may not have been complete. The roots of non-mycorrhizal plants did not show marked increase in weight with time as in the case of mycorrhizal plants whose roots were still increasing in length even at the 21$^{st}$ week.

Roots of mycorrhizal plants were well developed and highly branched contrary to those of uninfected plants which were less branched. This agree with the findings of Hayman and Mosse, (1971). The freshly dug mycorrhizal roots were more yellow than those from sterilised soils (Becker and Gerdemann, 1977).

Table 4a: Root Fresh Weight (g) per Onion Plant

| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0.03$^{NS}$ | 0.19$^{**}$ | 1.41$^{**}$ | 3.56$^{**}$ |
| Sterilised | 0.02 | 0.09 | 0.14 | 0.19 |
| Humic Nitosol | | | | |
| Unsterilised | 0.03$^{NS}$ | 0.26$^{**}$ | 1.42$^{**}$ | 2.09$^{**}$ |
| Sterilised | 0.03 | 0.08 | 0.05 | 0.04 |
| Mollic Andosol | | | | |
| Unsterilised | 0.04$^{NS}$ | 0.91$^{**}$ | 7.78$^{**}$ | 9.35$^{**}$ |
| Sterilised | 0.03 | 0.17 | 0.40 | 0.53 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.04$^{NS}$ | 0.21$^{*}$ | 0.85$^{**}$ | 2.64$^{**}$ |
| Sterilised | 0.03 | 0.12 | 0.15 | 0.16 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.03$^{*}$ | 0.61$^{**}$ | 7.21$^{**}$ | 11.62$^{**}$ |
| Sterilised | 0.05 | 0.27 | 1.00 | 2.41 |

Figures are means of four replicates comprising of a total of twelve plants. NS, Not significant. **,* significantly different from non-mycorrhizal plants at 1% and 5% respectively.

Table 4b: Root Dry Weight (g) per Onion Plant

| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0.002$^{NS}$ | 0.010$^{**}$ | 0.080$^{**}$ | 0.190$^{**}$ |
| Sterilised | 0.001 | 0.006 | 0.005 | 0.007 |
| Humic Nitosol | | | | |
| Unsterilised | 0.002$^{NS}$ | 0.014$^{*}$ | 0.080$^{**}$ | 0.120$^{**}$ |
| Sterilised | 0.001 | 0.005 | 0.007 | 0.004 |
| Mollic Andosol | | | | |
| Unsterilised | 0.002$^{*}$ | 0.037$^{**}$ | 0.400$^{**}$ | 0.500$^{**}$ |
| Sterilised | 0.001 | 0.010 | 0.020 | 0.045 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.001$^{NS}$ | 0.011$^{**}$ | 0.050$^{**}$ | 0.100$^{**}$ |
| Sterilised | 0.001 | 0.008 | 0.009 | 0.010 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.002$^{NS}$ | 0.024$^{**}$ | 0.350$^{**}$ | 0.550$^{**}$ |
| Sterilised | 0.001 | 0.016 | 0.056 | 0.140 |

Figures are means of four replicates comprising of a total of twelve plants. NS, Not significant. **,* significantly different from non-mycorrhizal plants at 1% and 5% respectively.

The root dry weight followed the same trend as that of the fresh weight.

4.5. Root: Shoot Ratio

It has been reported that mycorrhizal plants normally have a lower root:shoot ratio as compared to non-mycorrhizal (Sanders, 1975; Hayman and Mosse, 1971; Mosse and Hayman, 1971). Therefore the experiment was carried out to verify this fact in our Kenyan soils.

The root:shoot ratio was based on the dry weights of shoots and roots of respective plants at each harvest and the values are shown in Table 5. Mycorrhizal plants had higher root:shoot ratio values after four weeks of growth. Eight weeks of growth showed that the ratio increased for both mycorrhizal and non-mycorrhizal plants, though the uncolonized plants exhibited higher values except in the nitosol.

After 15 weeks of growth, there was a decrease in the ratio for mycorrhizal plants except in a few cases where there was a slight increase. This increase is accounted for by the decrease in shoot dry weight due to senescence. The root:shoot ratio for plants in sterilised soils remained higher except in the acrisol. Although the mycorrhizal roots weighed more and therefore were more extensive and exploited more soil, the root:shoot ratios of mycorrhizal plants were smaller than those

Table 5: Root:Shoot ratio of Onion Plants

| | WEEKS AFTER GERMINATION | | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0.13$^{NS}$ | 0.36$^{NS}$ | 0.22$^{NS}$ | 0.24$^{NS}$ |
| Sterilised | 0.10 | 0.38 | 0.24 | 0.35 |
| Humic Nitosol | | | | |
| Unsterilised | 0.14$^{NS}$ | 0.41$^{NS}$ | 0.22$^{NS}$ | 0.14$^{NS}$ |
| Sterilised | 0.13 | 0.32 | 0.37 | 0.27 |
| Mollic Andosol | | | | |
| Unsterilised | 0.10$^{NS}$ | 0.20$^{**}$ | 0.18$^{**}$ | 0.27$^{**}$ |
| Sterilised | 0.07 | 0.38 | 0.33 | 0.45 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.10$^{NS}$ | 0.37$^{NS}$ | 0.22$^{NS}$ | 0.14$^{**}$ |
| Sterilised | 0.08 | 0.38 | 0.30 | 0.50 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.11$^{NS}$ | 0.18$^{**}$ | 0.18$^{**}$ | 0.22$^{**}$ |
| Sterilised | 0.07 | 0.40 | 0.42 | 0.54 |

Figures are means of four replicates. NS, Not significant.

**,* significantly different at 1% and 5% respectively.

of non-mycorrhizal plants, in the same soil. This results confirms the findings of Hayman and Mosse, (1971).

4.6. Percentage Root Infection

Plants infected with vescular arbuscular fungi often take up more phosphorus from phosphorus deficient soils than uninfected plants (Gerdeman, 1975, Mosse, 1973). However, infection intensity is not always proportional to mycorrhizal effects. Therefore the experiment aimed at determining the rate of root colonization.

The values for percentage root infection are given in Table 6. It was observed that throughout the experiment, the plants in the sterilised soils remained uninfected. As for the plants in unsterilised soils, at first harvest, the percentage infection ranged from 43.5% to 84.5%. Though the Luvisol had the highest number of spores per gram of soil, it exhibited the least percentage root infection. This could be due to the slow germination of spores since the soil was sampled during the dry season.

By the 2$^{nd}$ harvest all the mycorrhizal plants had high root infections and ranged from 85.0% to 100%. This high infection was maintained upto the end of the experiment.

Table 6: Percentage Root Infection of Onion Plants

| | WEEKS | AFTER | GERMINATION |
|---|---|---|---|
| Plinthic Acrisol | | | |
| Unsterilised | 63.20 | 85.80 | 97.10 | 90.67 |
| Sterilised | 0 | 0 | 0 | 0 |
| Humic Nitosol | | | |
| Unsterilised | 68.20 | 85.00 | 95.60 | 92.30 |
| Sterilised | 0 | 0 | 0 | 0 |
| Mollic Andosol | | | |
| Unsterilised | 84.50 | 100.00 | 96.50 | 91.50 |
| Sterilised | 0 | 0 | 0 | 0 |
| Orthic Ferralsol | | | |
| Unsterilised | 55.60 | 90.10 | 96.83 | 93.80 |
| Sterilised | 0 | 0 | 0 | 0 |
| Chromic Luvisol | | | |
| Unsterilised | 43.60 | 93.00 | 95.00 | 94.12 |
| Sterilised | 0 | 0 | 0 | 0 |

The figures are means of four replicates comprising of 40 - 72 segments.

In all soils roots of onions were infected throughout the root length especially in the latter stages. Internal hyphae ran longitudinally through the roots. Presence of vessicles was evident from the second harvest. Quite often clumps of external hyphae attached to onion roots extended outwards into surrounding soil. It was observed that there was heavy infection in the upper roots as manifested by yellow pigmentation. The lower roots and those at the pot/soil interphase were uninfected and were white in appearance as shown on Plate 6.

4.7. Percentage Phosphorus in Plants

The experiment was carried out to determine the percentage phosphorus in both mycorrhizal and non-mycorrhizal plants. Table 7 shows the amount of phosphorus taken up by plants grown in unsterilised and sterilised soil.

After four weeks of growth mycorrhizal plants in two soils had significantly higher phosphorus content than non-mycorrhizal ones. In one soil, the non-mycorrhizal plants had more phosphorus than the infected plants and in the other two soils, there was no significant difference in phosphorus content. At $2^{nd}$, $3^{rd}$ and $4^{th}$ harvest, the mycorrhizal plants had significantly higher phosphorus than the non-mycorrhizal plants. In the case of mycorrhizal plants, there was a continous increase in phosphorus content with time

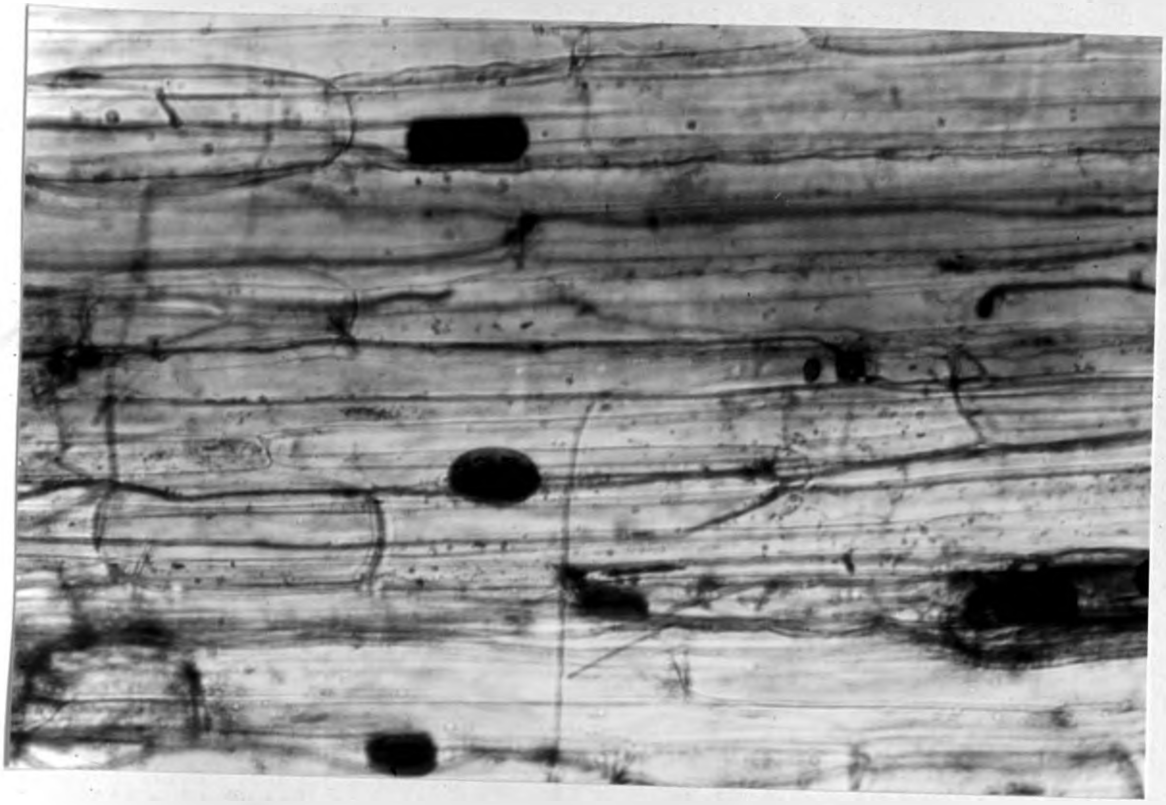


Plate 6: General view of root colonization by VAM fungi showing internal hyphae and vesicle.

Table 7: Percent Phosphorus in Tissue of Onion Plants

| Soil Acrisol | 4 WEEKS | 8 WEEKS | 15 WEEKS | 21 WEEKS AFTER GERMINATION |
|---|---|---|---|---|
| Plinthic Acrisol | | | | |
| Unsterilised | 0.08$^{NS}$ | 0.17$^{**}$ | 0.21$^{**}$ | 0.22$^{**}$ |
| Sterilised | 0.11 | 0.08 | 0.07 | 0.07 |
| Humic Nitosol | | | | |
| Unsterilised | 0.09$^{**}$ | 0.24$^{**}$ | 0.22$^{**}$ | 0.23$^{**}$ |
| Sterilised | 0.15 | 0.14 | 0.09 | 0.11 |
| Mollic Andosol | | | | |
| Unsterilised | 0.25$^{**}$ | 0.29$^{**}$ | 0.35$^{**}$ | 0.53$^{**}$ |
| Sterilised | 0.10 | 0.06 | 0.07 | 0.12 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0.09$^{NS}$ | 0.20$^{**}$ | 0.29$^{**}$ | 0.27$^{**}$ |
| Sterilised | 0.09 | 0.07 | 0.10 | 0.06 |
| Chromic Luvisol | | | | |
| Unsterilised | 0.19$^{*}$ | 0.29$^{**}$ | 0.39$^{**}$ | 0.51$^{**}$ |
| Sterilised | 0.14 | 0.09 | 0.09 | 0.10 |

Values are means of four replicates. NS, Not significant.

**,* significantly different at 1% and 5% respectively.

unlike in non-mycorrhizal plants whose phosphorus content was apparently constant. Thus mycorrhizal plants which took up more phosphorus were bigger. Expressed as percentage increase over the non-mycorrhizal plants (sterilized soils) the values obtained for phosphorus at the $21^{st}$ week of growth were 214.3, 109.1, 383.3, 350 and 410% corresponding to Acrisol, Nitosol, Andosol Ferralsol and Luvisol respectively. This findings are similar to those of Wamocho et al 1984. It has been observed that mycorrhizal plants take in more phosphorus than non-mycorrhizal plants and thus increased absorption is accompanied by increase in growth (Mosse, 1975).

4.8. Bulb Diameter

Phosphorus promotes development of root system and seed formation. It is also believed that phosphorus aid in bulb development. Since mycorrhizal plants absorb more phosphorus than the non-mycorrhizal ones, their bulb weight is greater and have larger bulbs diameter (Wamocho, 1984, Nelsen et al. 1981).

The experiment therefore investigated the difference between bulb diameter and bulb fresh weight of mycorrhizal and non-mycorrhizal plants. Table 8 shows the diameter of bulbs of onions grown in unsterilised and sterilised soil. All the plants in sterilised soils did not form bulbs except in andosol and luvisol where bulb formation started after 15 weeks of growth and even the bulbs formed were small.

Table 8: Onion Diameter (cm)

| | | WEEKS AFTER GERMINATION | | |
|---|---|---|---|---|
| Soil Type | 4 | 8 | 15 | 21 |
| Plinthic Acrisol | | | | |
| Unsterilised | 0 | 0.22$^{NS}$ | 0.56$^{**}$ | 1.11$^{**}$ |
| Sterilised | 0 | 0.20 | 0.20 | 0.20 |
| Humic Nitosol | | | | |
| Unsterilised | 0 | 0.25$^{*}$ | 0.58$^{**}$ | 1.30$^{**}$ |
| Sterilised | 0 | 0.20 | 0.20 | 0.20 |
| Mollic Andosol | | | | |
| Unsterilised | 0 | 0.45$^{**}$ | 1.45$^{**}$ | 2.56$^{**}$ |
| Sterilised | 0 | 0.20 | 0.29 | 0.36 |
| Orthic Ferralsol | | | | |
| Unsterilised | 0 | 0.25$^{*}$ | 0.52$^{**}$ | 0.98$^{**}$ |
| Sterilised | 0 | 0.20 | 0.20 | 0.20 |
| Chromic Luvisol | | | | |
| Unsterilised | 0 | 0.38$^{**}$ | 1.06$^{**}$ | 2.79$^{**}$ |
| Sterilised | 0 | 0.23 | 0.35 | 0.55 |

Figures are means of four replicates comprising of twelve bulks. NS, Not significant. **,* significantly different at 1% and 5% respectively.

After four weeks of growth, none of the mycorrhizal plants had formed bulbs but by the 8$^{th}$ week bulb formation had commenced and was quite noticeable for plants grown in Andosol and Luvisol. Fifteen weeks of growth produced pronounced bulbs on mycorrhizal plants. The bulb diameter increased even after 21 weeks of growth.

4.9. Bulb Fresh Weight

The fresh weight of the bulbs was determined after 21 weeks of growth as shown in Table 9. The mean fresh weight of a bulb ranged from 1.18 g to 14.8 g. Plants grown in three soils produced bulbs of low weight while plants in the other two soils produced bulbs of high weight. The plants raised in Luvisol and Andosol had more phosphorus in the tissues hence the high bulb fresh weight and the large bulb diameter. These results concur with those of Wamocho (1984).

4.10. Effects of High Phosphorus Levels on Growth of Host Plant

Many researchers have shown that infection with vescular arbuscular mycorrhizae causes increased growth of plants when the amounts of available phosphorus is a limiting factor (Gerdamann, 1964; Mosse, 1973). The increased growth is accompanied by higher concentrations of nutrients (Mosse, 1975). However, growth ratios between mycorrhizal and non-mycorrhizal plants decreases with increasing amounts of soil

Table 9: Fresh Weight of Bulb per Plant (g)

| Soil Type | Bulb Fresh Weight (g) |
|---|---|
| Plinthic Acrisol | 1.48 [a] |
| Humic Nicosol | 2.50 [a] |
| Mollic Andosol | 12.63 [b] |
| Orthic Ferralsol | 1.18 [a] |
| Chromic Luvisol | 14.47 [b] |

Numbers are means of four replicate consisting of 12 bulbs. Means followed by the same letter are not significantly different. Means followed by different letters are significantly are 5% and 1%.

phosphorus (Khan, 1975; Baylis, 1967) and infact at extreme quantities of soil phosphorus, the growth of non-mycorrhizal plants can exceed that of infected plants (Smith, 1982). It has also been observed that at high soil phosphorus levels the percentage root colonization is reduced (Ssali and Keya, 1986; Daft and Nicolson, 1969; Menge, 1978). Therefore, another experiment was carried out to study the effects of high phosphorus levels on growth of host plants.

Tables 10 a, 10 b, and 10 c show the heights, fresh and dry weights of shoot and root, percent phosphate concentration and root:shoot ratio of plants grown at different levels of phosphorus. In all the soils, both fresh and dry weight of shoot and root, and height and percentage phosphorus of the mycorrhizal plants was significantly higher than in non-mycorrhizal plants when no phosphorus was added to the soil and at 20 ppm. This results concur with those of Daft and Nicolson (1966), Mosse (1973, 1975).

At 50 ppm of phosphorus the values for the above parameters were variable. The percentage phosphorus root fresh and dry weight, shoot fresh and dry weight for plants were significantly greater than those of non-colonised plants at p (0.5) and even p (0.1). As for height, there was no significant difference except in Humic Nitosol.

At 100 ppm of phosphorus, there was no significant difference between colonised and uncolonised plants for the parameters measured, except in the Andosol where the uncolonised plants had higher shoot and root dry weight (Smith, 1982).

It was noted that the dry matter in both mycorrhizal and non-mycorrhizal plants increased with elevation of soil phosphorus. It was further observed that the mycorrhizal plants at 20, 50 and 100 ppm of phosphorus were similar and were significantly superior to plants at initial level of soil phosphorus. The exception was in the Ferralsol where mycorrhizal plants at 50 ppm performed better than those at 100 ppm. There was a general increase in phosphorus content of plant tissue as the soil phosphorus increased. Hence there is need for fertilizer application. In all three soils, plants grown at the initial soil phosphorus had the lowest amount of phosphorus in the tissue since the pool from which they were extracting phosphorus was limited. Mycorrhizal infection tended to decline with increasing additions of phosphate.

Table 10a: Growth Parameter and Mycorrhizal Infection of Onions Grown in a Nitosol

| P Level | Root Fresh Weight (g) | Root Dry Weight(g) | Shoot Fresh Weight(g) | Shoot Dry Weight(g) | Root:Shoot | % Root Infection | Plant Height (cm) | %P in Plant tissue |
|---|---|---|---|---|---|---|---|---|
| Unsterilised Soi P | 0.44** | 0.02** | 0.71** | 0.05** | 0.40 | 82.30 | 21.17** | 0.19** |
| Sterilised | 0.10 | 0.004 | 0.12 | 0.02 | 0.20 | 0 | 9.97 | 0.08 |
| Unsterilised 20 | 1.16** | 0.05** | 3.32** | 0.24** | 0.21 | 74.10 | 35.00** | 0.33** |
| Sterilised | 0.20 | 0.10 | 0.22 | 0.03 | 0.33 | 0 | 12.51 | 0.11 |
| Unsterilised 50 | 1.01** | 0.04* | 3.59** | 0.25* | 0.16 | 71.30 | 38.20* | 0.39* |
| Sterilised | 0.67 | 0.03 | 1.59 | 0.12 | 0.25 | 0 | 27.80 | 0.31 |
| Unsterilised 100 | 1.75NS | 0.07NS | 5.23* | 0.37NS | 0.19 | 65.80 | 41.62NS | 0.44* |
| Sterilised | 2.02 | 0.09 | 3.68 | 0.31 | 0.29 | 0 | 36.07 | 0.32 |

Figures are means of three replicates comprising of twelve plants.

NS, Not Significant .**.* significantly different at 1% and 5% respectively.

Table 10b: Growth Parameters and Mycorrhizal Infection of Onions Grown in a Ferralsol

| P Level (in ppm) | Root Fresh Weight(g) | Root Dry Weight(g) | Shoot Fresh Weight(g) | Shoot Dry Weight(g) | Root:Shoot | % Root Infect-ion | Plant Height (cm) | % P in Plant tissue |
|---|---|---|---|---|---|---|---|---|
| Unsterilised Soil P | 0.58** | 0.03** | 1.22** | 0.11** | 0.27 | 84.50 | 27.21** | 0.23** |
| Sterilised | 0.22 | 0.01 | 0.34 | 0.03 | 0.33 | 0 | 14.68 | 0.10 |
| Unsterilised 20 | 1.41** | 0.07** | 3.80** | 0.27** | 0.26 | 74.40 | 36.43** | 0.44** |
| Sterilised | 0.54 | 0.02 | 0.71 | 0.06 | 0.33 | 0 | 22.83 | 0.13 |
| Unsterilised 50 | 1.64* | 0.08** | 4.61** | 0.31** | 0.26 | 61.50 | 38.57NS | 0.45** |
| Sterilised | 0.93 | 0.04 | 2.51 | 0.18 | 0.22 | 0 | 34.03 | 0.38 |
| Unsterilised 100 | 1.11NS | 0.05NS | 2.62NS | 0.27NS | 0.19 | 59.00 | 34.67NS | 0.58** |
| Sterilised | 1.32 | 0.05 | 4.35 | 0.29 | 0.17 | 0 | 40.40 | 0.50 |

Figures are means of three replicates comprising of twelve plants.

NS, Not Significant. **,* significantly different at 1% and 5% respectively.

Table 10c: Growth Parameters and Mycorrhizal Infection of Onions Grown in an Andosol

| P Level (in ppm) | Root Fresh Weight(g) | Root Dry Weight(g) | Shoot Fresh Weight(g) | Shoot Dry Weight(g) | Root:Shoot | % Root Infect-ion | Plant Height (cm) | % P in Plant tissue |
|---|---|---|---|---|---|---|---|---|
| Unsterilised Soil P | 1.86** | 0.07** | 3.72** | 0.26** | 0.27 | 92.10 | 37.77** | 0.32** |
| Sterilised | 0.26 | 0.01 | 0.25 | 0.02 | 0.39 | 0 | 12.41 | 0.10 |
| Unsterilised 20 | 2.92** | 0.13** | 6.93** | 0.75** | 0.17 | 78.00 | 43.39** | 0.42** |
| Sterilised | 0.61 | 0.03 | 0.57 | 0.05 | 0.60 | 0 | 17.58 | 0.12 |
| Unsterilised 50 | 2.82* | 0.14* | 6.00NS | 0.75* | 0.19 | 68.10 | 40.40NS | 0.47NS |
| Sterilised | 2.03 | 0.08 | 6.63 | 0.42 | 0.19 | 0 | 43.30 | 0.45 |
| Unsterilised 100 | 3.65* | 0.18* | 7.92* | 0.99NS | 0.18 | 65.10 | 43.35NS | 0.50NS |
| Sterilised | 4.98 | 0.20 | 11.02 | 1.04 | 0.19 | 0 | 46.78 | 0.47 |

Figures are means of three replicates comprising of twelve plants.

NS, not Significant. **,* Significantly different at 1% and 5% respectively.

4.11. Rate of Root Colonization

Most soils contain native mycorrhizal fungal spores (Khan, 1971a) and the root infection by these fungi depends both on spore density and rate of germination. Low spore population may result in low levels of infection (Powell, 1980).

The experiment was therefore carried out to determine the rate of onion root colonization by mycorrhizal fungi in five Kenyan soils.

Figures 1a to 1e show the extent of root infection with time. It was observed that by the $14^{th}$ day after seeding no germination had occured. At the $18^{th}$ day, spores germination had started and root infection had commenced. The percentage root infection increased in a geometric manner.

Percent root colonization is depicted in Fig 1. All graphs had a lag phase except for the andosol. The initial lag phase of infection was more pronounced in soils containing low spore population. This could imply that considerable time is required for the few spores to germinate and initiate root infection. Maximum root infection was attained by the $39^{th}$ day of growth

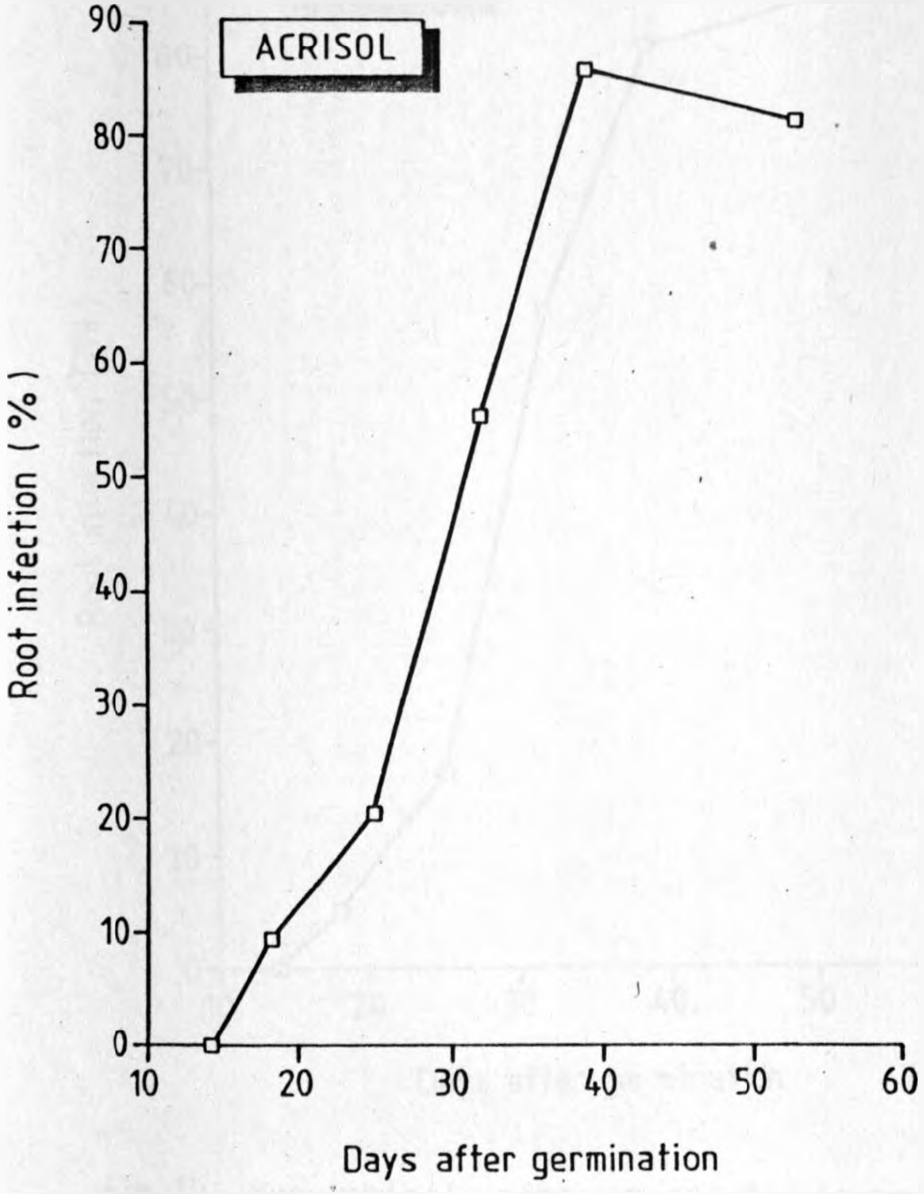



Fig. 1a. Mycorrhizal infection and development in onions grown in an Acrisol.

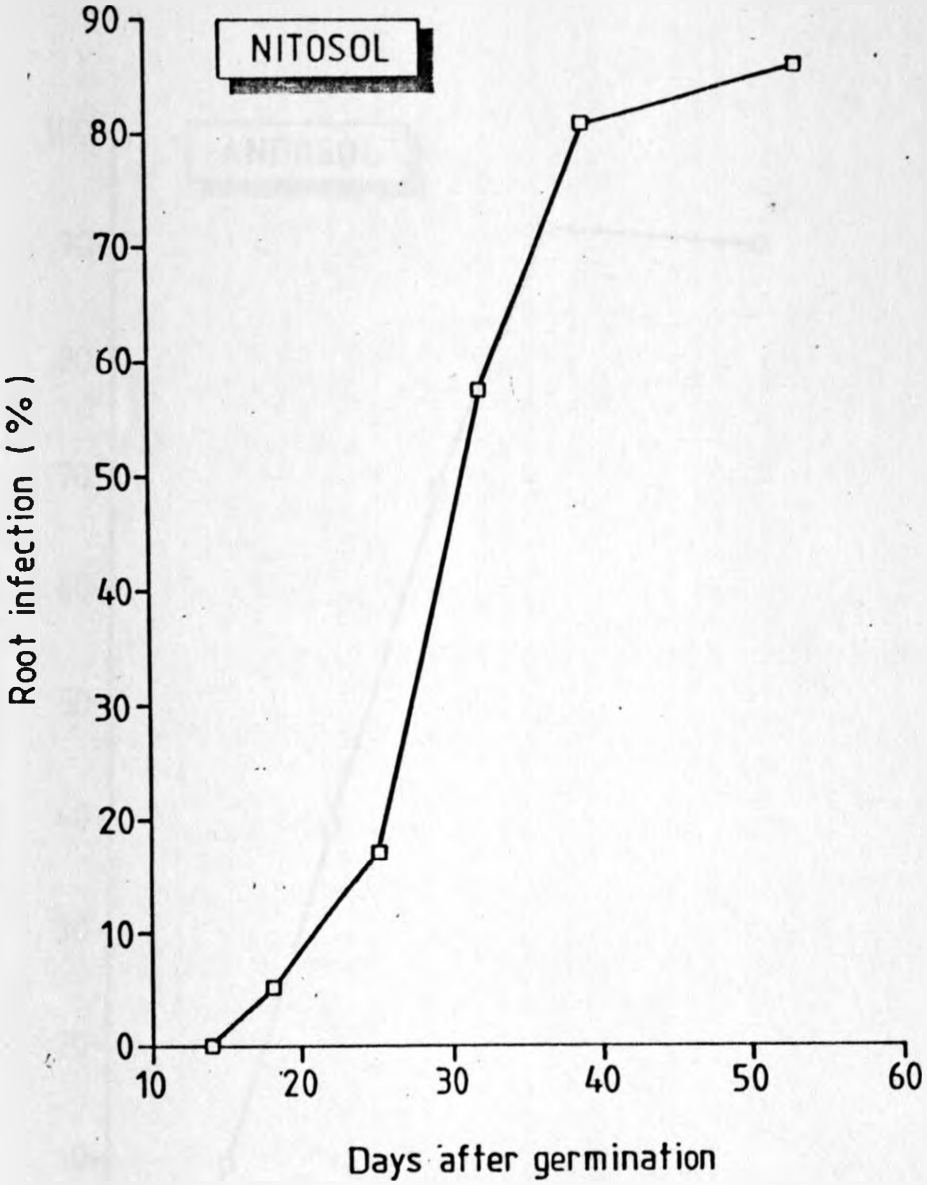



Fig. 1b. Mycorrhizal infection and development in onions grown in a Nitosol.

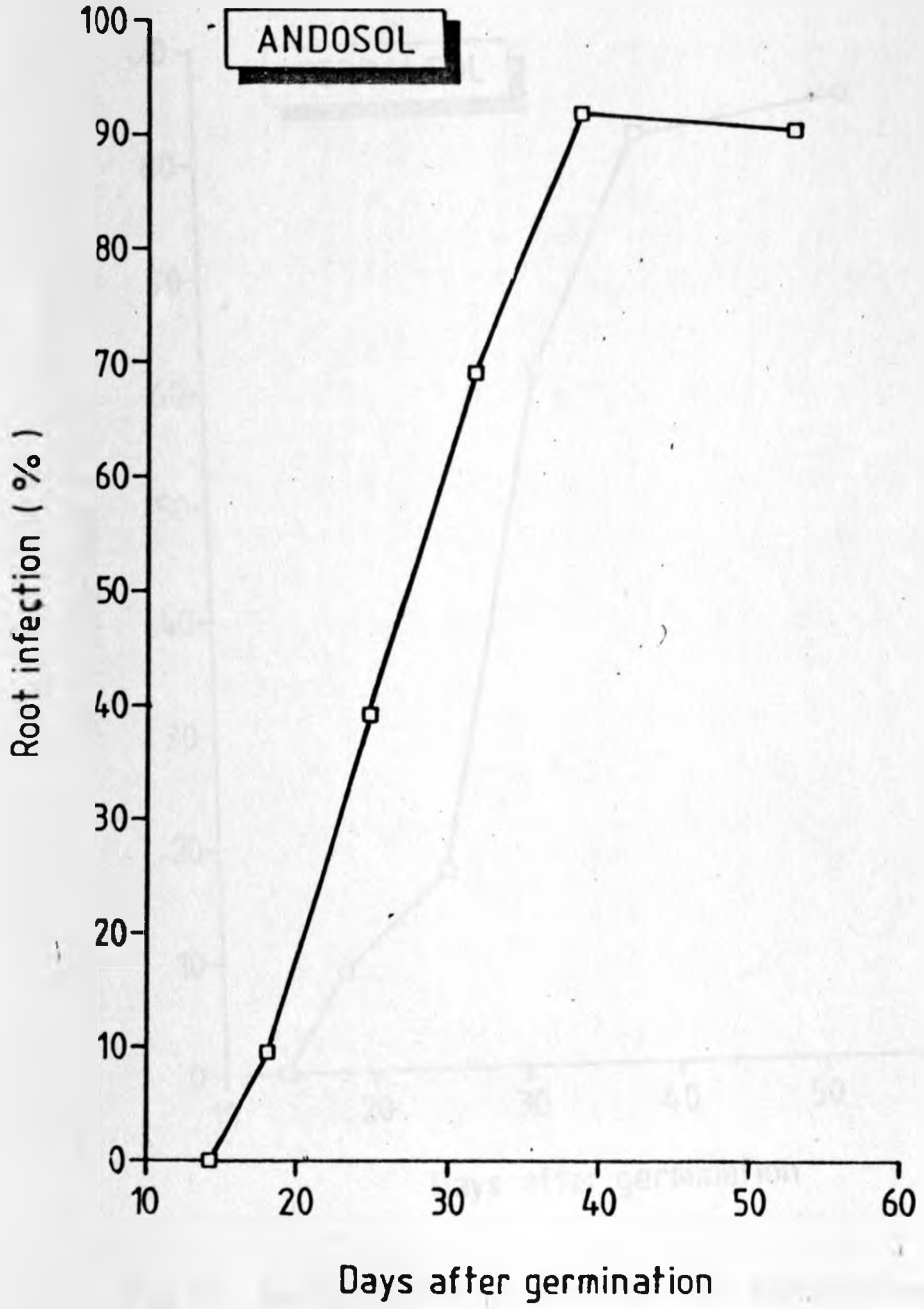



Fig. 1c. Mycorrhizal infection and development in onions grown in an Andosol.

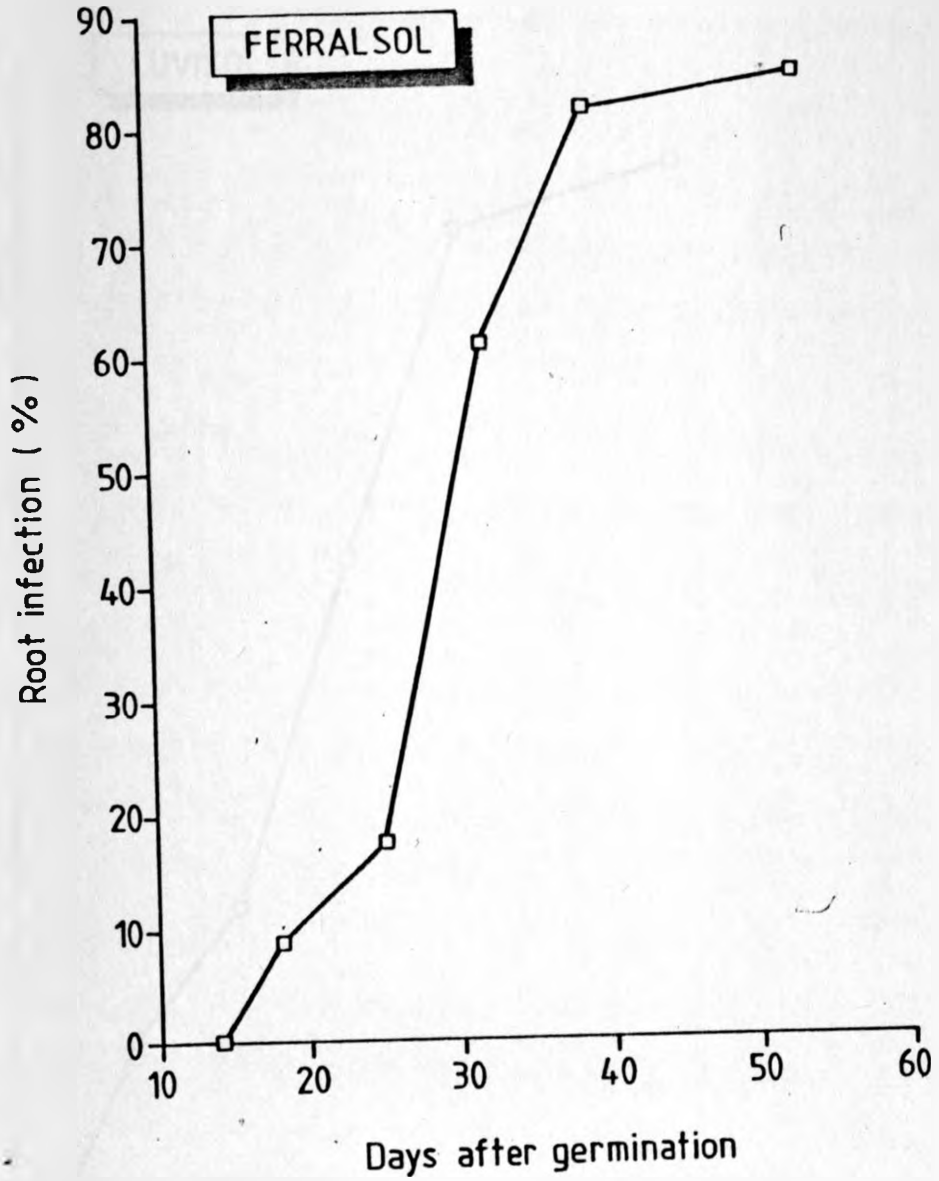



Fig. 1d. Mycorrhizal infection and development in onions grown in a Ferralsol.

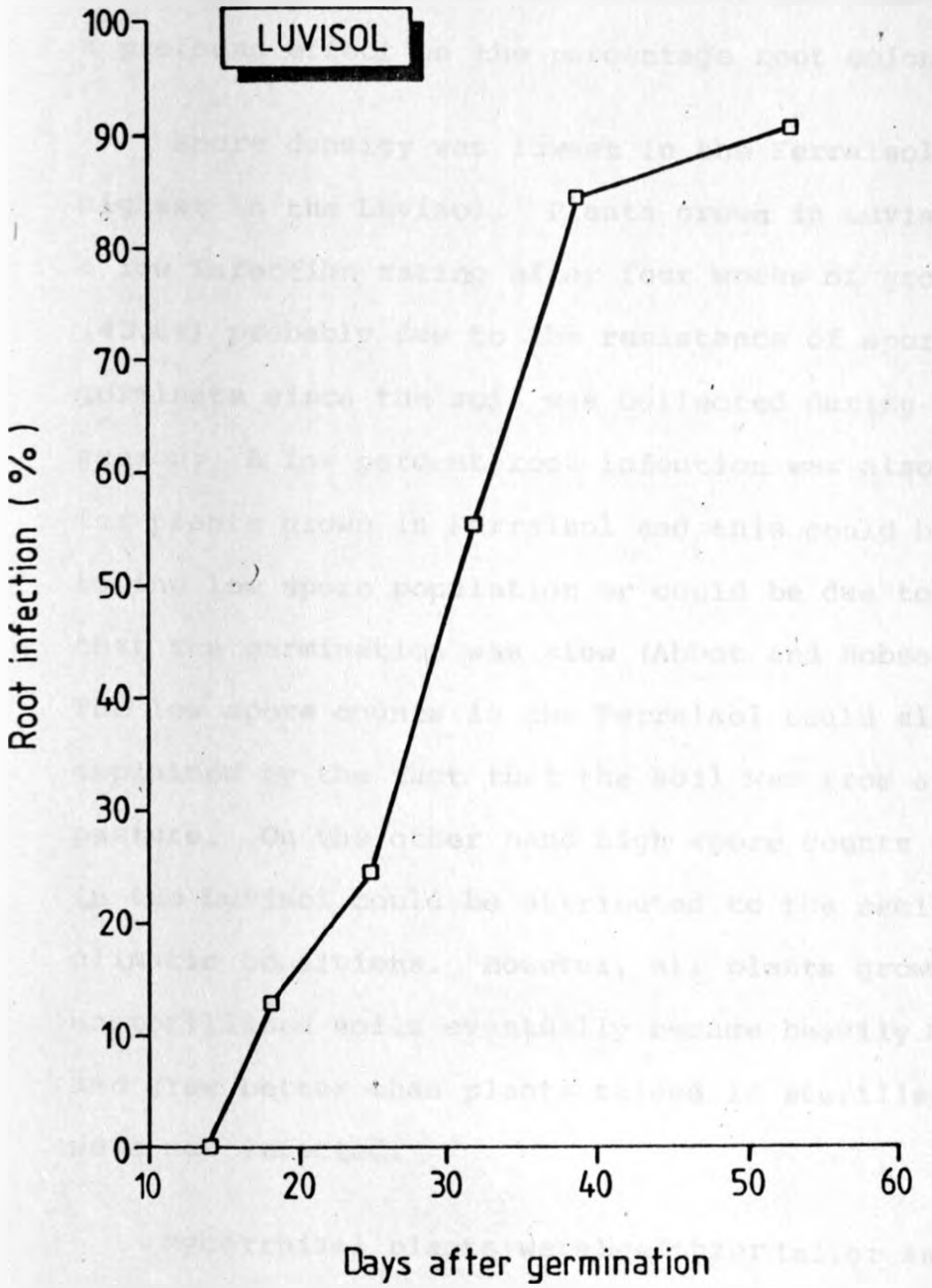



Fig. 1e. Mycorrhizal infection and development in onions grown in a Luvisol.

# 5. DISCUSSION

The study showed that the spore density varied amongst the soils depending on the locality. This variation and climatic conditions at the sites had a profound effect on the percentage root colonization.

Spore density was lowest in the Ferralsol and highest in the Luvisol. Plants grown in Luvisol had a low infection rating after four weeks of growth (43.6%) probably due to the resistance of spores to germinate since the soil was collected during the dry season. A low percent root infection was also recorded for plants grown in Ferralsol and this could be ascribed to the low spore population or could be due to the fact that the germination was slow (Abbot and Robson, 1982). The low spore counts in the Ferralsol could also be explained by the fact that the soil was from a field under pasture. On the other hand high spore counts exhibited in the Luvisol could be attributed to the semi-arid climatic conditions. However, all plants grown in unsterillised soils eventually became heavily mycorrhizal and grew better than plants raised in sterilised soils which were not infected.

Mycorrhizal plants were healthier taller and had more leaves. They gave high mean shoot fresh and dry weight. Similarly mycorrhizal plants had higher

fresh, and dry root weight per plant. On the contrary, the non-mycorrhizal plants were stunted, and had fewer leaves. Height of non-mycorrhizal plants was relatively constant except for plants raised in the Luvisol and Andosol where there was some increase in height and plant weight.

Root and shoot weights of non mycorrhizal plants were relatively constant throughout the growth period. Roots of mycorrhizal plants were thick, highly branched and the presence of yellow pigment was authetic indicating effective mycorrhizal infection. This pigment has been observed in maize (Gerdemann,1961), and Onion and Maize (Becker and Gerdemann, 1977). Roots of non-mycorrhizal plants were less branched and had no yellow pigment. Thus, mycorrhiza enhances growth (Mosse, 1959, 1973, 1977; Gerdemann, 1964, 1968, Sanders, 1977, Asai, 1943).

The differences in growth between mycorrhizal and non mycorrhizal plants became apparent after eight weeks of growth. This implies that at the first harvest, though there was heavy infection, there was no corresponding increase in weight leading to the conclusion that the mycorrhizal fungi were not yet functional. However, in Andosol, the differences in weight were noted at first harvest and this could be due to the mycorrhizal fungi being effective since the percent root infection was quite high (84.5%).

The root:shoot ratio of the non-mycorrhizal onions were on the average higher than for mycorrhizal ones confirming the observation of Hayman and Mosse (1971), Mosse and Hayman, (1971), Abbot and Robson, (1977). The mycorrhizal plants recorded a significantly higher % P in the tissue than the non-mycorrhizal ones. Thus mycorrhizal plants absorbed more P. than the uninfected plants (Mosse, 1973; 1975; Gerdamann, 1964; 1968; Daft and Nicolson, 1966; Allen, 1980). Improved uptake of P is attributed to the hyphae of mycorrhizal fungi which increases surface area for absorption and the amount of soil colonized (Sanders and Tinker, 1973). It has been reported that hyphae from host root can grow as far as 7cm into the soil (Hattigh and Gerdemann, 1973) and per cm of infected root there are as much as 80cm of hyphae (Sanders and Tinker, 1973).

The VAM fungi has additional advantage since the hyphae are continously growing and the growth is not restricted to tip of the hyphae, but, fine rhizoid like hyphae arise latterally from fully mature hyphae. Also the fungus has few or no septa and migration of cytoplasm having nutrients is faster.

Loss of leaves or the withering of tops of leaves in non-mycorrhizal plants can be explained in terms of nutrient redistribution in the plant. Non mycorrhizal

plants take up less P, hence older leaves lose P to young leaves faster. Hopkinson (1966) considers that leaf senescence to some degree is controlled by the availability of nutrients, such as phosphorus, from the soil. The stimulating effects on the development and set of bulbs in mycorrhizal plants can be attributed to the availability of phosphorus. Phosphorus promotes the development of the root system, aids in seed formation and hastens rippening. Nelsen et al. (1981) obtained 64% increase in bulb weight by mycorrhiza at a P rate of 30 kg/ha.

The overall performance of the mycorrhizal plants in the Andosol was similar to that of Luvisol but was significantly superior to that of plants in the Nitosol, Acrisol and Ferralsol whose performance were also comparable. These variation could be due to difference in percentage base saturation, pH or fungal species efficiency. Therefore, full response for an initially limiting nutrient such as phosphorus is not obtained becuase a second nutrient such as potasium may become limiting.

The germination of the spores display a similar pattern. Infectivity (aggressiveness) of the spores in the andosol is high, hence does not have a lag phase in the typical sigmoid curve of percent infection agaist time. The lag phase not only depends on the amount of spores present, but also, the rate at which

the spores germinate in the soil and initiate root colonization (Abbot and Robson, 1982). All the other four soils had a lag phase depicting time for initiation of germination and the lag phase varied depending on spore infectivity.

In the latter stages of infection it appears that the rate of colonization of a root system and rate of root growth come into equilibrium, for the curve of the percentage of the root colonized against time almost shows a plateau following phase of rapid colonization. The percentage infection varies since the method employed does not take into account the size of infected area.

The results from the interactions of P with mycorrhiza indicated that at low to moderate P levels all the mycorrhizal plants had significantly higher root and shoot weight, height and % phosphorus than the non mycorrhizal plants. As the available phosphorus level rose, so did the weight of mycorrhizal and non-mycorrhizal plants, and infact at 100 ppm of phosphorus there was no significant difference between the mycorrhizal and non-mycorrhizal plants. These findings are similar to those of Daft and Nicolson, (1966); Murdoch et al. (1967) and Mosse, (1973). However, the mycorrhizal plants at 50 ppm of p were performing as well as non mycorrhizal plants at 100 ppm of P. Hence it would appear that the mycorrhiza can save P-fertiliser of between 50 ppm and 100 ppm.

Mycorrhizal plants at 20 ppm are generally not significantly different from those at 100 ppm of P, though plants at high P-level have high P-content. This implies that there is luxury consumption of P and some other element is limiting the productivity. Vescular arbuscular mycorrhizal infection does not apparently change the external morphology of roots, the internal morphology can be readily observed after clearing and staining the infected roots (Phillips and Hayman, 1970).

Mycorrhizal infection was extensive in all the soils. There was some reduction in the percent root infection with increase in phosphate. It seems that the phosphate content of these soils was still less than the amount which decreased infection in other experiments (Daft and Nicolson, 1969a; Mosse, 1973) or alternatively most of the added phosphate ions rapidly become bound to soil colloids or fixed as iron and aluminium phosphates rendering them unavailable (Tinker, 1975b). This is more poignant in the Nitosol in which Ssali and Keya (1986) recorded a 0-40% reduction in infection with 150 kg of P per hectare.

Root:shoot ratio in onions were higher for non mycorrhizal plants than mycorrhizal plants when no P was added to the soil and at 20 ppm P. As the P content in the soil rose, leading to increased absorption by plants, the ratio decreased such that at 100 ppm both

mycorrhizal and non mycorrhizal plants had similar ratios (Mosse, 1973; Daft and Nicolson, 1969b). In some previous studies, mycorrhizal plants had considerably lower root to shoot weight values than non mycorrhizal plants. The reduction in root:shoot ratio with increased amount of P possibly reflects increased P-status in the tissue leading to growth of the tops.

The % P in mycorrhizal plants is higher at low to moderate P-levels in the soil. However as the P in soil increases, there is increased absorption of P by both mycorrhizal and non mycorrhizal plants. The uptake of phosphate especially from low phosphate soils, is thought to be controlled by slow rates of movement of these ions in the soil so that the limiting factor is not the ability of the root to take them up, but the ability of the phosphate ions to reach the absorbing surface. At high P concentration in the soil the roots are quite accessible to the P and formation ofa depletion zone is unlikely. Therefore the mycorrhizal plants will not have any advantage. At high P mycorrhizal infection is almost nil.

Since vescular arbuscular mycorrhizal benefit host growth more under low phosphate, this gain is not apparent with the application of soluble phosphate. Hence mycorrhizal fungi may not be of importance in agriculture when high soil fertility is maintained.

However, there are instances where a high degree of mycorrhizal dependence exists regardless of high soil fertility. Certain citrus varieties remain stunted if they remain non-mycorrhizal even with the addition of high levels of fertilizer (Mehraveran, 1977). Infact in some New Zealand forest soils plants lacking V A mycorrhiza do not survive (Baylis, 1959). Dependence on mycorrhiza may be related to limited root hairs development (Baylis, 1972) although there are cases where plants with well developed root hairs still benefits from mycorrhizal infection (Mosse et al. 1973).

## 6. CONCLUSION AND RECOMMENDATIONS

The study showed that native mycorrhiza are important in plant growth, and onions are dependent on vescular arbuscular mycorrhizal fungi for normal growth and development. Without vescular arbuscular mycorrhizal fungi, no proper growth could be achieved. Mycorrhizal plants took up more phosphorus than non-mycorrhizal ones. Thus, vescular arbuscular mycorrhiza fungi enhances P-uptake. The study further demonstrated that effects of vescular arbuscular mycorrhiza becomes apparent first where there is fast infection. This in turn depends on both spore population and their infectivity.

The influence of phosphorus level on VAM fungi and onion growth showed that at low P concentration the beneficial effect of vescular arbuscular mycorrhizal fungi was not as strong as at medium P (20-50 ppm). However, at very high P (100 ppm) the vescular arbuscular mycorrhizal fungi confer no advantage to the plants. Performance of mycorrhizal plants at 50 ppm was as good as that of non-mycorrhizal plants at 100 ppm. Thus vescular arbuscular fungi could save P-fertilizer equal to 50 ppm (or 100 kg of P per hectare). Therefore, in the management of nutritionally deficient soils, where there is need to conserve the dwindling and costly fertilizer reserves, we must consider the three dimensional system of soil, mycorrhiza and plant relation rather than soil and plant only.

It was recommended that such studies be extended to important food crops and plantation crops of commercial value like Tea, Coffee and fruit trees. Since the mycorrhizal effects will depend on the interaction of the host plant, VA fungus and soil, research for practical application can be achieved by judicious selection of a more effecient VAM fungus which will modify the nutrient absorption by the plant.

Though there is a striking relationship between phosphate and VAM mycorrhiza as shown in this study it should be emphasised that VAM fungi are not solely involved in phosphate uptake. There have been reports of increased absorption of other nutrients such as Zinc, Copper, Sulphur and Potasium. However this study did not investigate the effect of mycorrhizal infection on these nutrients.

Maintanance of soil fertility in natural ecosystems requires either additions of nutrient elements to replace those lost through leaching or an efficient means of recycling. For phosphorus, there is no natural process and mycorrhiza could partly offer an answer to this problem since the association is thought to have solved as a means for the extraction of P from the soil efficiently.

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