
**The Role of Indigenously-Associated Arbuscular Mycorrhizal Fungi as Biofertilisers and
Biological Disease-control Agents in Subsistence Cultivation of *Morogo***

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DEDICATION

I am proud to dedicate this work to my two sons, Kabelo and Katlego. I feel a deep sense of gratitude for their constant demonstration of love and patience during my studies outside the home.

"One never notices what has been done; one can only see what remains to be done"

- Marie Currie -

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DECLARATION

The research as presented in this dissertation is, to the best of my knowledge and belief, original and has not been previously submitted for degree purpose to any other university. Appropriate acknowledgements in the text have been made where the use of work conducted by other researchers have been included.

Mohlapa Junior Sekoele

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ABSTRACT

The study examined interactions between *morogo* plants, arbuscular mycorrhizal fungi (AMF) and *Fusarium* species. *Morogo* refers to traditional leafy vegetables that, together with maize porridge, are dominant staple foods in rural areas of the Limpopo Province such as the Dikgale Demographic Surveillance Site (DDSS). *Morogo* plants grow either as weeds (often among maize), occur naturally in the field or are cultivated as subsistence crops by rural communities.

Botanical species of *morogo* plants consumed in the DDSS were determined. Colonisation of *morogo* plant roots by AMF and *Fusarium* species composition in the immediate soil environment were investigated in four of eight DDSS subsistence communities. Isolated AMF were shown to belong to the genera *Acaulospora* and *Glomus*. Twelve *Fusarium* species were isolated from soil among which *Fusarium verticillioides* and *Fusarium proliferatum* occurred predominantly.

Greenhouse pot trials were conducted to examine the effect of AMF on *morogo* plant growth (cowpea; *Vigna unguiculata*) and *Fusarium proliferatum* levels in soil. Interaction between plants and AMF, as well as tripartite interactions of cowpea plants, AMF and *Fusarium proliferatum* were investigated. Non-inoculated cowpea plants served as controls for the following inoculations of cowpea in pots: (i) *Fusarium proliferatum*; (ii) commercial AMF from Mycoroot (PTY) Ltd. (a mixture of selected indigenous *Glomus* spp referred to *commercial* AMF for the purpose of this study); (iii) indigenous AMF obtained from DDSS soil (referred to *local* AMF for the purpose of this study); (iv) commercial AMF plus *Fusarium proliferatum*; (v) local AMF plus *Fusarium proliferatum*.

Results showed reduced root colonization by local as well as commercial AMF when *Fusarium proliferatum* were present. Local AMF significantly enhanced cowpea growth while commercial AMF apparently reduced the level of *Fusarium proliferatum* in the rhizosphere and surrounding soil. Results suggest that AMF may have potential as biological growth enhancers and bioprotective agents against *Fusarium proliferatum*.

Keywords: Arbuscular mycorrhizal fungi (AMF), *Fusarium*, *Morogo*, cowpea (*Vigna unguiculata*), biological growth enhancer, biocontrol agent, subsistence farming, traditional / indigenous knowledge.

OPSOMMING

Die studie het interaksies tussen *morogo* plante, arbuskulêre mikorisa fungi (AMF) en *Fusarium* spesies ondersoek. *Morogo* is tradisionele blaargroentes wat, tesame met mieliepap, die oorwegende stapelvoedsels in landelike gebiede van die Limpopo Provinsie, soos die "Dikgale Demographic Surveillance Site" (DDSS), is. *Morogo* plante groei óf as onkruid (dikwels tussen mielies), kom natuurlik in die veld voor óf word as onderhoudsgewasse deur landelike gemeenskappe verbou.

Botaniese spesies van *morogo* plante wat deur DDSS gemeenskappe geëet word, is bepaal. Kolonisasie van *morogo* plantwortels deur AMF en *Fusarium* spesies samestelling in die onmiddellike grondomgewing is in vier van agt DDSS onderhoudsgemeenskappe ondersoek. Daar is gevind dat geïsoleerde AMF tot die genera *Acaulospora* en *Glomus* behoort. Twaalf *Fusarium* spesies is uit die grond geïsoleer waaronder *Fusarium verticillioides* en *Fusarium proliferatum* oorwegend voorgekom het.

Glashuis potproewe is uitgevoer om die uitwerking van AMF op *morogo* plante (Akkerbone; *Vigna unguiculata*) en *Fusarium proliferatum* vlakke in grond te ondersoek. Interaksies tussen akkerbone en AMF, sowel as driedigedige interaksies tussen akkerbone, AMF en *Fusarium proliferatum* is ondersoek. Nie-geïnokuleerde akkerbone plante het as kontrole gedien vir die volgende inokulasies van akkerbone in potte: (i) *Fusarium proliferatum*; (ii) kommersiële AMF van Mycoroot (EDMS) Bpk. (n mengsel van geselekteerde *Glomus* spp. verwys na as kommersiële AMF vir die doel van hierdie studie); (iii) inheemse AMF uit DDSS grond verkry (verwys na as plaaslike AMF vir die doel van hierdie studie); (iv) kommersiële AMF plus *Fusarium proliferatum*; (v) plaaslike AMF plus *Fusarium proliferatum*.

Resultate dui op verminderde wortelkolonisasie deur plaaslike sowel as kommersiële AMF wanneer *Fusarium proliferatum* teenwoordig is. Lokale AMF het die groei van akkerbone betekenisvol verbeter terwyl kommersiële AMF die vlakke van *Fusarium proliferatum* in die risosfeer en omringende grond verlaag het. Volgens die resultate wil dit voorkom of AMF potensiaal mag hê as biologiese groeiversterker en bio-beskermingsmiddel teen *Fusarium proliferatum*.

Sleutelwoorde: Arbuskulêre mikorisa fungi (AMF), *Fusarium*, *Morogo*, Akkerbone (*Vigna unguiculata*), biologiese groeiversterker, bio-beskermingsmiddel, onderhoudsboerdery, tradisionele / inheemse kennis

LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
CFU	Colony forming units
CLA	Carnation Leaf Agar
cm	Centimetre
Com	Commercial
dH ₂ O	Distilled water
DDSS	Dikgale Demographic Surveillance Site
Fus	<i>Fusarium</i>
HCl	Hydrochloric acid
HSD	Honest significant difference
INVAM	International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi
K	Potassium
KCl	Potassium chloride
KH ₂ PO ₄	Potassium phosphate
km	Kilometre
KNO ₃	Potassium nitrate
KOH	Potassium hydroxide
Loc	Local
M-	Away from maize plants
M+	In close proximity of maize plants
Mg	Magnesium
MgSO ₄ .7H ₂ O	Magnesium sulphate heptahydrate
mm	Millimetre
N	Nitrogen
nm	Nanometre
Na	Sodium
P	Phosphorus
PCNB	Pentachloronitrobenzene
PDA	Potato Dextrose Agar
PVA	Polyvinyl alcohol
PVLG	Polyvinyl-lacto-glycerol

SNA	Synthetic Nutrient Agar
µm	Micrometre

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CHAPTER 1

INTRODUCTION

1.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that depend on host cells for their carbon supply (Driver, *et al.*, 2005). They form symbiotic associations with a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity. The symbiosis develops within the plant root system, where the fungus colonises the apoplast and cortical cells of the root (Bergero *et al.*, 2003). The symbiotic associations of roots and fungi are characterised by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil (Sylvia, 2006).

Laboratory studies indicated that the presence of these symbiotic fungi aid establishment and growth of a diversity of plant species in soils with little available phosphorus (Demir, 2004; Kapoor *et al.*, 2004; Pattinson *et al.*, 2004). AMF increase plant uptake of minerals especially phosphorus (Demir, 2004), improve soil structure (Lendzemo *et al.*, 2005), restore plant communities (Pattinson *et al.*, 2004), increase seedling survivorship (Fisher & Jayachandran, 2002), enhance tolerance of biotic and abiotic stresses (Tian *et al.*, 2004; Yano & Takaki, 2005), and alter plant water relations and responses to drought (Hernández-Sebatia *et al.*, 1999; Al-Karaki *et al.*, 2004; Augé, 2004; Wu & Xia, 2006; Subramanian *et al.*, 2006). Mycorrhiza not only increase growth, but also changes the anatomy/morphology of host stems and leaves. These changes alter the ability of plants both to survive stress and to gain access to resources that ultimately improve the fitness of the plant (Allen, 1991).

A major role of mycorrhizal fungi may be protection of the root system from endemic pathogens such as *Fusarium spp.* Studies have shown that AMF protect plants from the deleterious effects of root pathogenic fungi (Chakravarty & Mishra, 1986; Newsham, 1995; Abdalla & Abdel-Fattah, 2000; Karagiannidis, 2002). AMF play a key ecological role in disease prevention by modifying exudate composition, stimulating disease protective response through its own infection of the root tissue, inhibiting competing microbial populations directly through synthesis of antibiotics, limiting

access of plant pathogens to root tissue by physically occupying the root surface (Tate, 2000), enhancing or altering plant growth, nutrition, and morphology, as well as promoting growth of microbiota that can suppress plant pathogens (Whipps, 2004).

1.2 Problem statement

Together with maize porridge, *morogo* vegetables have been the dominant staple in the rural areas of the Limpopo Province. Most people in the rural villages of Limpopo rely on subsistence farming without employing any form of irrigation, pest control or fertilisers. They lose much of their crop to diseases, insect pests, drought, low soil fertility, and other abiotic stresses. Low soil fertility is a problem in some areas of the Limpopo Province, especially the Dikgale Demographic Surveillance Site (where this study was conducted). The climate of the Dikgale Demographic Surveillance Site (DDSS) is dry with low rainfall. Rates of unemployment and illiteracy are high and the population lives mostly on subsistence farming. Continuous cultivation of low fertility soils without adequate soil nutrient replenishment probably results in decline of crop productivity. This is due to traditional farming systems and poor land management among the resource-poor subsistence farmers.

Plant roots in native vegetation are commonly colonised by arbuscular mycorrhizal fungi. By increasing yield and controlling pests, mycorrhizal fungi could decrease the dependence on chemical fertilizers through more efficient nutrient and water uptake from soil (Carlile *et al.*, 2001). Inoculation of soil with appropriate arbuscular mycorrhizal fungi could be beneficial to subsistence farmers where *morogo* plants establish mutualistic relationships with indigenous AMF.

In the DDSS indigenous vegetables are abundant immediately after the rainy season and very scarce during the dry season. Green leafy vegetables (GLV) are rich sources of vitamins such as β -carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as iron, calcium and phosphorus (Gupta *et al.*, 2005). GLV are also recognized for their characteristic colour, flavour and therapeutic value. Increasing the utilisation of *morogo* in the diet can eradicate micronutrient malnutrition and also prevent the degenerative diseases.

1.3 Research objectives

The aim of the present study was to isolate and identify mycorrhizal fungi growing indigenously in association with *morogo* crops and evaluate the effect thereof on crop yield and the level of *Fusarium spp.*

Specific objectives of this study were:

- ❖ Survey of study area and documentation of indigenous knowledge by means of questionnaire.
- ❖ Isolation of mycorrhizal fungi growing indigenously with *morogo* crops.
- ❖ Identification and quantification of mycorrhizas associated indigenously with *morogo*.
- ❖ Isolation of *Fusarium* species associated with *morogo*.
- ❖ Identification and quantification of *Fusarium* species associated with *morogo*.
- ❖ Evaluation of plant growth and mycorrhizal root colonization.
- ❖ Evaluation of the effect of AMF on the level of *Fusarium* in the soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Beneficial mycorrhizal fungi are considered an important cornerstone of sustainable agricultural systems. Since mycorrhizal associations aid host plants in utilising available soil water and fertility more efficiently, they serve as biofertilisers and increase drought resistance and plant productivity (Davies *et al.*, 2005). Mycorrhizal fungi may reduce the incidence and severity of root diseases (Sylvia, 2006), serving as biological disease-control agents.

Mycorrhizas are symbiotic associations that form between the roots of most plant species and fungi. The symbiosis develops within the plant root system where the fungus colonises the apoplast and cortical cells of the root (Bergero *et al.*, 2003). Mycorrhizas are considered to be classic examples of mutualistic symbioses (Jones & Smith, 2004). These symbioses are characterised by bi-directional movement of nutrients where organic carbon resulting from photosynthesis flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil (Sylvia, 2006). The fungi form an extensive network of thread-like hyphae in the soil that are very efficient at exploiting nutrient reserves.

According to Sylvia (2006) the following types of mycorrhizas can be distinguished:

Ectomycorrhiza

Ectomycorrhizas (EM) have hyphae between root cortical cells producing a network structure called the Hartig net. Ectomycorrhizas are found on woody plants ranging from shrubs to forest trees. Ectomycorrhizal fungi belong to the divisions Basidiomycota and Ascomycota.

Arbuscular mycorrhizas

These are endomycorrhizas; the fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within cortical cells. Arbuscular mycorrhizas (AM) are characterised by the development of highly branched arbuscules within the root cortical cells. Arbuscular mycorrhizal fungi are Glomeromycetes and are all classified in the order Glomerales (Kirk *et al.*, 2001).

Ericaceous mycorrhizas

Ericaceous mycorrhizas have hyphae that can penetrate cortical cells (endomycorrhizal) but the fungi do not form arbuscules (Sylvia, 2006). Ericaceous mycorrhizal associations occur on plants that belong to the division Magnoliophyta, order Ericales.

Orchidaceous mycorrhizas

These are characterized by the fungus growing into the plant cell, invaginating the cell membrane and forming hyphal coils within the cell. The coils are active for only a few days, after which they lose turgor and degenerate and the nutrient contents are absorbed by the developing orchid (Sylvia, 2006). Orchidaceous mycorrhizal fungi belong to the division Basidiomycetes.

Mixed infections

Mixed infections occur in which a host supports more than one type of mycorrhizal association. Plants such as *Salix* (Willows) and *Eucalyptus* can have both AM and EM associations on the same plant (Sylvia, 2006).

Of the above described types of mycorrhizas, arbuscular mycorrhizal symbiosis is the most widespread and the most ancient (Bergero *et al.*, 2003). During 400 million years of co-evolution, plants and arbuscular mycorrhizal fungi have become highly interdependent, both ecologically and physiologically (Bécard *et al.*, 2004). AMF colonise plant roots and extend hyphae beyond the reach of host plant roots, leading to substantial increases in the nutrient uptake of host plant (Wolfe & Klironomos, 2005). AM fungi depend on the organic carbon provided by their plant host to complete their developmental cycle. Mycorrhiza formation appears to be obligate for vegetative growth and sporulation of AM fungi. This leads to the assumption that AM fungal fitness is completely reliant on carbon supplied by autotrophic plants (Jones & Smith, 2004). AM fungi and plants express specific elements of their genetic program to live together and complement each other (Bécard *et al.*, 2004). The AM type of symbiosis is very common as the fungi involved can colonise a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type (Sylvia, 2006). About 90% of all vascular plant species as well as many non-vascular lower plants have AM fungal symbionts, to the extent that mycorrhizas have been described as the main absorbing organs of plants (Carlile *et al.*, 2001).

Mycorrhizas result in benefits to plant growth, health and survival. Benefits to the plant are derived when the relationship is fully established with intraradical colonisation and extraradical colonisation of the soil. Variations in responsiveness can occur depending on host genotype and

AM fungal combination and the environmental conditions of the tests; therefore caution is needed in predicting benefit from inoculating with AM fungi under any set of conditions when different genotypes are grown (Linderman & Davies, 2004).

Although AMF are indigenous to soils throughout the world and indicate a general lack of host specificity, they do exhibit environmental specificity. The study by Davies *et al.* (2002) showed that inoculation of plants with an arbuscular mycorrhizal fungal isolate from an arid region enhanced the ability of AMF to impart drought resistance of the host plant species. Conversely, drought caused reductions in all mycorrhizal parameters with an arbuscular mycorrhizal fungal isolate from the more humid region.

2.2 The role of arbuscular mycorrhizal fungi as biofertilisers

The primary function of mycorrhiza is the acquisition of mineral nutrients from the soil. Increased plant uptake of nutrients results in improved growth of plants. Effects of mycorrhizas in increasing nutrient uptake will be most marked for nutrients which move to roots principally by diffusion and for host plants with coarse roots and few root hairs (Wallander, 2000).

Mycorrhizal stimulation of uptake is attributed to uptake of nutrients by fungi from soil beyond the depletion zones that can develop around roots, production of degradative extracellular enzymes or organic acids by the fungi, and the ability of fungi to translocate nutrients faster than they could diffuse through soil (Wallander, 2000).

2.2.1 Increase in nutrient acquisition, especially of elements that are immobile in soils.

In the case of a poorly-mobile ion such as phosphate, a sharp narrow depletion zone develops close to the root. Mycorrhizal hyphae can readily bridge this depletion zone and grow into soil securing an adequate supply of phosphorus for the host plant (Sylvia, 2006). Studies have shown that AMF increase plant uptake of minerals, especially phosphorus (Hovsepian & Greipsson, 2004; Demir, 2004; Walling & Zabinski, 2004). The greater phosphorus concentrations in mycorrhizal plants compared with that of non-mycorrhizal plants of similar size may also have an effect on photosynthesis because the photosynthetic process is known to be positively influenced by phosphorus (Demir, 2004). When the phosphorus concentration of plants increases, the photosynthetic rate and its substances also increase and this positively affects the plants.

2.2.2 AMF plays a role in the uptake of heavy metals and micronutrients

AMF can improve or restrict the uptake of heavy metals and micronutrients by plants (Karagiannidis *et al.*, 2002; Hovsepyan & Greipsson, 2004).

2.2.2.1 Mycorrhizas improve the uptake of micronutrients.

Micronutrients such as zinc and copper have limited diffusion in solution in many soils. AMF facilitates heavy metal uptake by forming chelates that solubilises metals and increase their bio-availability in soil. This can happen with the assistance of helper bacteria such as *Bacillus thuringiensis*. These bacteria increase the physiological and metabolic status of AMF (Vivas *et al.*, 2003).

A study by Hovsepyan and Greipsson (2004), has shown that arbuscular mycorrhizal plants had higher concentrations of zinc (Zn) and copper (Cu) compared to plants with suppressed AMF activity. These results strongly suggest that AMF mediated Zn and Cu uptake. An investigation by Ryan and Angus (2003) has shown that high levels of AM fungal colonisation enhanced Zn uptake of autumn-sown wheat and field pea crops.

Liu *et al.* (2005) conducted a glasshouse pot experiment to study the effect of arbuscular mycorrhizal colonisation by *Glomus mosseae* (BEG167) on the yield and arsenate (As) uptake of tomato plants in soil experimentally contaminated with five As levels. AM colonization of tomato plants increased plant biomass, As concentration and As uptake at lower levels of soil As contamination.

Davies *et al.* (2001) showed that mycorrhizal sunflower had greater chromium-accumulating ability than non-arbuscular mycorrhizal plants at the highest concentrations of Cr(III) and Cr(IV). Mycorrhizal fungi helped to partially alleviate chromium (Cr) toxicity as indicated by greater plant growth, net photosynthesis and reduced visual symptoms of stress. AM enhanced plant accumulation and tolerance to chromium.

According to Davies *et al.* (2001) mycorrhiza can potentially increase chromium uptake by:

- (i) secreting metal-chelating molecules (e.g., organic acids, phytosiderophores) into the rhizosphere to chelate and solubilise soil-bound metals (i.e., greater mobilization of Cu, Zn and manganese (Mn) from soil),

- (ii) reducing soil-bound metal ions by specific plasma membrane bound metal reductases,
- (iii) solubilising heavy metals by acidifying rhizosphere soil environment with proton extrusion, and
- (iv) extraradical hyphae of mycorrhiza increasing root absorption surface area and ability of roots to access normally non-available sites.

Solubilised metal ions may then enter the root via extracellular (apoplastic) or intracellular (symplastic) pathways.

2.2.2.2 AMF restricts heavy metal uptake

Contrary to the findings that AMF improve the uptake of metals, Karagiannidis *et al.* (2002) showed that a reduced concentration of the microelements Mn, Zn, iron (Fe) and Cu was found in the leaves of AM plants. He attributed that to the speculation that under conditions of excess metals, soil supply metals are bound in the root system and not transported to the aerial parts of the plant.

Hovsepyan and Greipsson (2004)'s results suggest that AMF restricts lead (Pb) and Mn uptake of plants. Lead concentrations were generally higher in non mycorrhizal plants than in mycorrhizal plants. Furthermore, Mn concentrations were significantly higher in plants non mycorrhizal plants than in mycorrhizal plants. Thus, plants with indigenous AMF colonisation accumulated significantly less Pb and Mn than plants with suppressed AMF activity. Therefore, AMF provides protection against Pb and Mn toxicity.

Plants of the Zn, cadmium (Cd) and Pb hyperaccumulator *Thlaspi praecox* Wulfen inoculated or not inoculated with an indigenous AM fungal mixture were grown in a highly Cd, Zn and Pb contaminated substrate in order to evaluate the functionality of symbiosis and assess the possible impact of AM colonisation on heavy metal uptake and tolerance (Vogel-Mikus *et al.*, 2005). Colonised plants showed significantly improved nutrient and decreased Cd and Zn uptake. Reduced heavy metal uptake, especially at higher soil metal contents, indicated a changed metal tolerance strategy in colonised *T. praecox* plants.

The role of AMF in heavy metal uptake is metal specific (Hovsepyan & Greipsson, 2004). Uptake of metals by mycorrhizal plants does not have a generalised pattern and depends on factors such as soil properties, the host plant, concentrations of the metals in the soil, and resident AMF groups (Davies *et al.*, 2001; McGrath *et al.*, 2001).

2.2.3 AMF enhance tolerance of biotic and abiotic stresses

2.2.3.1 Acidity

Rohyadi *et al.*, (2004) showed the potential role of AM fungi in assisting the plants to grow better in acid soils. Their work demonstrated that depressed growth of non-mycorrhizal cowpea at pH 4.7 was reversed to a greater extent by inoculating with *Gigaspora margarita*. The work showed that cowpea was intolerant to very low soil pH, but very responsive to AM associations. AM formation increased total mineral uptake by plants mostly associated with increased plant size. The improvement of cowpea growth by *G. margarita* was attributed to increased nutrient acquisition, particularly of phosphorus. It was concluded that the function of AM fungi under acidic conditions is strongly dependent on the fungal species, since *G. margarita* was much more effective in improving the growth and element uptake of cowpea plants than *Glomus etunicatum* at pH as low as 4.7.

The work by Yano and Taki (2005), demonstrated that the mycorrhizal symbiosis can improve root development and shoot growth in sweet potato plants grown in acidic soil. The improvements were significant only in arbuscular mycorrhizal plants grown in stressed environments, thus indicating the role of mycorrhizal symbiosis in alleviating the impact of acid soil.

2.2.3.2 Salinity

In the study by Tian *et al.* (2004), although mycorrhizal colonization was reduced with increasing sodium chloride (NaCl) levels, the dependency of cotton plants on mycorrhizal fungi was increased. The symbiotic association between mycorrhizal fungi and cotton plants was strengthened in a saline environment once the association was established. This indicated the ecological importance of AM associations for plant survival and growth of plants under salinity stress. AM fungi increased phosphorus uptake, and salinity stress in plants was thereby alleviated.

2.2.3.3 Drought

Root colonisation by AMF can affect the water relations and drought resistance of host plants. AM symbiosis improves plant drought resistance (Augé, 2004). Drought resistance can occur via drought avoidance or drought tolerance. Drought avoidance involves the maintenance of high

internal water potential. Drought tolerance involves the survival of low internal water potential. Mycorrhizal fungi can improve host plant water relations in a number of different ways. These include increased stomatal conductance and transpiration rates, acceleration of recovery from stress, and other aspects of host physiology, particularly hormonal relations involving abscisic acid and cytokinins (Augé, 2004).

Al-Karaki *et al.* (2004) conducted a study to determine the effects of the AM fungus inoculation on growth, grain yield and mineral acquisition of two winter wheat cultivars grown in the field under well-watered and water-stressed conditions. The improved growth, yield, and nutrient uptake in wheat plants reported in the study, demonstrated the potential of mycorrhizal inoculation to reduce the effects of drought stress on wheat grown under field conditions in semiarid areas of the world.

Wu and Xia (2006) studied the influence of AM fungus *Glomus versiforme* on plant growth, osmotic adjustment and photosynthesis of *Citrus tangerine* under well-watered and water stress conditions. AM significantly stimulated plant growth and biomass regardless of the soil water status. The results showed that AM colonization changed the plant growth, osmotic adjustment and photosynthesis characters of *Citrus tangerine*. The results suggested that the benefit of AM colonisation under water stress conditions was due to the enhancement of osmotic adjustment.

The effects of root colonisation by AM fungus *Glomus intraradices* Schenck and Smith on growth, flower and fruit production, and fruit quality in field-grown tomato plants exposed to varying intensities of drought was studied (Subramanian *et al.*, 2006). In all cases, colonisation improved drought resistance of field-grown tomato plants. Mycorrhizal response was more pronounced under severe drought than well watered conditions. Improved nutritional status in conjunction with maintenance of leaf water status may have assisted the plants to translocate minerals and assimilates to the sink and alleviated the impacts of drought on fruit production. Tomato fruit quality was improved by mycorrhizal colonization. AM colonisation enhanced nutritional status and leaf relative water content (RWC) and enabled the host plant to withstand varying intensities of drought under field conditions.

In the study by Hernández-Sebatia *et al.* (1999) AM symbiosis by *G. intraradices* increased the RWC of whole strawberry plants under normal growth conditions. The effect was related to increased water content in mycorrhizal root systems, where the root osmotic potential was maintained similar to non-mycorrhizal roots, suggesting that mycorrhizal roots must have a higher

concentration of water soluble compounds or a different distribution in the cell compartments than non-AM roots.

Pinior *et al.* (2005) investigated the mechanism and the extent to which AM can prevent drought damage and whether physiological analyses revealed enhanced drought tolerance of the rose plants. Micropropagated rose plants were inoculated with the AM fungus *Glomus intraradices* and subjected to different drought regimens. The results demonstrated the positive effect of mycorrhiza on photosynthetic yield under drought stress. Due to more balanced physiological processes, the mycorrhizal symbiosis could lead to an improved photosynthetic performance and thus to enhanced plant survival under drought.

In many arid and semi-arid regions of the world, drought limits crop productivity (Al-Karaki *et al.*, 2004). The incorporation of factors enabling plants to withstand drought stress would be helpful to improve crop production under drought conditions. Inoculation of plant roots with arbuscular mycorrhizal fungi may be effective in improving crop production under drought conditions.

2.2.4 AMF restore plant community

Pattinson *et al.* (2004) investigated the influence of AM on the growth and survival of seedlings in an extremely impoverished and highly disturbed soil. By using plants and fungi adapted to the soil, they demonstrated that plant growth and survival followed a pattern typically found in mineral-rich soils. These results strongly suggest that the presence of AM is probably essential for the return of complex communities formally present at disturbed sites, regardless of the level of available phosphate in soil.

Johnson *et al.* (2003) examined plant community responses to interactions between AM fungi and availability of atmospheric carbon dioxide and soil nitrogen. In their experimental system, plant species richness was greatest when AM fungi were present, soil N was low and atmospheric CO₂ was elevated. It was concluded that mycorrhizas could be important mediators of plant community responses to atmospheric CO₂, and that soil N further regulates those responses.

Plant roots can be linked by a common mycorrhizal network of AM fungi. A common mycorrhizal network can be defined as a below-ground system of interconnected hyphae and roots (Southworth *et al.*, 2005). Nutrients such as carbon, nitrogen and phosphorus might move through the common mycorrhizal network from plant to plant (He *et al.*, 2003). If mycorrhizal colonisation

results in an equalisation of resource availability, it would be expected to reduce dominance of aggressive species, so promoting coexistence and greater biodiversity (Read, 1997).

2.2.5 AMF increase seedling survivorship

Seedlings of two plant species from South Florida, *Amorpha crenulata* and *Jacquemontia reclinata* were grown in pots with various native soil treatments under greenhouse conditions (Fisher & Jayachandran, 2002). AMF significantly increased the dry weight and total P content of seedlings growing on native soil. It was concluded that nursery-grown seedlings might have improved survivorship when they were later out planted if they were first colonized by AMF (Fisher & Jayachandran, 2002). This was also found by Wilson and Hartnett (1997) in legume seedlings.

2.3 The role of AMF as biological disease-control agents

Azcón-Aguilar and Barea (1996) defined biological control as “the directed, accurate management of common components of ecosystems to protect plants against pathogens”. Plant pathogens are destructive agents that can cause mortality, reduced fitness of individual plants, rapid declines of populations of host species, or dramatic shifts in the structure or composition of plant communities (Gilbert, 2002).

The ecological interactions associated with biological disease control emphasize four key areas that must be considered (Whipps, 2004):

- (i) the aetiology and epidemiology of the pathogen,
- (ii) the growth and method of cultivation of the crop,
- (iii) the physical, chemical and microbiological environment, and
- (iv) biocontrol agents available.

Mechanisms by which AM fungi could control root pathogens include (Whipps, 2004; Azcón-Aguilar & Barea, 1996; Declerck *et al.*, 2002):

- (i) Improved nutrient status of the host plant

The plant may be more resistant to or tolerant of pathogen attack because increased nutrient uptake, particularly of P, made possible by the AM symbiosis results in more vigorous plants.

(ii) Damage compensation

Improved growth due to AM colonisation may allow better compensation for damage caused by pathogens. By compensating for the loss of root biomass or function caused by pathogens, AM fungi increase host tolerance of pathogen attack.

(iii) Direct competition

AM fungi and root pathogens may compete for photosynthates reaching the host plant roots. When AM fungi have primary access to photosynthates, the higher carbon demand may inhibit pathogen growth. Competitive interactions may occur directly between the pathogen and the mycorrhizal fungus, leading to pressure for infection sites or space on the roots.

(iv) Microbial changes in the mycorrhizosphere

AMF formation induces exudation of a variety of compounds by roots of host plant. The root exudates impact the soil microbial community in their immediate vicinity, influence resistance to pests, support beneficial symbioses, alter the chemical and physical properties of the soil, and inhibit the growth of competing plant species (Bertin *et al.*, 2003).

(v) Activation of plant defence mechanisms

In host-pathogen interactions a range of defence mechanisms is activated in response to a microbial attack (Vierheilig, 2004). AMF make the root more responsive to pathogen attack. AMF initiate a host defence response which is subsequently suppressed. Plant resistance responses include formation of structural barriers to prevent pathogen ingress; production of enzymes that degrade the cell wall and enzymes associated with the production of phenolics, phytoalexins, and structural barriers; as well as the production of phenolics and phytoalexins. Phenolics and phytoalexins are toxic to the pathogens; they accumulate with pathogen attack and are released at sites of infection.

This research addresses the hypothesis that AMF can help control the levels of *Fusarium* on plant roots. The species of the genus *Fusarium* are considered among the most important food contaminants in the world. Fusaria are ubiquitous, i.e., they are widely distributed and they are found in every climatic agricultural region (Lerda *et al.*, 2005). *Fusarium* species produce mycotoxins that are harmful to mammals and cause plant diseases that adversely affect crop production (Yates *et al.*, 2003). Important mycotoxins produced include moniliformin, fusaric acid, and fumonisin B group of toxins (Kroschel & Elzein, 2004). The most important producers of fumonisin B₁ are *Fusarium moniliforme*, *F. proliferatum*, *F. nygamai*, and *F. napiforme* (Marin *et*

al., 1998; Nelson *et al.*, 1992). According to Kellerman *et al.* and Harrison *et al.* (quoted by Shephard *et al.*, 2002) fumonisin B₁ has been linked to various disease syndromes in animals, such as leucoencephalomalacia in horses and pulmonary oedema in swine. Fumonisin B₁ has also been implicated in oesophageal and gastric cancers in humans (Chu & Li, 1994).

The interaction between the AM fungus *Glomus mosseae* and the two pod rot pathogens *Fusarium solani* and *Rhizoctonia solani* and subsequent effects on plant growth and yield of peanut plants were investigated by Abdalla and Abdel-Fattah (2000). Infection by the pathogens *F. solani* and *R. solani* reduced growth and yield of peanut plants at all stages. Pre-inoculation of plants with *G. mosseae* reduced the impact of the pathogens. Peanut plants inoculated with the AM fungus had a lower incidence of pod and root rot disease than non-mycorrhizal plants. Results suggested that the AM fungus *G. mosseae* could act as a bioprotective agent against *F. solani* and *R. solani*, the peanut pod and root disease pathogens (Abdalla & Abdel-Fattah, 2000).

In the study by Karagiannidis *et al.* (2002) AMF *Glomus mosseae* enhanced tolerance by eggplant and tomato plants of the disease caused by the pathogenic fungus *Verticillium dahliae*. Inoculation of tomato and eggplant seedlings with AMF significantly increased their height and their fresh and dry weight. The combination of the AM and the pathogenic fungus on the other hand significantly reduced height and fresh and dry shoot weight in eggplant and tomato (except plant height) in comparison to the mycorrhizal plants, but significantly increased plant height and fresh weight in both the species when compared with the controls. Substantially lower values for these three characteristics were recorded for both the species in *Verticillium* inoculated plants when compared with the controls. That led to the conclusion that the beneficial effect of the AMF superseded the pathogenic effect of *V. dahliae* (Karagiannidis *et al.*, 2002).

Newsham *et al.* (1995) conducted a study in which seedlings of the annual grass *Vulpia ciliate sub sp. ambigua* were inoculated with both the root pathogen *Fusarium oxysporum* and an AM fungus (*Glomus sp.*). Inoculation with AMF had not increased plant P concentrations, but had protected the plants from the deleterious effects of *F. oxysporum* infection on shoot and root growth, apparently by suppressing pathogen development in roots. The effects of AMF on plant performance were negligible in the absence of *F. oxysporum*. Their results suggested that the main benefit supplied by the AM fungi to *V. ciliate* is in the protection from pathogenic fungi, rather than the improved P uptake.

Microdochium patch (*Fusarium patch*) disease is considered the most important turf grass pathogen in the United Kingdom. Gange and Case (2003) conducted a survey of pesticide records

of golf courses to ascertain the incidence of *Microdochium* patch. A negative correlation was found to exist between AM fungal abundance and disease incidence. Addition of AM fungi to a putting green produced some evidence that this resulted in a reduction in pathogen attack. It was concluded that AM fungi might have potential for use in a biocontrol program against *Microdochium* patch in fine turf.

The interaction between four AM fungi, *Glomus* sp., *G. proliferum*, *G. intraradices* and *G. versiforme*, and the root-rot fungus *Cylindrocladium spathiphylli* and subsequent effects on growth and phosphorus nutrition of banana were investigated (Declerck *et al.*, 2002). Root infection by *C. spathiphylli* reduced the growth of banana plants, but preinoculation with AM fungi significantly reduced that detrimental effect. Lower disease severity was observed for the plants inoculated with one of the four AM fungi. Root damage caused by *C. spathiphylli* was decreased in the presence of AM fungi.

In an investigation by Chakravaty and Mishra (1986) the wilting of *Cassia tora* caused by *Fusarium oxysporum* was reduced significantly when inoculated with AMF. In that study, plant growth was enhanced in mycorrhizal plants. There was also reduction in the density of *F. oxysporum* in the rhizosphere of mycorrhizal plants. It could be concluded from the results that *G. fasciculatus* and *G. tenuis* acted to some degree as biocontrol agents against *F. oxysporum* wilting of *C. tora*.

2.4 Morogo plants

Morogo is the Pedi/Tswana name for green leafy vegetables that grow as weeds, naturally or cultivated as crop. *Morogo*, together with maize porridge has been a dominant staple in the rural villages of the Limpopo Province. *Morogo* plants are known to be good sources of calcium, magnesium, iron, zinc, vitamin C and carotene (Steyn *et al.*, 2001). Green leafy vegetables are also recognised for their characteristic colour, flavour and therapeutic value (Gupta *et al.*, 2005). *Morogo* plants are not only of dietary significance, but also provide people with their basic needs in terms of medicine and as a source of income (Nesamvuni *et al.*, 2001; Shackleton, 2003).

The scarcity of some of these *morogo* plants is becoming a major problem in many rural areas of Southern Africa due to droughts, population pressure and overexploitation (Nesamvuni *et al.*, 2001). *Morogo* plants may also be lost to fungal diseases. The interaction of *morogo* plants and AMF as well as the tripartite interaction of *morogo* plants, AMF and *Fusarium* species has not

been studied. AMF may benefit plants in terms of nutrient acquisition (Hovsepian & Greipsson, 2004; Demir, 2004; Walling & Zabinski, 2004), maintenance of plant water status (Hernández-Sebatia *et al.*, 1999; Augé, 2001; Al-Karaki *et al.*, 2004; Subramanian *et al.*, 2006; Pinior *et al.*, 2005; Wu & Xia, 2006;) and protection from pathogens (Chakravaty and Mishra, 1986; Newsham *et al.*, 1995; Abdalla & Abdel-Fattah, 2000; Declerck *et al.*, 2002; Karagiannidis *et al.*, 2002; Gange & Case, 2003). The objectives of the current study are therefore to (i) isolate and identify AMF growing indigenously with *morogo* plants, (ii) evaluate plant growth and mycorrhizal colonisation, and (iii) evaluate the effect of AMF on the level of *Fusarium* in soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

The Dikgale Demographic Surveillance Site (DDSS) is a rural setting situated in the Limpopo Province, South Africa, about 40 km east of Polokwane, the capital of the province, and 15 km from the University of Limpopo. The site covers an area of 71 km², situated between latitudes 23.46° and 23.49° S longitudes 29.42° and 29.47° E and lies at an average altitude of 1400 m above sea level (Alberts *et al.*, 2005).

DDSS is composed of eight villages namely, Madiga, Mantheding, ga-Ntsime, ga-Maphoto, Moduane, Sefateng, ga-Tjale, and Maselaphaleng. The villages are situated close to one another. Each village has a central residential area comprising demarcated housing stands, with communal open fields used for grazing. The infrastructure is poor.

The climate is dry with low rainfall. Most of the rainfall occurs between November and April and the average rainfall is 401-500 mm (Alberts *et al.*, 2005). The temperature ranges from a minimum of 6°C in winter to an average maximum of 26°C in summer (Alberts *et al.*, 2005). The population lives mostly from subsistence farming.

3.2 Sampling sites

Sampling was done from only four villages of the DDSS namely, Madiga, Mantheding, Moduane and Sefateng. The four villages were selected because they are the largest of the eight villages. Soil samples were collected from each of the individual sites.

3.3 Sampling

Soil and *morogo* (*Amaranthus thunbergii* and *Cleome gynandra*) sampling was carried out in May 2005, February 2006 and April 2006. *Morogo* plants were carefully lifted with a fork from the ground and a soil corer of 10 cm diameter was used to dig out soil to a depth of 30 cm. Soil

samples were collected around the roots of six *morogo* plants (*Amaranthus* spp.) selected randomly at each village. Each composite sample, consisting of six sub-samples, from each village was carefully transferred to a marked plastic bag and kept in a cooler box during transport to the laboratory. Upon arrival at the laboratory, soil samples were stored in the fridge at 4°C for two to three weeks before analysis. *Morogo* samples were stored at 4°C, for two days before analysis.

3.4 Mycorrhiza

Mycorrhizal spores were isolated from soil particles, enumerated and identified. Assessment of colonisation by arbuscular mycorrhizal fungi (AMF) was done on roots of *Amaranthus thunbergii* after staining the root samples.

3.4.1 Isolation of mycorrhizal spores

The wet sieving and sucrose-density gradient centrifugation method was used (Brundrett *et al*, 1996). 50 g of the composite soil sample was placed in a beaker and 200 ml of water was added to the beaker with soil. The suspension was stirred to free the spores from the soil. The mixture was passed through sieves of different mesh sizes (250, 150, 125, and 63 µm). Sievings were collected into centrifuge tubes and mixed with about 40 ml of 70 % sugar solution. The mixture was centrifuged at 2000 rpm for three minutes using Precision Duraforce 100 Centrifuge. The supernatant was sieved through the 32 µm-sized mesh sieve and washed thoroughly with sterilised deionised water to replace sucrose and alleviate osmotic stress on spores. The pellets left in the centrifuge tubes were discarded. The spores were collected into Petri dishes and stored at 4 °C until they were examined.

3.4.2 Enumeration of spores - quantification of mycorrhizas

A 50 g soil sample was weighed and spores extracted using the above method. The spore extract was transferred to a Petri dish and contents shaken to distribute spores. Direct counting of spores was done because the spores were few enough to be counted directly (INVAM, 2006a). Only shiny, bright spores that looked alive, contained many lipids and were not parasitised were counted.

3.4.3 Identification of spores

Diagnostic slides for spore identification were prepared following the procedure by Schenck and Pérez (1990). Spores were picked up individually and mounted on to microscope slides using polyvinyl-lacto-glycerol (PVLG) alone and PVLG plus Meltzer's reagent as mountants (Schenck & Pérez, 1990). Spore characteristics were examined using Motic Compound Microscope and pictures captured using Motic MC Camera 1.1. The spore characteristics examined were spore dimensions, spore colour, hyphal attachment, occlusions and wall structures. Identifications were based on current species descriptions and identification manuals (Schenck & Pérez, 1990; INVAM, 2006b).

3.4.4 Staining roots for mycorrhizal colonisation

Young "feeder" root samples were stained and examined for colonisation. The procedure followed was the slow method of Smith and Dickson (1997). During this procedure, root samples were rinsed with water and cleared in 10 % potassium hydroxide (KOH) overnight. Cleared roots were rinsed thoroughly with water and bleached with 0.1 N hydrochloric acid (HCl). The roots were then stained by covering them with 0.05 % Trypan blue in lactoglycerol solution for two days. Roots were destained in 0.1 N HCl and transferred to lactoglycerol until they were examined.

3.4.5 Root colonisation assessment – quantification of mycorrhizas

The gridline intersection method (Brundrette *et al.*, 1996; Smith & Dickson, 1997), under dissection microscope, was used to assess the root colonisation. The number of times a root intersected with the grid as well as the number of intersects in which the root contained mycorrhizal structures were determined. The ratio of those two values was used to obtain the percent colonisation.

3.5 *Fusarium*

Fusaria were isolated from air, plant organs and soil samples. The isolated species were purified, identified and quantified.

3.5.1 Isolation of *Fusarium* from air, plant and soil samples.

3.5.1.1 From air

The exposed Petri dish technique was used. Petri dishes containing *Fusarium* selective modified Nash-Snyder PCNB agar medium (Nelson *et al.*, 1983) were exposed for five minutes. The Petri dishes were then incubated at 25 °C for 10 days. All *Fusarium*-like colonies that developed during incubation were transferred, by cutting pieces of agar, to Carnation Leaf Agar (CLA) plates.

3.5.1.2 From leaves

Morogo leaves were put in 1.0 % peptone water containing 0.01 % Tween 80. One leaf was added to 99.0 ml of Tween-peptone water (to prepare the 10^{-2} dilution), processed, and agitated on a rotary shaker for 10 minutes at 200 rpm. Tween was added to break down the surface tension between *Fusarium* colonisers and the leaf surface. The mixture was shaken on a rotary shaker to wash off surface fungal colonisers into the diluents. A volume of 1.0 ml of the peptone water-leaf mixture was drawn and added to 9.0 ml peptone water to prepare the 10^{-3} dilution. The dilution procedure was repeated to yield the 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} dilutions. A 0.1 ml aliquot from each dilution was then spread onto a Petri dish containing *Fusarium* selective modified Nash-Snyder PCNB agar medium (Nelson *et al.*, 1983). Plates were incubated at 25 °C for 14 days. All *Fusarium*-like colonies that developed during incubation were transferred to Carnation Leaf Agar (CLA) plates and incubated at 25 °C under light of wavelength 498 nm from fluorescent tubes for 7 to 10 days.

3.5.1.3 From roots

Roots were washed in running tap water for 10 minutes, rinsed in sterile water and then blot-dried. 10 mm long roots pieces, three per plate were placed on the *Fusarium* selective modified Nash-Snyder PCNB agar medium (Nelson *et al.*, 1983). Plates were incubated at 25 °C under light from fluorescent tubes for 14 days. All *Fusarium*-like colonies that developed during incubation were transferred to carnation leaf agar (CLA) plates.

3.5.1.4 From soil

The serial dilution plating method was used. Soil was allowed to air-dry before the suspension was prepared in order to reduce bacterial contamination (Nelson *et al.*, 1983). A 1.0 g soil sub-sample was added to 9.0 ml sterile water to prepare the 10^{-1} dilution. A volume of 1 ml of the resulting suspension was transferred to 9.0 ml sterile water to prepare the 10^{-2} dilution. The procedure was repeated to yield 10^{-3} , 10^{-4} and 10^{-5} dilutions. A 0.1 ml aliquot from each dilution was then spread on to a Petri dish containing *Fusarium* selective modified Nash-Snyder PCNB agar medium (Nelson *et al.*, 1983). Plates were incubated at 25°C under light from fluorescent tubes for 14 days. All *Fusarium*-like colonies that developed during incubation were transferred to Carnation Leaf Agar (CLA) plates.

3.5.2 Purification of *Fusarium* cultures

Single-spore isolation method (Nelson *et al.*, 1983) was used. Cultures on CLA plates were flooded with 10 ml sterile deionised water to prepare a suspension of conidia. The suspension was poured over the solidified 2 % water agar plates to cover the surface. Excess suspension was discarded. Plates were incubated at 25 °C for 24 hours. Plates were examined under a stereomicroscope and small squares of agar containing single germinating conidia were transferred aseptically to CLA, Potato Dextrose Agar (PDA), and Synthetic Nutrient Agar (SNA) plates. Plates were incubated at 25 °C under light from fluorescent tubes for 10-14 days.

3.5.3 Identification of *Fusarium*

Fusarium species were identified from the CLA, PDA and SNA culture media. Identification was carried out on the basis of macroscopic characteristics such as colony colour, morphology, and microscopic characteristics including conidia size and morphology (Nelson *et al.*, 1983).

3.5.4 Quantification of *Fusarium*

All *Fusarium*-like colonies, based on the colour and growth pattern of mycelia (Nelson *et al.*, 1993) that developed during incubation after isolation from plant parts, soil and air were counted. The number of colony-forming units (CFU) was confirmed after identification.

3.6 Soil physical and chemical analyses

Soil physical and chemical analyses were done by the Eco-analytica Laboratory, North-West University. Soil samples were analysed for particle size distribution according to the procedures advocated by the American Society for Testing and Materials (ASTM, 1961). Soil samples were chemically analysed by means of a 1:2 (soil: water) extraction procedure as described by Peech (1965). Soil pH and electrical conductivity (EC) were determined in the 1:2 (v/v) water extraction with a WTW LF92 conductivity meter at 25°C.

3.7 Identification of *morogo* plants

Morogo plants sampled from the study area were identified scientifically by the South African National Botanical Institute (SANBI), Pretoria, South Africa. Local villagers of the DDSS provided traditional names for the different *morogo* types.

3.8 Questionnaires

Questionnaires were developed to survey the study area and collect data on socio-economic status and *morogo* plant usage. The first part of the questionnaires aimed at obtaining information on number of household members, age, occupation and diet. The second part comprised questions about *morogo* plants: the parts eaten, cultivation, harvesting, storage, preparation methods, and frequency of consumption.

3.9 Greenhouse trials

Greenhouse trials were performed in order to examine the potential of AMF to improve *morogo* plant growth and to control *Fusarium proliferatum*. *Morogo* host plants that were used were cowpeas. In order of preference, cowpea is ranked number three by the villagers of the DDSS. The second most preferred type of *morogo*, *Amaranthus spp*, died off before the end of the investigation. *Cleome gynandra* which is the most preferred type, belongs to the family Capparaceae that is known to be non-mycorrhizal (Brundrett, 1999).

3.9.1 Experimental design

In this investigation, the following six treatments were included: 1) uninoculated control, 2) inoculated with a commercial arbuscular mycorrhizal fungal inoculum from Mycoroot (PTY) Ltd that consisted of a mixture of selected indigenous *Glomus* spp. (referred to as commercial AMF in this study), 3) inoculated with indigenous AMF that was baited from DDSS soil (referred to as local AMF in this study), 4) inoculated with a commercial arbuscular mycorrhizal fungal inoculum from Mycoroot (PTY) Ltd that consisted of a mixture of selected indigenous *Glomus* spp. and *Fusarium proliferatum*, 5) inoculated with local AMF from DDSS soil and *F. proliferatum*, 6) inoculated with *F. proliferatum*. Three replicates for each treatment were made. The total number of pots was 18, i.e., six treatments x three replicates. Two plants were grown in each pot.

3.9.2 Culturing of AMF

Two types of inocula were used separately as described above. Local AMF cultures were obtained using natural soil as inoculum for increasing propagule numbers. Pot cultures were initiated using sorghum (*Sorghum sudanese*) as trap plants and sterilised sand as the growth medium. Sterilised sand was mixed with natural soil on a 1:1, v/v ratio and surface sterilised sorghum seeds were sown. Cultures were grown for eight weeks. Aerial parts of sorghum plants were cut and soil allowed to dry out. The dried soil was put in ziploc bags and stored at 4 °C for two weeks. Spores were isolated from the soil to initiate single-spore cultures. A minimum of 20 spores was used to inoculate sterilised sand and surface sterilised sorghum seeds were sown. Cultures were allowed to grow for another eight weeks. As in the first round of planting, aerial parts of plants were cut, soil allowed to dry and dried soil put in ziploc bags and stored at 4 °C for two weeks. Spores were extracted from the soil and replanted to produce the local AMF inoculum.

3.9.3 Surface sterilisation of sorghum and cowpea seeds

To get rid of microorganisms on the surface of seeds, sorghum (*Sorghum sudanese*) seeds used for trap plants and cowpea (*Vigna unguiculata*) seeds were soaked in 10 % household bleach (sodium hypochlorite) for 10 minutes (Miyasaka *et al.*, 2003). After soaking seeds were rinsed three times with sterile water.

3.9.4 Sterilisation or pasteurisation of sand

Sand used to initiate pot cultures was autoclaved on a three day consecutive cycle at 121 °C for 15 minutes. Sand used for growing *morogo* plants (cowpea) was pasteurised for 60 minutes at 70-80 °C using electrode boiler model AL-60 steam generator.

3.9.5 Culturing of *Fusarium*

Fusarium proliferatum (PPRI 7935) isolated in a previous study from *morogo* growing in Gyani was obtained from the National Collection of Fungi: Culture Collection, Pretoria. The culture was maintained on PDA at 4 °C. The fungus was subcultured on PDA and incubated at 25 °C under light of wavelength 498 nm from fluorescent tubes for seven days before inoculation.

3.9.6 Inoculation of soil with AMF

To investigate the effect of AMF on plant growth and the potential of AMF on controlling *Fusarium proliferatum*, soil in which cowpea seeds were sown was inoculated with AMF. 5 g of the commercial mycorrhizal inoculum was placed about 1 cm below the seed at planting. In pots inoculated with local AMF, a minimum of 20 spores put on sterile filter paper, was placed about 1 cm below the seeds at planting.

3.9.7 Inoculation of soil with *Fusarium*

Seven weeks after potting, three sets of plants were inoculated with *Fusarium proliferatum*. A delayed inoculation time with *F. proliferatum* was chosen because bioprotection by AMF occurs mainly when symbiosis is well established before the pathogen attack (Rosendahl, 1985). *F. proliferatum* was grown on (PDA) slants at 25°C for seven days. The inoculum was prepared by washing the growing mycelia with sterile water (10 ml per test tube) to suspend conidia. A volume of 1 ml of the resulting suspension was transferred to 9.0 ml sterile water to prepare the 10⁻² dilution. The procedure was repeated to yield 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions. Several 0.01 ml aliquots from each dilution were then deposited on microscope slides. Conidia were counted under the compound microscope and the concentration of conidial suspension was estimated. The concentration of the suspension was approximately 2.74 x 10⁵ conidia ml⁻¹. A sterile tube of 8 mm diameter open at both ends was inserted into the soil next to each plant and 8 ml of the conidial

suspension was injected with a sterile syringe through the tube into the soil. Control plants were in the same manner given 8 ml of sterile water.

3.9.8 Growth substrate

The potting medium was a pasteurised river sand with low nutrient content. The texture was mostly fine sand (96.2 % sand, 0.3 % silt and 3.5 % clay).

3.9.9 Growth conditions

Planted pots were placed in the greenhouse with temperature maintained at 25 °C during the day and 20 °C during the night. Plants were watered as required with approximately 200 ml per pot. A volume of 200 ml of one quarter strength Long Ashton nutrient solution (Hewitt, 1966) per pot was given to plants every third week.

3.9.10 Evaluation of plant growth and mycorrhizal root colonisation

To evaluate the level of mycorrhizal root colonisation and the effect thereof on plant growth and *F. proliferatum* levels, the following procedures were followed: Plants were harvested ten weeks after planting. Immediately after harvesting root systems were washed carefully in tap water to remove adhering soil particles. Two small fractions of the root system were taken from the composite sample. One fraction was kept for determination of mycorrhizal colonisation by clearing and staining with Trypan blue. The other fraction kept for the recovery of *Fusarium*. Dry weights of roots and shoots were determined after drying at 70°C for 24 hours. Dry weight of leaves was determined after freeze-drying at -60°C for 24 hours. Shoot height was measured at harvest.

3.9.11 Identification and quantification of *Fusarium proliferatum*

Identification (Nelson *et al.*, 1983) and quantification of *F. proliferatum* was done after the pathogen was recovered from soil, roots, and leaves.

3.9.12 Fumonisin determination

To evaluate the levels of fumonisin produced by *F. proliferatum*, root samples were ground using mortar and pestle. The ground root samples were added to 70% methanol water and shaken on a

rotary shaker at 200 rpm for 10 minutes. The mixture was filtered through filter paper and the supernatant was kept for the determination of fumonisin. The presence of fumonisin was detected using the commercial Veratox Quantitative Fumonisin test, ELISA kit, Neogen (USA), following the manufacturer's instructions.

3.9.13 Leaf elemental analysis

Leaf composition of phosphorus, carbon, nitrogen and potassium was analysed by the Agricultural Research Council (ARC) Institute for Soil, Climate and Water, Pretoria.

3.10 Statistical analyses

Statistical analyses were performed on all data sets using STATISTICA 7 (StatSoft, Inc ©). Parametric and non-parametric statistical analyses were performed on all data sets. Data were tested for normality using the Shapiro-Wilk's test. For normally distributed (parametric) data, a one-way breakdown analyses of variance (ANOVA) was performed, where after a Tukey's honest significant difference (HSD) test was performed to determine statistical significance between the various samples. For non-parametric data, Kruskal-Wallis ANOVA was used to determine statistical significant difference. All tests were performed at 5 % probability of error ($P \leq 0.05$). Correlations between variables were investigated using correlation matrices.

CHAPTER 4

INDIGENOUS KNOWLEDGE SYSTEMS

Some people at the Dikgale Demographic Surveillance Site (DDSS) are not aware of the nutritional value of naturally occurring foods, particularly *morogo* plants and they regard them as inferior to use. *Morogo* plants are good sources of calcium, magnesium, iron, potassium, zinc, vitamin C, folate and carotene (Steyn *et al.*, 2001, Nesamvuni *et al.*, 2001). These plants are not only of dietary significance, but also provide people with their basic needs in terms of medicine and as a source of income (Nesamvuni *et al.*, 2001; Shackleton, 2003).

Morogo is the Pedi / Tswana name for green leafy vegetables that grow as weeds mostly among maize plants, occur naturally among field flora or are cultivated as crop. *Morogo* plants are usually cultivated in home gardens and schemes. The plants survive without any form of irrigation or pest control. The best yields of *morogo* are obtained during rainy seasons. Young leaves are picked mainly by women approximately one month after germination. *Morogo* crops are picked in bulk, sun-dried and stored for winter season. Drying is done either before or after cooking. *Morogo* that is dried before cooking is known as *legwahla* (the traditional references made hereafter are in Pedi). *Mokhuša* is the name given to *morogo* that is cooked before drying. Dried *morogo* is kept in closed plastic or metal containers, for as long as it is needed.

Morogo, together with maize or sorghum porridge has been, and is still, a dominant staple in the rural villages of the Limpopo Province. The names of *morogo* plants that are usually used for consumption in the (DDSS) are presented in Table 4.1. *Morogo* plants are consumed once to three times a week with a serving size of one cup. Young children and teenagers do not like eating *morogo*; they eat *morogo* only when there is nothing available for them as a side dish to porridge.

Traditionally, fresh *morogo* leaves and *legwahla* (*morogo* dried before cooking) are cooked with water, tomato and salt only. Cooking times vary from 20 to 60 minutes for fresh leaves depending on the type of *morogo*. *Legwahla* and *mokhuša* cooks in about 20 minutes. Tomato, onion, cooking oil, water and salt are usually added when cooking *mokhuša*.

Table 4.1 - Morogo plants that are consumed at the DDSS

Local name	Botanical name	Other name
Leroto	<i>Cleome gynandra</i> ^a	Spider-wisp
Lehlanye	<i>Vernonia fastigiata</i> ^a	
Thepe	<i>Amaranthus thunbergii</i> ^a , <i>A. hybridus</i> ^{b c} , <i>A. viridis</i> ^{b c}	Amaranth
Monawa	<i>Vigna unguiculata</i> ^b	Cowpea
Mophotse / mphodi	<i>Cucurbita spp.</i> ^b	Pumpkin
Moraka	<i>Lagenaria siceraria</i> ^b	Calabash
Mophara	*	
Thelele / Motelele	<i>Corchorus tridens</i> ^a	Wild jute
Mokgadi wa segasega	*	
Monyaku	<i>Cucumis myriocarpus</i> ^c	Bitter apple / gooseberry cucumber
Motšhatšha (lerotse)	<i>Citrullus lanatus</i> ^a	Wild watermelon
Motšhatšha (legapu)	*	
Tshehlo	<i>Tribulus terrestris</i> ^c	Devil's thorn

^a South African National Botanical Institute (SANBI)

^b van Wyk and Gericke (2000): identification was based on information in this source

^c Bromilow (2001): identification was based on information in this source

* SANBI specialist was not available to do the identification

Leroto (*Cleome gynandra*)

Leroto is the most preferred type of *morogo*. It is available during rainy seasons. It is usually cultivated amongst maize plants. It is usually mixed with *thepe* to dilute its bitter taste. Fresh *leroto* takes about an hour to cook. *Leroto* is usually preserved as *mokhuša*.

Thepe (*Amaranthus spp.*)

Thepe grows naturally or as weeds. It is the second most preferred type of *morogo* because of its versatility in the sense that it can be mixed with any other type of *morogo*. On their own, *thepe* leaves are cooked fresh for about 30 minutes immediately after being picked. They are dried only when mixed with other types of *morogo* leaves. *Thepe* is used by some people as a purgative to facilitate evacuation of the bowel.

Monawa (Vigna unguiculata)

Monawa is usually cultivated for its leaves as well as for its seeds (beans). Fresh leaves taste better when cooked on the second day after picking. Leaves can be preserved as *mokhuša*. Dry seeds are cooked and served in different ways: *monawa* seeds are cooked and served as relish to porridge; seeds are mixed with maize meal during cooking and served as main dish (*sekgotho*); *monawa* seeds are mixed with maize seeds during cooking (*lewa*); *monawa* seeds are cooked, mashed and served as main dish (*semotane*). The roots of *monawa* are used as medicine to suppress pains, in women after giving birth. The said pains are referred to as *tšhilwane*.

Mphodi / mophotse

Mphodi is cultivated for its leaves, fruit and seeds. The leaves are cooked in water for 15 to 20 minutes. They become hard and unpalatable when cooked for a longer period. The fruit of *mphodi* is a pumpkin. Seeds are cooked with small amount of water and salt, allowed to dry out and either served with porridge or as snack.

Monyaku

This type of *morogo* grows naturally in the field. The leaves are a bit coarse. They are eaten fresh and can be preserved as *legwahla*. *Monyaku* is usually consumed when other *morogo* types are scarce. The leaves are cooked in water for 15 to 20 minutes.

Moraka (Lagenaria siceraria)

Moraka is cultivated for its leaves, fruit and outer covering of fruit. The leaves look similar to those of *monyaku* but have a smoother texture than *monyaku* leaves. Leaves are usually dried before cooking. *Moraka* plants produce sweet potato-like fruit (*leraka*). The fruit is boiled before eating and served as snack. When old, the covering of the fruit hardens and dries to form a container called calabash. Depending on the shape of the fruit, the dried fruit is either cut open by a knife at one end or cut into two equal parts. The contents of the covering are removed and discarded. Three types of calabashes can be identified according to shape and size: (i) *sego* – round to oval shaped, with capacity of one to two litres – used as a jug for drinking water or as dish for serving porridge; (ii) *sephoko / mokgopu* – looks like a rounded cup with a long handle, with a capacity of a cup being 250 ml and 500 ml – used for serving traditional beer; (iii) *kgapa* – round to oval shaped, with capacity of two to five litres – used as a container for traditional beer or for storing dried beans and mielie seeds.

Mophara

Mophara is cultivated for its leaves and fruit. The leaves of this *morogo* type look like those of *mphodi* but are smaller than *mphodi* leaves. Leaves are dried before cooking. The plant bears cucumber-like fruit known as *diphara* (singular, *phara*) which are oval to round shaped. Fruit are eaten raw as a snack.

Motšhatšha -legapu

Motšhatšha is cultivated for its leaves, fruit and seeds. *Motšhatšha* leaves are usually dried before cooking. This type of *morogo* plants produce watermelons which are smaller and more rounded than oval. The flesh of the fruit is white when ripe. Seeds are cooked with small amount of water and salt, allowed to dry out and either served with porridge or as snack.

Motšhatšha -lerotse (*Citrullus lanatus*)

Leaves look the same as, but bigger than those of legapu. Leaves are also dried before cooking. The plant bears fruit known as *marotse* (singular, *lerotse*) which are oval to round shape. The fruit is boiled until done, then thickened with maize meal to the consistency of a soft porridge and allowed to simmer for about 15 minutes. This fruit-maize meal dish is referred to as *kgodu* or *semphemphe*. The dish is served hot as dessert with sugar. Seeds are also cooked and served as snack or as relish to porridge.

Lehlanye (*Vernonia fastigiata*)

Lehlanye leaves are dried before cooking. The leaves have a bitter taste and are often mixed with either *tshehlo* or *thepe* leaves. *Lehlanye* plants grow naturally in the field.

Mokgadi wa segasega

Mokgadi wa segasega also grow naturally and wind over fences or trees. The leaves are cooked fresh. Some people at the DDSS believe that this type of *morogo* can be used to treat hypertension.

Tshehlo (*Tribulus terrestris*)

Tshehlo is not a preferred type of *morogo* to majority of people of the DDSS. *Tshehlo* is usually consumed when other *morogo* types are scarce. *Tshehlo* plants grow naturally in the field

Thelele / Letelele (Corchorus tridens)

Thelele leaves are usually dried before cooking. If cooked fresh, *thelele* leaves smell like *dagga* (*Cannabis sativa*) and taste bad. Cooked *thelele* is believed to relieve constipation. *Thelele* also grows naturally.

CHAPTER 5

RESULTS

In this study, isolation, identification and quantification of arbuscular mycorrhizal fungi (AMF) and *Fusarium* spp. growing in association with *morogo* plants in four villages of the Dikgale Demographic Surveillance Site (DDSS) were done. Growth, mycorrhizal colonisation and the effect of AMF on the level of *Fusarium* infection of greenhouse *morogo* plants were examined.

5.1 MYCORRHIZAS

Arbuscular mycorrhizal fungi spores were extracted from soil samples, identified and enumerated. Mycorrhizal colonisation was assessed from the roots of *morogo* plants (*Amaranthus thunbergii*).

5.1.1 Quantification of mycorrhizas

To quantify AMF, spores in soil samples were counted and percentage root colonised was determined.

5.1.1.1 Spore densities in soil

Arbuscular mycorrhizal fungal spores were present in all soil samples. The soil contained total AMF spores ranging from 2.8 to 9.8 per g soil with mean values of replicates ranging from 3.1 to 8.4 spores per g soil (Table 5.1).

Table 5.1 - Mean arbuscular mycorrhizal spore number per gram dry matter of soil at different villages of the DDSS (n=3)

Village	Spore size (µm)			Total spores / g soil
	63-125	125-250	250+	
Mantheding	7.04	1.29	0.1	8.43 ± 0.03 ^a
Moduane	2.91	0.15	0.02	3.08 ± 0.24 ^b
Sefateng	4.55	0.61	0.04	5.21 ± 0.51 ^{ab}
Madiga	5.44	0.74	0.12	6.30 ± 1.25 ^{ab}
%	86.6	12.1	1.2	

Values shown (last column) are mean ± standard error. Values with the same combination of superscript letters indicate no significant differences among villages.

Within the AMF spores obtained, 86.6 % (997 spores) were small with diameters less than 125 µm (Table 5.1). 12.1 % (140 spores) were in the 125 - 250 µm range. 1.2 % (14 spores) were larger than 250 µm.

It was evident from the mean spore densities that Mantheding had the highest overall spore numbers and Moduane had the lowest (Table 5.1). As shown in Table 5.1, the difference in spore numbers was significant between Mantheding and Moduane ($P = 0.008$).

5.1.1.2 Assessment of mycorrhizal colonisation

Percentage colonisation ranged from 22.9 % to 48.9 % with mean values of replicates ranging from 24.48 % to 39.24 % (Table 5.2). It was evident from the mean percentage colonisation that Sefateng had the lowest colonisation. Madiga showed the highest percentage colonisation followed by Mantheding then Moduane. No significant differences ($P \leq 0.05$) in percentage colonisation among villages were found. This indicates that the root samples from these villages are similar with respect to mycorrhizal colonisation.

Table 5.2 - Percentage root colonisation

Village	Spores / g dry soil
Mantheding	33.5 ± 3.45 ^a
Moduane	28.48 ± 2.85 ^a
Sefateng	24.48 ± 2.07 ^a
Madiga	39.20 ± 6.72 ^a

Values shown are mean ± standard error. Values with the same combination of superscript letters indicate no significant differences among villages.

5.1.1.3 Correlations of spore densities with percentage mycorrhizal colonisation

No significant correlation was observed between spore numbers and percentage colonisation (Fig. 5.1).

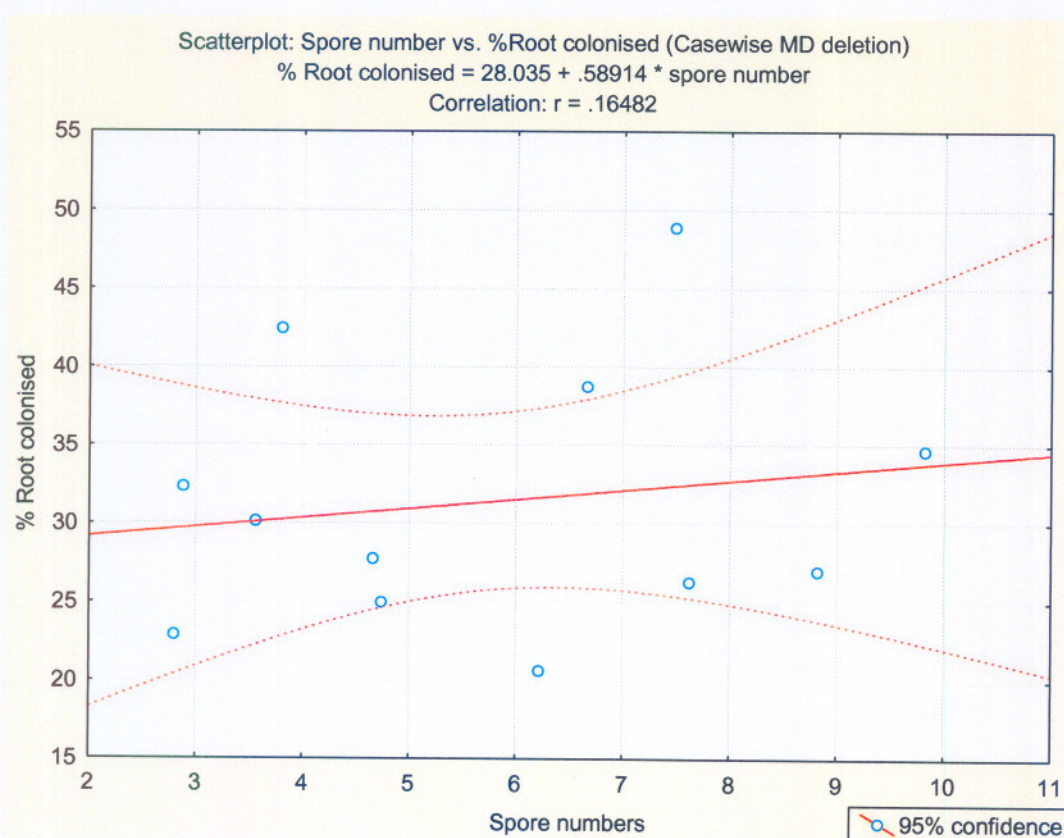


Figure 5.1 - Relationship between spore numbers / g soil and % root colonisation.

5.1.1.4 Soil Factors and their relationships between spore densities and between % root colonisation

Soil analysis results (Fig. 5.2) showed the lowest fertility for Mantheding and the highest for Moduane. Phosphorus (P) level was highest for Sefateng and lowest for Madiga. Correlation matrices for spore densities with soil fertility factors indicated that spore numbers were not significantly correlated ($P \leq 0.05$) with soil P, potassium (K), and sodium (Na) levels. A significant negative correlation was observed between spore numbers and soil magnesium (Mg) levels at $P \leq 0.05$ (Fig. 5.3). A significant negative correlation was also observed between % root colonisation and soil P levels at $P \leq 0.05$ (Fig. 5.4).

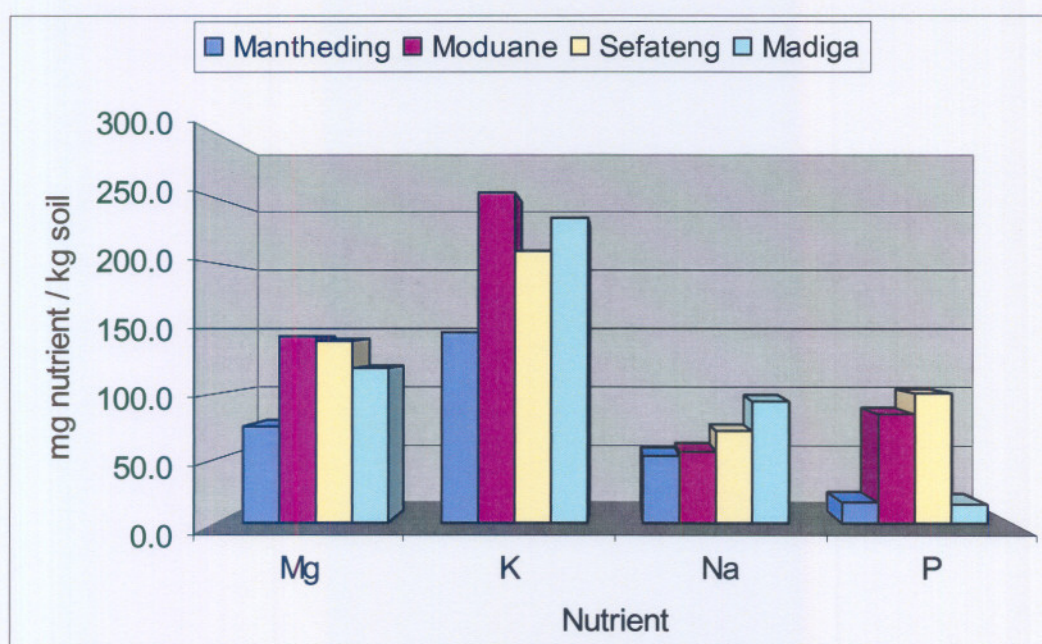


Figure 5.2 - Soil nutrient status (Mg, K, Na, P).

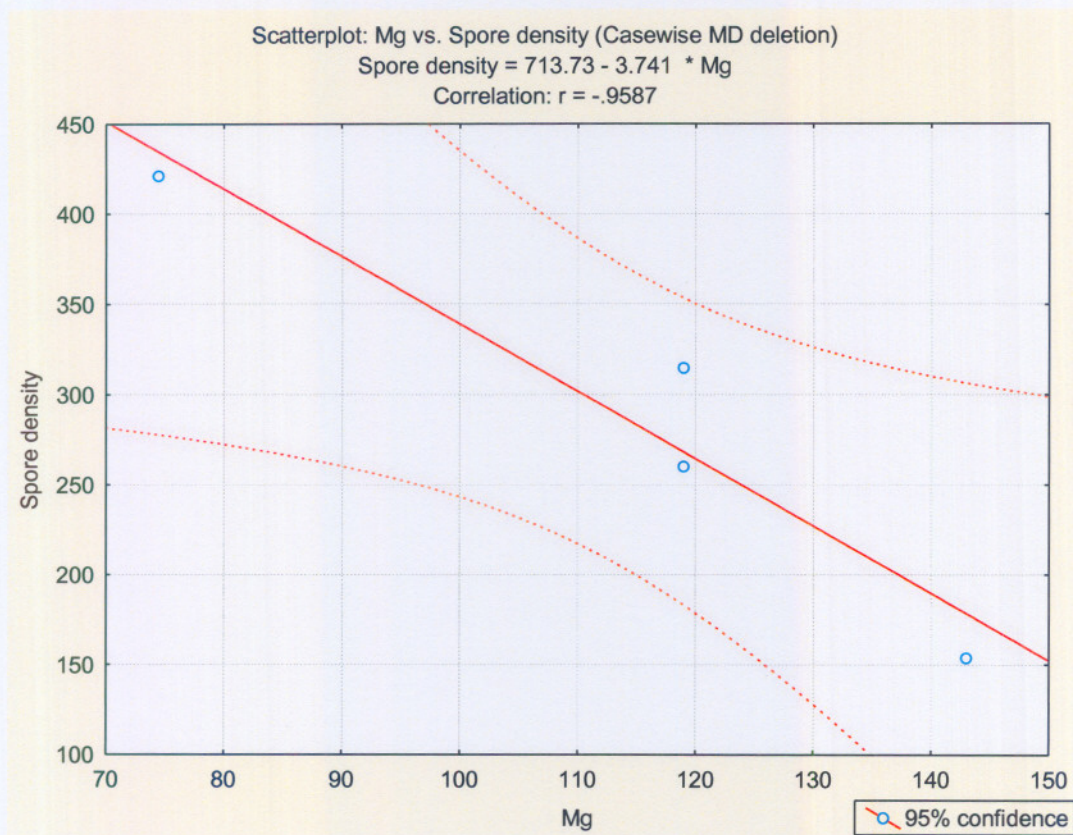


Figure 5.3 - Relationship between spore numbers / g soil and mg magnesium / kg soil.

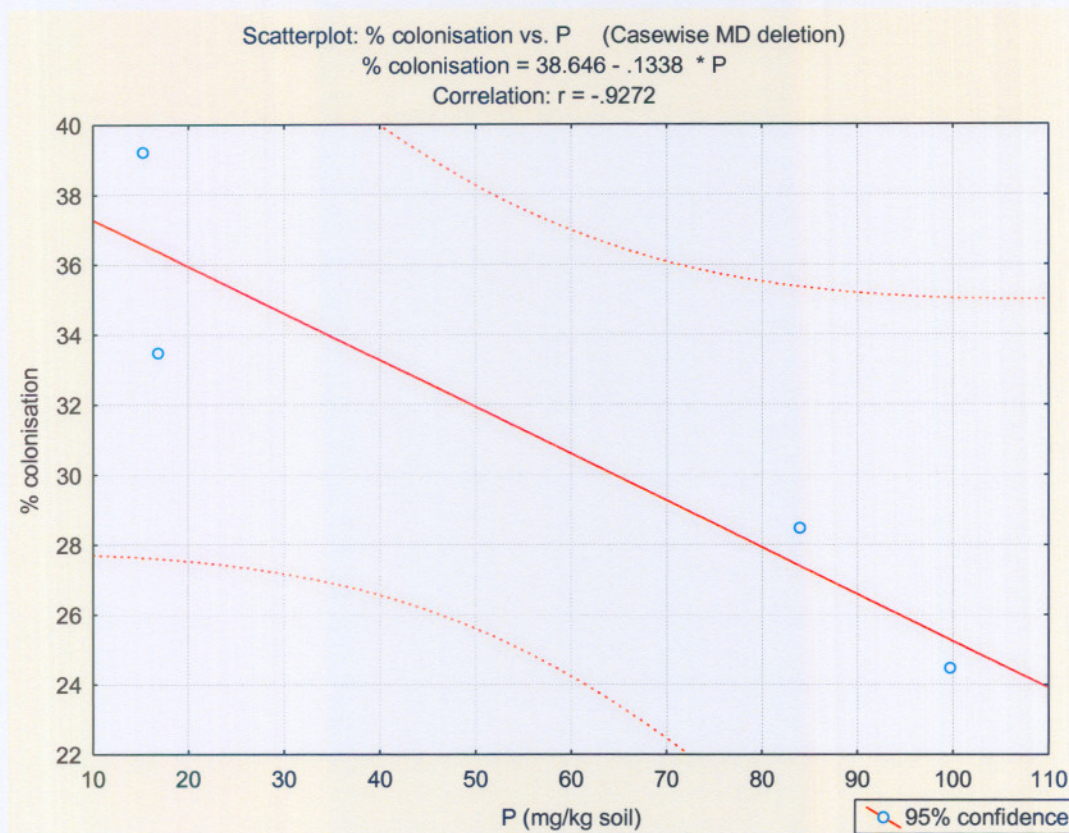


Figure 5.4 - Relationship between percentage colonisation and phosphorus levels.

5.1.2 Identification of mycorrhizal spores

Two genera of arbuscular mycorrhizal spores, *Glomus* and *Acaulospora*, were identified. Predominant AMF were tentatively identified to species level by applying the morphological characteristics of the AM fungal spores.

Isolate 1

Spores were brown to dark red-brown in colour. Their shape was globose. Spore sizes ranged from 88 to 150 μm (Plate 5.1a). The spore wall consisted of three layers; L1, L2 and L3. The outer layer (L1) was hyaline, 1-3 μm thick; the middle layer (L2) was 6-10 μm thick and the inner layer (L3) was orange-brown, 1-2 μm thick.

Tentative identification

The spores fit the description of *Glomus globiferum* Koske & Walker (INVAM, 2006b).

Isolate 2

The colour of spores ranged from yellow-brown to orange-brown. Spores were globose to subglobose. Spore sizes ranged from 120-220 μm . The spore wall consisted of three layers. L1 measured 2-3 μm thick and was mucilagenous and often degrading from the spore. L2 measured 1-1.7 μm and was generally rigid. L3 measured 2-4 μm (Plate 5.1b).

Tentative identification

Spores fit the description of *Glomus mosseae* (Nicol. & Gerd) Gerd & Trappe (INVAM, 2006b).

Isolate 3

The colour of spores ranged from pale yellow-brown to yellow-brown. Spores were globose. Their sizes ranged from 90-150 μm . The spore wall consisted of four layers. The outer layer was hyaline, mucilagenous, usually degrading and sloughing off. It measured 2-5 μm thick. The second layer was thicker measuring 1.4-5 μm . The third layer measured 0.5-5 μm . The innermost layer measured 0.3-0.9 μm (Plate 5.1c).

Tentative identification

Spores were tentatively designated to *Glomus luteum* (INVAM, 2006b).

Isolate 4

Colour ranged from yellow-brown to red-brown. Spore sizes ranged from 100-120 μm . Spores were globose to subglobose. The spore wall consisted of three layers. The outer layer (L1) was hyaline, mucilagenous, degrades and measured 2-3 μm thick. L2 measured 3-5 μm . The inner layer, L3 often appeared to be consisting of two sub layers. The sub layers either remained adherent or separate and together they had a thickness of 3-7 μm (Plate 5.1d).

Tentative identification

Spores fit the description of *Glomus intraradices* Schenck & Smith (INVAM, 2006b).

Isolate 5

Spores were globose with diameters ranging from 190-210 μm . Spores were light brown. The spore wall consisted of one layer that was 4-5.2 μm (Plate 5.1e).

Tentative identification

Spores were tentatively designated to *Glomus lacteum* Rose & Trappe (INVAM, 2006b).

Isolate 6

The colour of spores ranged from orange brown to dark brown. Spores were globose to subglobose with diameters ranging from 160-220 μm . The spore wall consisted of three layers. L1 was hyaline and its thickness ranged from 2-3 μm . L2 measured 2.5-3.5 μm and L3 1.8-2.4 μm . Two flexible inner walls, germinal wall 1 and germinal wall 2, were observed. Germinal wall 1 consisted of two layers, L1 which measured 0.5-1.6 μm and L2 which measured 1.5-2 μm . Germinal wall two also had two layers L1 and L2 which measured 1-2 μm , and 1.5-1.7 μm , respectively (Plate 5.1f).

Tentative identification

Spores fit the description of *Acaulospora capsicula* Blaszkowski (INVAM, 2006b).

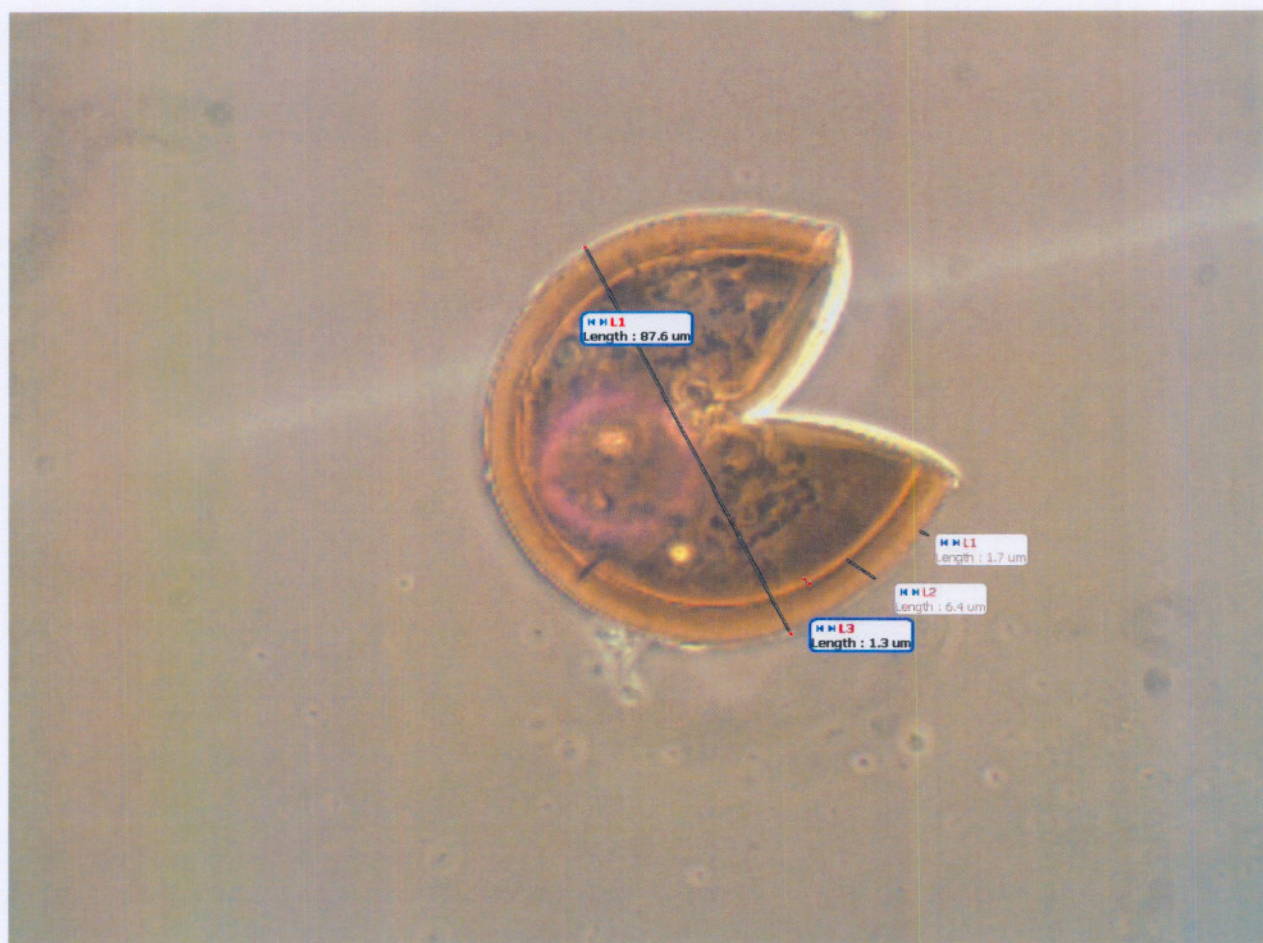


Plate 5.1a - Isolate 1 identified as *G. globiferum* Koske & Walker.

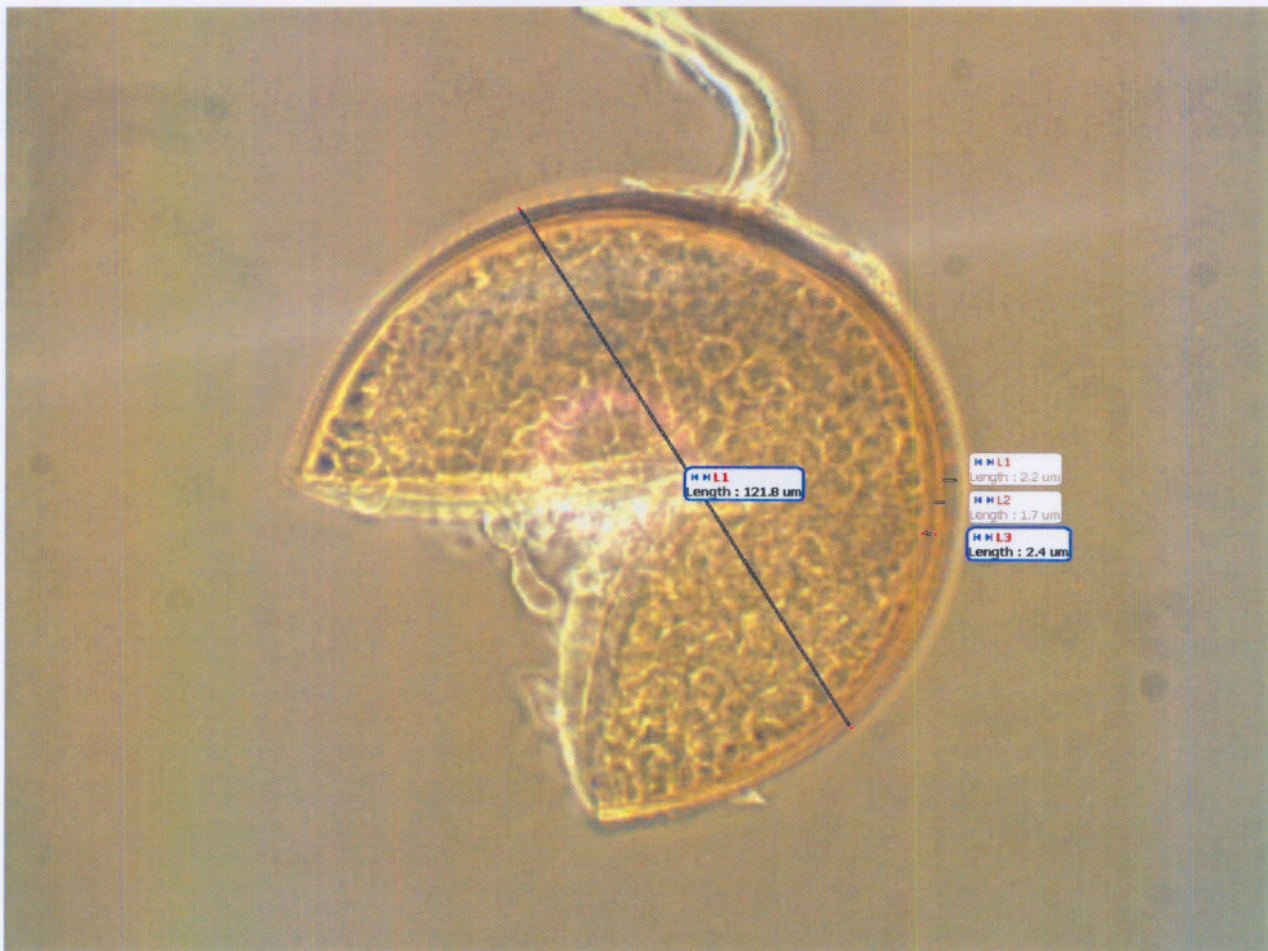


Plate 5.1b - Isolate 2 identified as *G. mosseae* (Nicol. & Gerd) Gerd & Trappe.

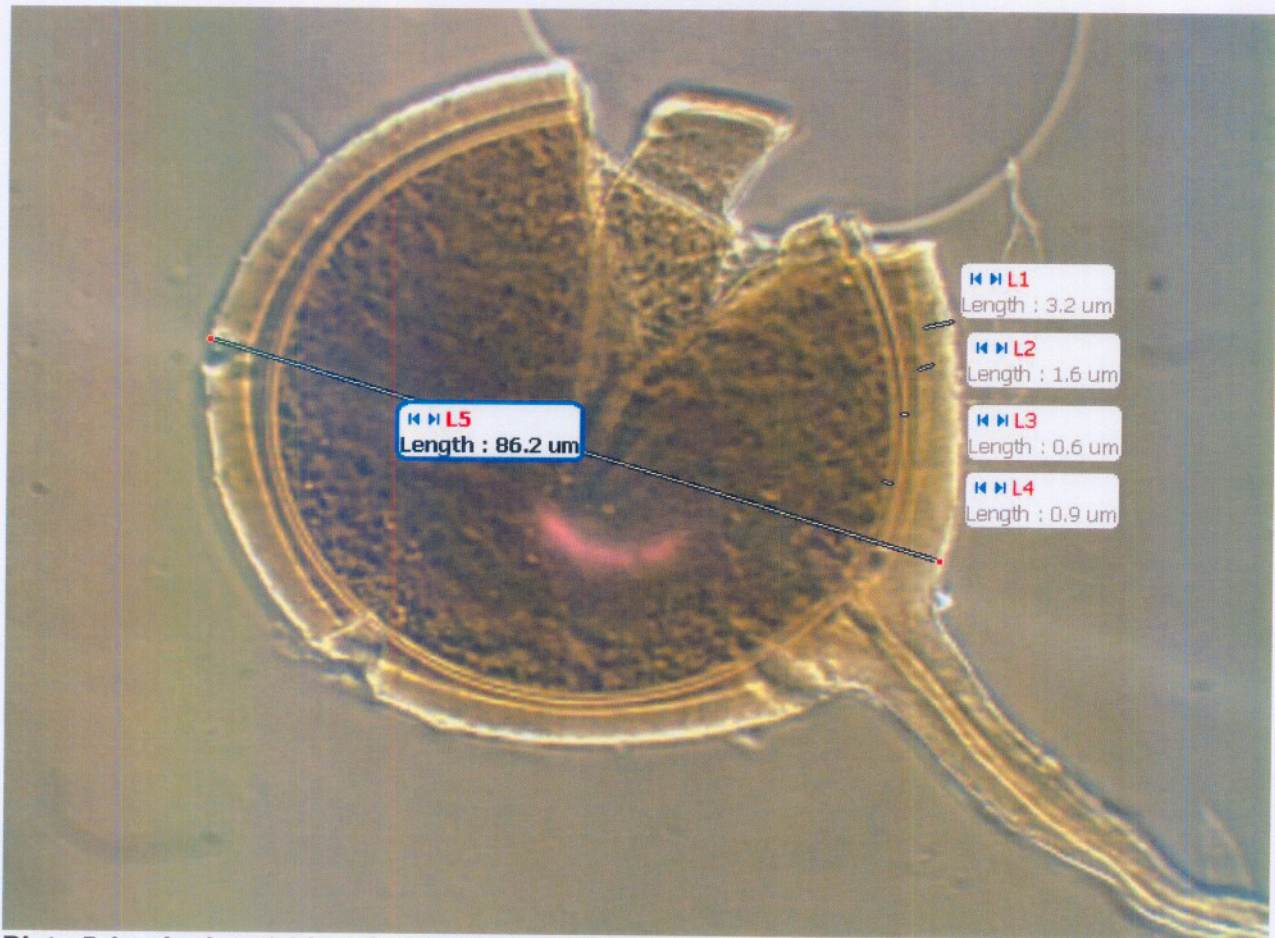


Plate 5.1c - Isolate 3 identified as *G. luteum*.



Plate 5.1d - Isolate 4 identified as *G. intraradices* Schenck & Smith.

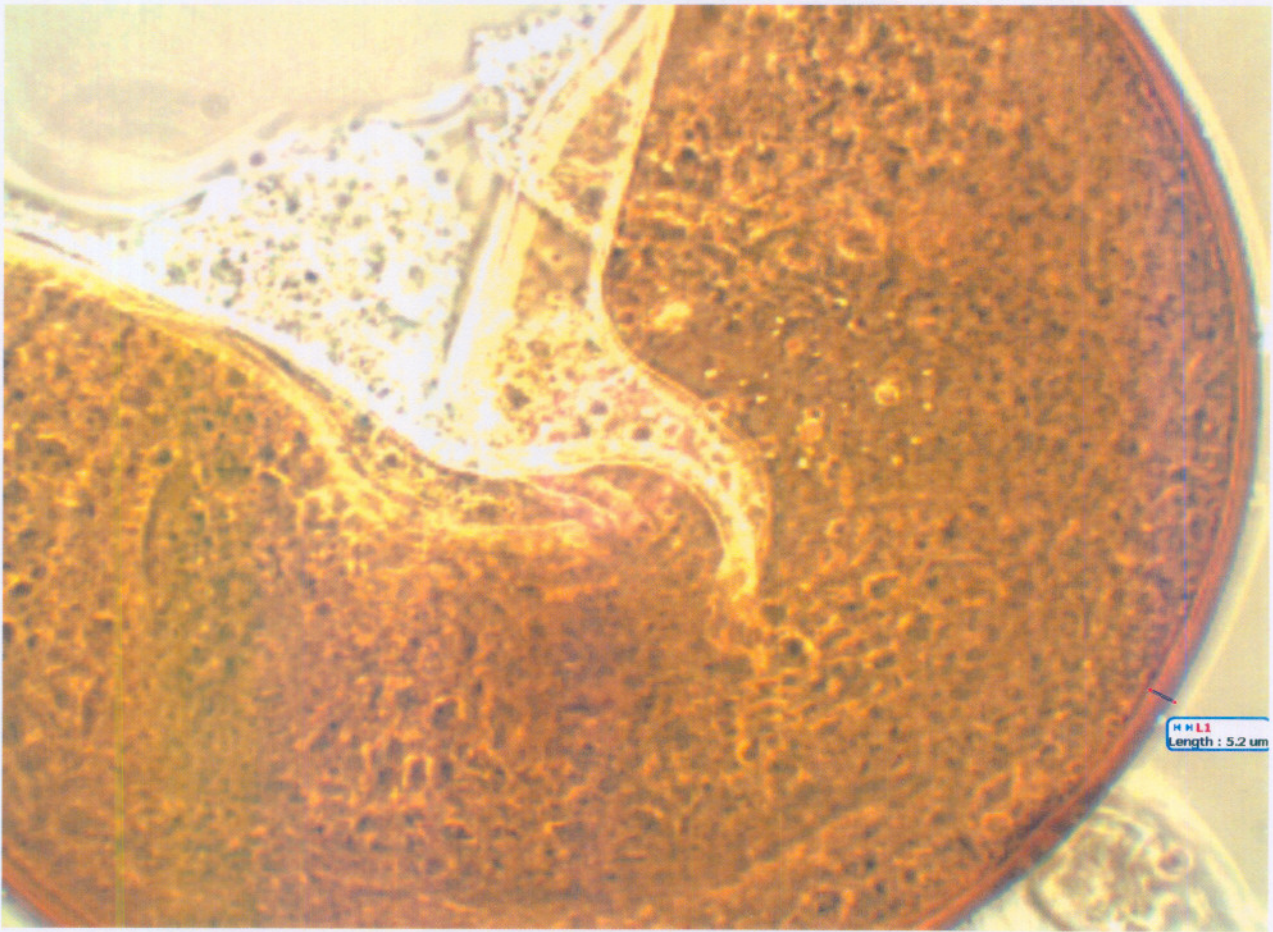


Plate 5.1e - Isolate 5 identified as *G. lacteum* Rose & Trappe.



Plate 5.1f - Isolate 6 identified as *Acaulospora capsicula* Blaszkowski.

5.2 *Fusarium*

Fusarium species isolated from air, soil and *morogo* samples from the DDSS were identified and quantified. *Morogo* species from which fusaria were isolated included *Amaranthus thunbergii* Moq, *A. thunbergii* sensu Suess. & Podlech and *Cleome gynandra*.

5.2.1 Identification of *Fusarium*

Table 5.3 summarises the *Fusarium* species that were identified from the DDSS.

Table 5.3 - *Fusarium* species identified for each of the four villages

Species	Mantheding	Moduane	Sefateng	Madiga
<i>F. chlamydosporum</i>	x	nd	x	nd
<i>F. avenaceum</i>	x	x	nd	nd
<i>F. oxysporum</i>	x	x	x	nd
<i>F. equiseti</i>	x	x	x	nd
<i>F. semitectum</i>	nd	x	x	x
<i>F. proliferatum</i>	x	x	x	x
<i>F. subglutinans</i>	x	x	nd	x
<i>F. verticillioides</i>	x	x	x	x
<i>F. poae</i>	x	nd	x	nd
<i>F. solani</i>	nd	nd	x	nd
<i>F. scirpi</i>	nd	nd	x	nd
<i>F. reticulatum</i>	nd	x	nd	nd

x: detected

nd: not detected

5.2.2 Quantification of *Fusarium*

The colony forming units obtained from *morogo* leaves, soil and air are summarised in Tables 5.4 and 5.5. Statistical analysis of the fusarial counts indicated no significant difference ($P \leq 0.05$) between the villages with respect to *Amaranthus thunbergii*. The significant difference in fusarial counts was observed between Mantheding and Madiga with respect to *Cleome gynandra* grown in close proximity with maize plants (MZ+). *C. gynandra* plants grown away from maize at Moduane and Madiga had significantly higher fusarial counts than *C. gynandra* plants in close proximity with

maize plants. There was no significant difference in total numbers of CFU isolated from leaves among villages.

Table 5.4 - Fusarial counts (CFU x 10³ / ml) from leaves of *Amaranthus thunbergii* and *Cleome gynandra* obtained from four villages of the DDSS

Village	<i>A. thunbergii</i> MZ-	<i>A. thunbergii</i> MZ+	<i>C. gynandra</i> MZ-	<i>C. gynandra</i> MZ+
Mantheding	2.4 ± 1.97 ^a	20.4 ± 20.0 ^a	3.7 ± 1.8 ^a	0.5 ± 0.4 ^a
Moduane	0.2 ± 0.13 ^a	1.4 ± 0.97 ^a	4.5 ± 1.3 ^a	1.1 ± 0.59 ^{ab}
Sefateng	2.1 ± 1.3 ^a	3.3 ± 2.1 ^a	2.0 ± 0.71 ^a	7.8 ± 3.5 ^b
Madiga	0.5 ± 0.27 ^a	4.6 ± 2.4 ^a	7.5 ± 2.3 ^a	1.1 ± 0.41 ^{ab}

Shown are means ± standard errors (n=10). Means ± standard errors with same superscript letters (within columns) are not significantly different at $P \leq 0.05$. MZ-: away from maize plants; MZ+: in close proximity with maize plants.

The lowest number of *Fusarium* colonies was isolated from air (MZ-) of Sefateng. Soil (MZ-) from Sefateng showed the highest number of *Fusarium* colonies (significant at $P \leq 0.05$).

Table 5.5 - Fusarial counts from soil (CFU x 10³ / g) and air (CFU) of the DDSS

Village	Soil MZ-	Soil MZ+	Air MZ-	Air MZ+
	CFU x 10 ² / g	CFU x 10 ² / g	CFU	CFU
Mantheding	32 ± 2.52 ^a	40 ± 1.53 ^b	13.7 ± 2.3 ^a	33 ± 0.58 ^a
Moduane	30 ± 1.0 ^a	28 ± 1.53 ^a	14 ± 3.51 ^a	27.7 ± 1.67 ^a
Sefateng	60 ± 1.73 ^b	30 ± 2.0 ^a	2 ± 0.67 ^b	29 ± 0.58 ^a
Madiga	29 ± 1.53 ^a	32 ± 1.53 ^a	18 ± 2.08 ^a	68.7 ± 19.9 ^a

Shown are means ± standard errors (n=3). Means standard ± errors with same superscript letters (within a column) are not significantly different at $P \leq 0.05$. MZ-: away from maize plants; MZ+: in close proximity with maize plants.

5.3 Identification of *morogo* plants

Citrullus lanatus (Thunb.) Matsum. & Nakai, *Vernonia fastigiata* Oliv. & Hiern, *Corchorus tridens* L., *Cleome gynandra* L., *Amaranthus thunbergii* Moq., and *A. thunbergii* sensu Suess. & Podlech were identified by the South African National Botanical Institute (SANBI), Claremont, South Africa. Some references (van Wyk & Gericke, 2000; Bromilow, 2001) were used to identify species such

as *Vigna unguiculata*, *Cucurbita* spp, *Lagenaria siceraria*, *Cucumis myriocarpus* and *Tribulus terrestris*.

5.4 Greenhouse trials

To investigate the effect of colonisation of cowpea roots on plant growth and on population of *Fusarium proliferatum*, greenhouse experiments were performed.

5.4.1 Mycorrhizal colonisation

At the end of the experiment, ten weeks after planting, all plants inoculated with AMF were colonised by AMF and intraradical structures (vesicles and hyphae) where observed (Plate 5.2). AM colonisation of plants inoculated with AMF (local or commercial) alone was significantly higher ($P < 0.05$) than those inoculated with both AMF (local or commercial) and the pathogen *Fusarium proliferatum* (Fig. 5.5, Table 5.6). Plants that were not inoculated with AMF registered only 0 – 2 % colonisation.

Table 5.6 - Mean percent colonisation (n=3) of plants for different treatments

Loc. AMF	Loc. AMF + Fus	Com. AMF	Com. AMF + Fus	Fus	Control
79.1 ± 0.61 ^a	33.9 ± 1.95 ^b	37.4 ± 0.35 ^b	21.5 ± 1.20 ^c	0.0 ^d	2.0 ± 0.48 ^d

Means with same superscript letters are not significantly different at $P < 0.05$. Loc.: local, Com.: commercial, Fus: *Fusarium*.

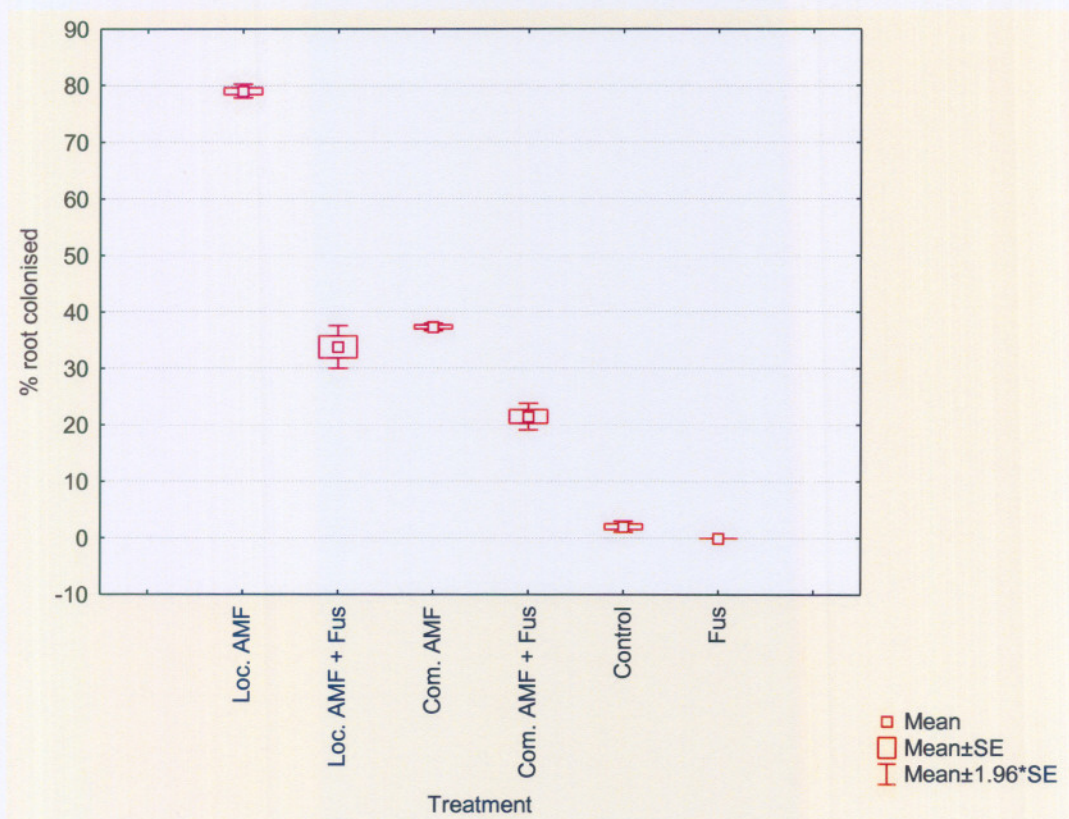


Figure 5.5 - Mean mycorrhizal colonisation and standard error (SE) at 95% confidence interval.

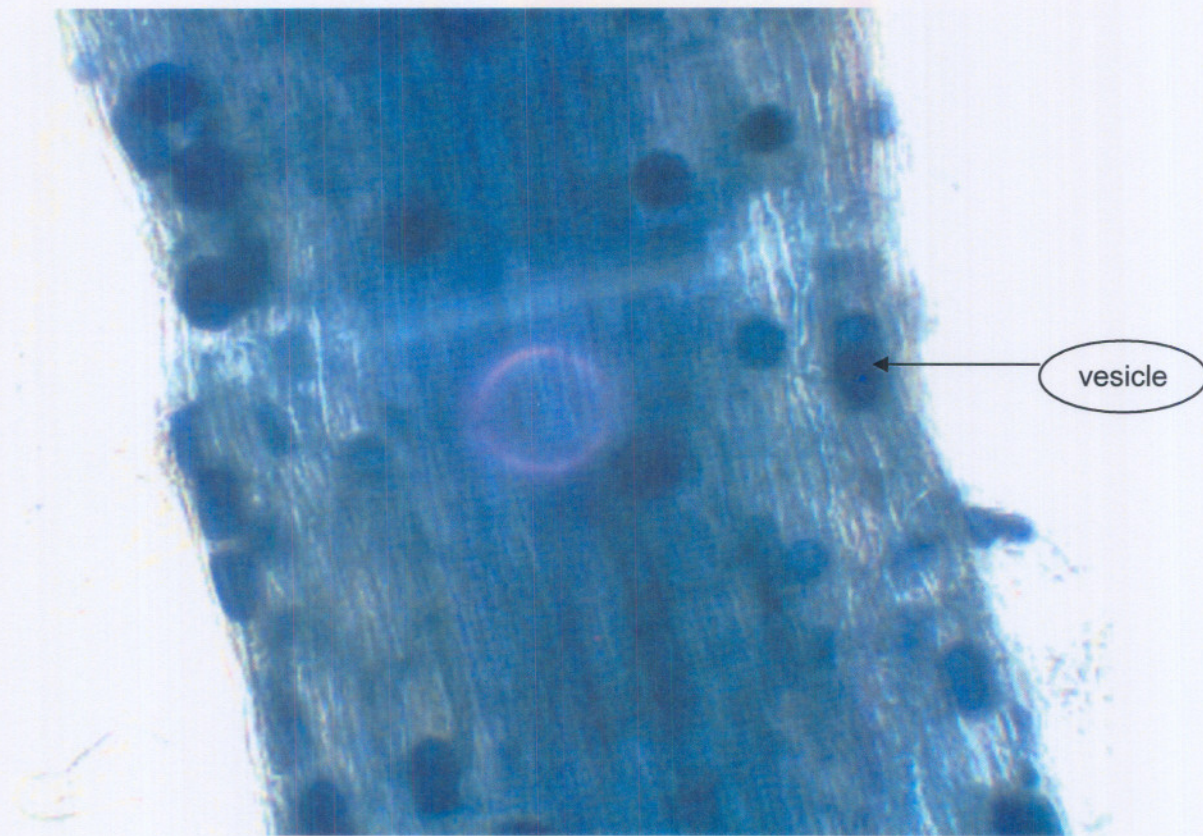


Plate 5.2a - Root from cowpea plant stained with Trypan blue to show vesicles (100x).

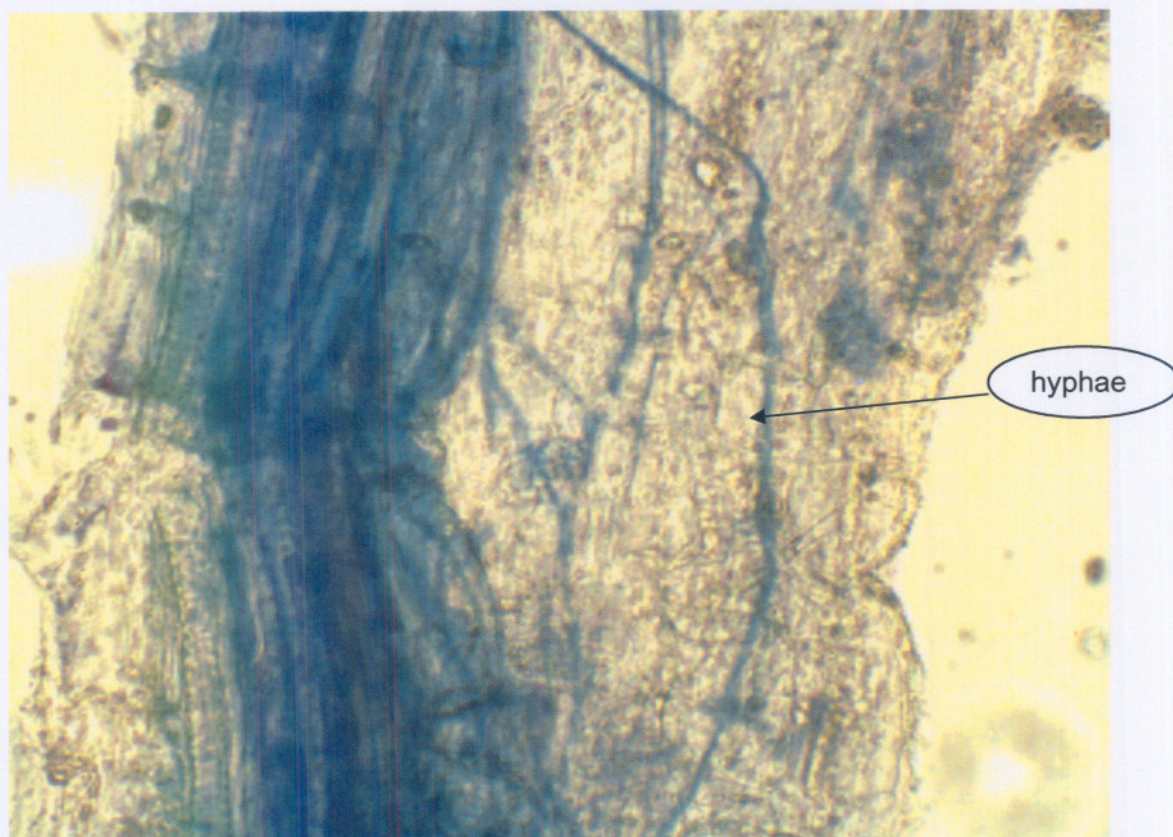


Plate 5.2b - Root from cowpea plant stained with Trypan blue to show intraradical hyphae (100x).

5.4.2 *Fusarium* recovered from plant organs and soil

No *Fusarium* was isolated from the leaves of plants from all the treatments. *Fusarium proliferatum* occurred only in the inoculated plant pots: there was apparently no cross-contamination between treatments. A minimum of one and a maximum of five *Fusarium proliferatum* colonies were isolated per 30 mm of root from plants inoculated with the pathogen. The colony forming units of *Fusarium* isolated from root surfaces and soil samples are presented in Tables 5.8. Both commercial and local AMF markedly affected the level of *Fusarium* in the soil. The level of *F. proliferatum* was significantly lower on the root surface of plants that had been treated with the commercial AMF. Fumonisin was detected in samples infected with *Fusarium* at 0.16-0.68 µg/g.

Table 5.7 - Numbers of colony forming units (CFU) of *Fusarium* in soil and on root surface and fumonisin concentrations ([Fum]) in root samples

Treatment	CFU x 10 ³ /g dry soil	CFU/30 mm root	[Fum] (µg/g)
Loc. AMF	0 ^a	0 ^a	0
Loc. AMF + Fus	5.7 ± 0.61 ^b	3 ± 0.58 ^{bc}	0.684
Com. AMF	0 ^a	0 ^a	0
Com. AMF + Fus	4.0 ± 0.26 ^b	1 ± 0.33 ^{ab}	0.155
Fus	16.0 ± 3.61 ^c	5 ± 1.15 ^c	0.191
Control	0 ^a	0 ^a	0

Shown are means ± standard errors (n=3). Means ± standard errors with same superscript letters (within a column) are not significantly different at $P \leq 0.05$.

5.4.3 The effect of AMF on *Fusarium*

Table 5.8 shows that the mean dry mass (root + shoot) of plants inoculated with both local AMF and *Fusarium* was significantly higher than the mean dry mass of plants inoculated with *Fusarium* only. The dry mass of plants inoculated with both commercial AMF and *Fusarium* was also higher than the dry mass of plants inoculated with *Fusarium* only, but not significantly at $P \leq 0.05$.

Table 5.8 - Mean plant height and dry mass for different treatments

Treatment	h (mm/plant)		Dry mass (g/pot)	
	stem	shoot	root	shoot + root
Loc. AMF	139.3 ± 1.8 ^c	1.860 ± 0.033 ^a	0.585 ± 0.013 ^d	2.445 ± 0.046 ^d
Loc. AMF + Fus	148.0 ± 1.2 ^d	2.028 ± 0.068 ^a	0.455 ± 0.036 ^{ac}	2.483 ± 0.103 ^d
Com. AMF	116.3 ± 3.3 ^a	0.815 ± 0.008 ^{bc}	0.422 ± 0.018 ^{ac}	1.237 ± 0.011 ^a
Com. AMF + Fus	120.3 ± 1.4 ^a	0.832 ± 0.008 ^{bc}	0.322 ± 0.050 ^{abc}	1.154 ± 0.055 ^{ac}
Fus	102.0 ± 1.2 ^b	0.685 ± 0.016 ^c	0.310 ± 0.019 ^{ab}	0.995 ± 0.024 ^{bc}
Control	101.0 ± 4.8 ^{ab}	0.767 ± 0.082 ^c	0.382 ± 0.057 ^a	1.149 ± 0.057 ^{ab}

Shown are means ± standard errors in brackets (n=3). Means ± standard errors with same superscript letters are not significantly different at $P < 0.05$.

The mean stem height of plants inoculated with both local AMF and *Fusarium* was significantly higher than the mean stem height of plants inoculated with *Fusarium* only. The mean stem height of plants inoculated with both commercial AMF and *Fusarium* was significantly higher than the mean stem height of plants inoculated with *Fusarium* only.

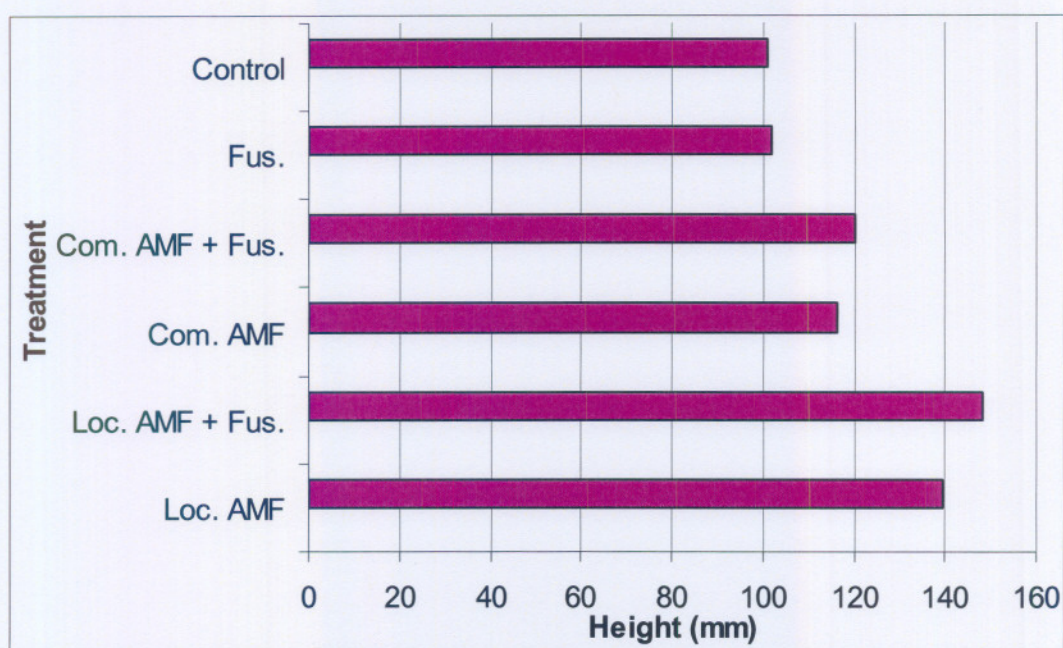


Figure 5.6 - Mean plant height (n=3).

5.4.4 The effect of AMF on plant growth

Figure 5.7 shows that both the root and shoot dry masses were significantly higher for plants inoculated with local AMF than for those not inoculated with any of the fungi (control). The mean stem height of local AMF plants was significantly higher than the mean stem height for the control (Table 5.8). Plants inoculated with commercial AMF had higher root and shoot dry masses as well as longer stem height than control, but the differences were not significant.

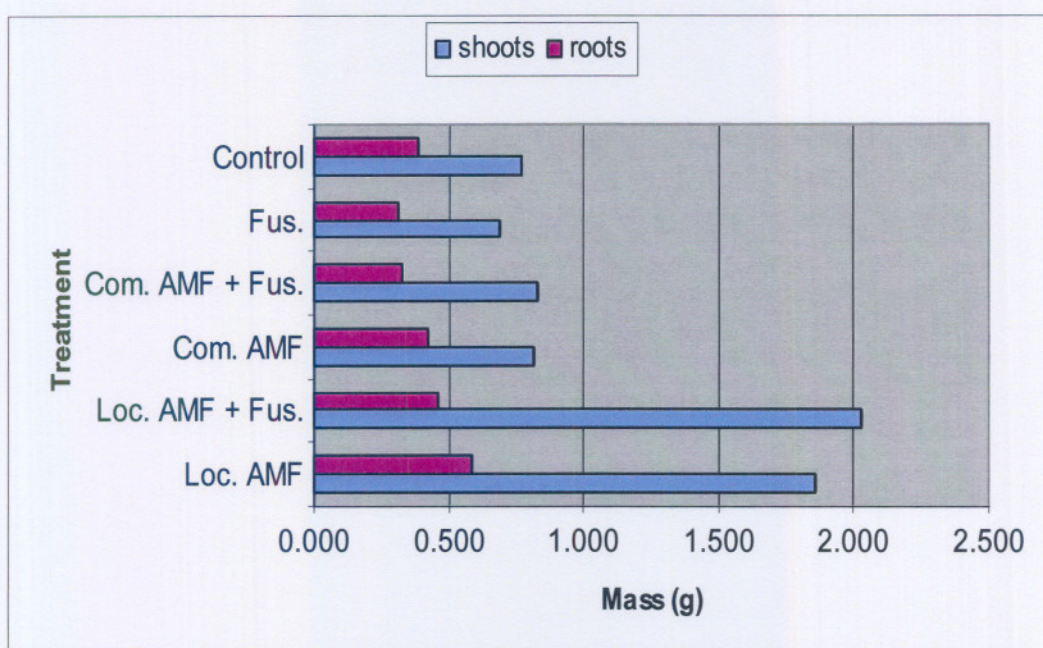


Figure 5.7 - Mean shoot and root dry mass (n=3).

5.4.5 The effect of *Fusarium* on plant growth

Fusarium-inoculated plants showed lower shoot and root dry masses as compared to control, but the differences were not significant at $P \leq 0.05$ (Table 5.8). Plants challenged with *Fusarium* had longer stems than control plants (difference not significant).

5.4.6 The relationship between shoot dry mass and root dry mass

Figure 5.8 shows that there was a significant positive correlation between the shoot dry mass and root dry mass of plants.

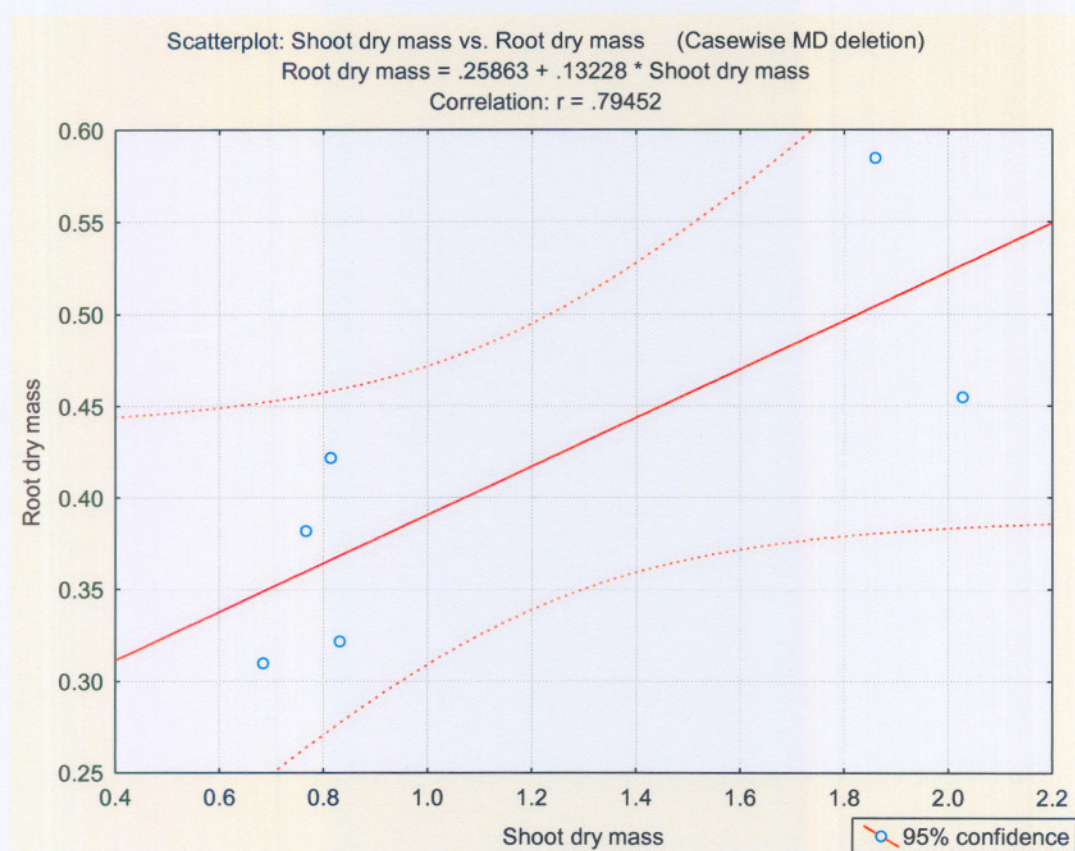


Figure 5.8 - Relationship between shoot dry mass and root dry mass.

5.4.7 The relationship between mycorrhizal colonisation and plant dry mass

Figure 5.9 shows that there was a significant ($P \leq 0.05$) positive correlation between the mycorrhizal colonisation and plant dry mass.

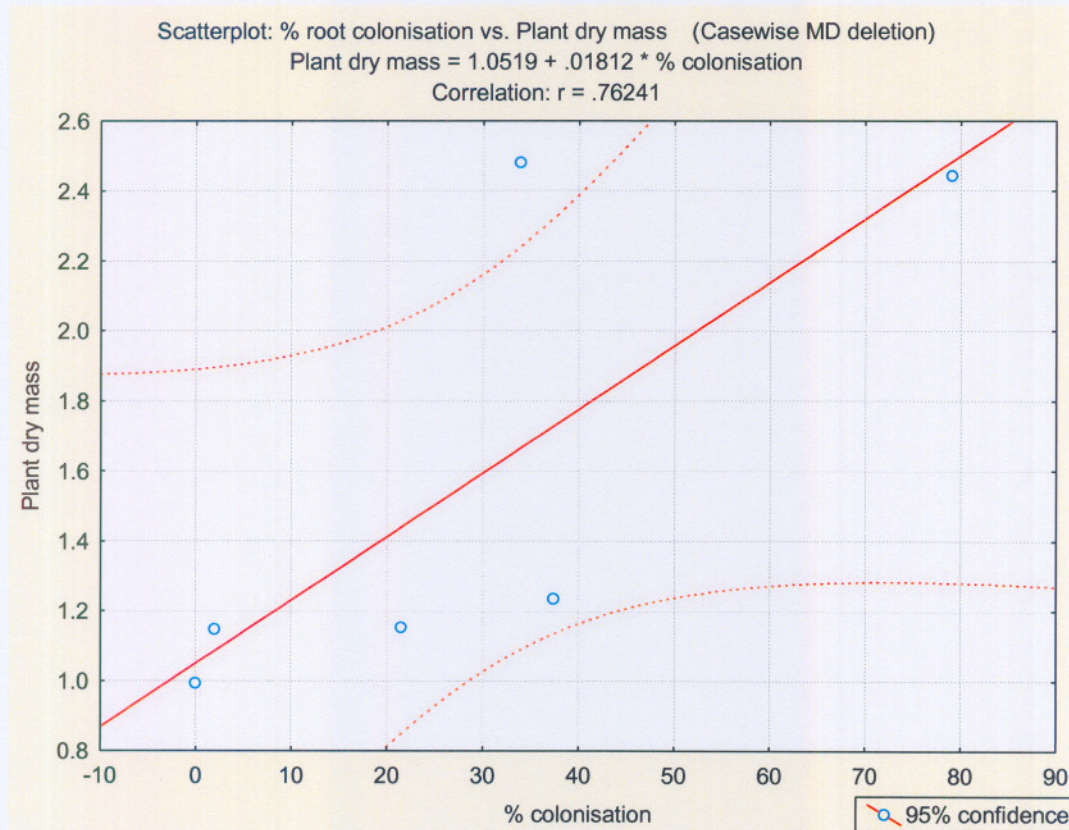


Figure 5.9 - Relationship between % colonisation and plant dry mass.

5.4.8 Leaf elemental composition

Figure 5.10 - 5.12 show the percentage leaf composition of phosphorus, carbon (C), nitrogen (N) and potassium (K), respectively. Leaf P concentration was not significantly correlated with percentage root colonisation ($r = -0.44$, $P \leq 0.05$). Leaf N, K and P of plants inoculated with both AMF and *F. proliferatum* were lower than of those inoculated with AMF only. Commercial AMF plants showed the lowest percentage of leaf C and the highest percentage of leaf N. Percentage leaf C of local AMF plants was higher than that of control plants.

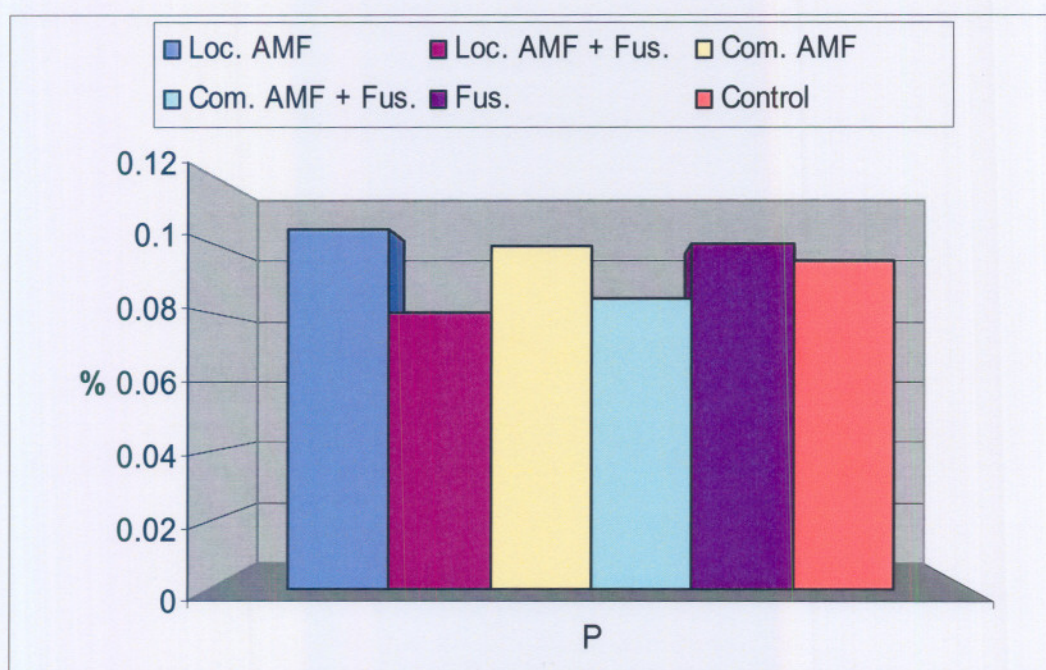


Figure 5.10 - Leaf P composition.

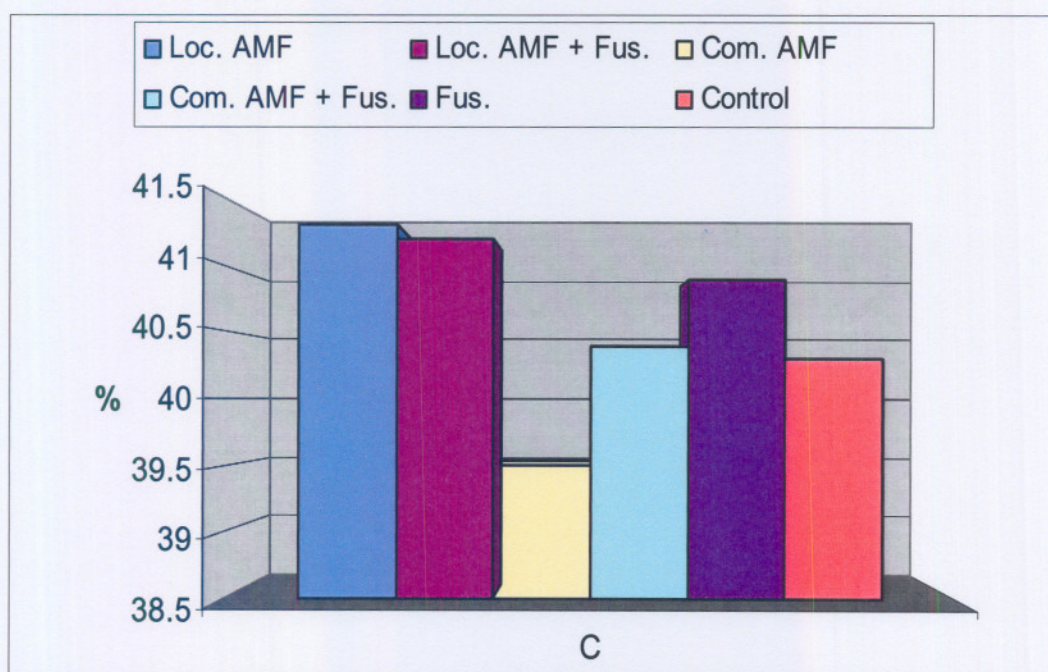


Figure 5.11 - Leaf C composition.

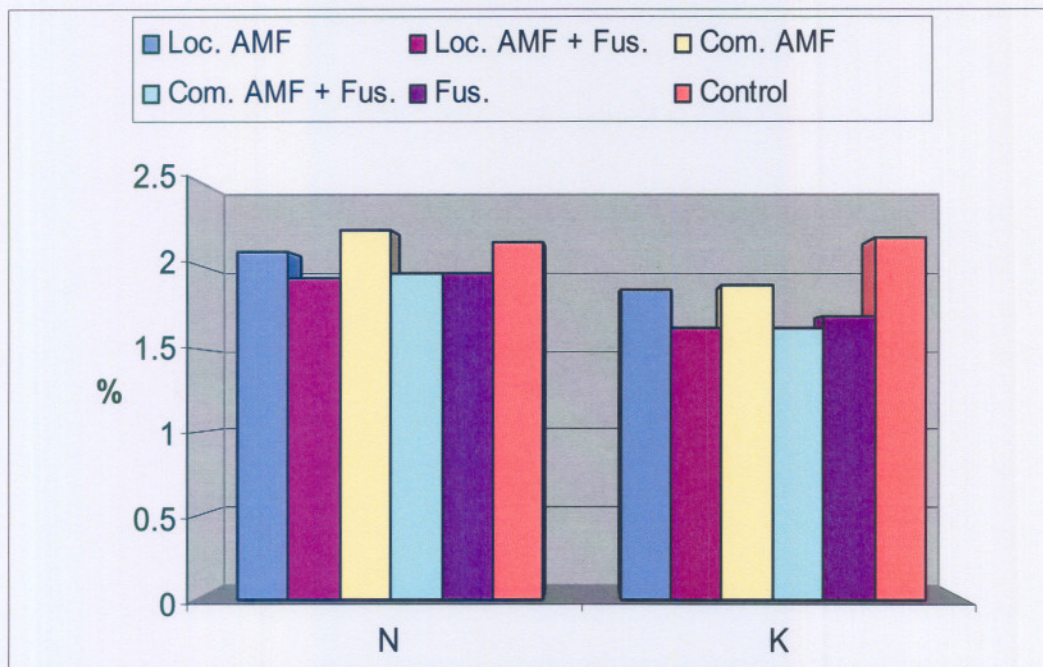


Figure 5.12 - Leaf N and K composition.

CHAPTER 6

DISCUSSION

6.1 Mycorrhiza

The occurrence of arbuscular mycorrhizal fungi (AMF) indigenously-associated with traditional *morogo* plants was investigated.

6.1.1 Quantification of mycorrhizas

Spore densities and mycorrhizal root colonisation were used as parameters to evaluate the levels of arbuscular mycorrhizal fungi (AMF) associated with traditional *morogo* plants.

6.1.1.1 Spore densities in soil

Arbuscular mycorrhizal fungi (AMF) are indigenous to soils throughout the world, despite having relatively poor dispersal mechanisms and being totally dependent on a living host (Hetrick, 1984). Spore densities are known to vary greatly in different ecosystems (Johnson & Wedin, 1997; Picone, 2000). The spore densities found in this study were relatively low, ranging from 2.8 to 9.8 spores g⁻¹ dry soil. Although soil sieving and centrifugation procedures are reliable extraction techniques to use when evaluating the arbuscular mycorrhizal spore population, it should be noted that the total number of spores has probably been underestimated. Underestimation may be due to the fact that very small spores may be lost during the extraction process (Hall, 1984).

Studies have shown that arbuscular mycorrhizal spores occur in suitable microsites in the rhizosphere (Tews & Koske, 1986; St. John & Koske, 1988). Variability shown by the spore density data (Table 5.1) in this study may be accounted for by the aggregated distribution of spores in the soil. According to Uhlmann *et al.* (2004), differences in vegetation cover between sites also control the distribution of AMF. A study by Still (1991) has also shown that slight differences in soil pH can result in relatively large differences in spore numbers.

6.1.1.2 Mycorrhizal root colonisation

High colonisation levels reflect the highly mycotrophic nature of the plant community in these villages. The demonstrated absence of significant differences in the percentage root colonisation of *morogo* plants from the different villages (Table 5.2) indicates that root samples from those villages were similar with respect to mycorrhizal colonisation due to similar environments of the villages. The slight variability observed may be a result of ecological specificity and interspecific competition (Gemma *et al.*, 1989).

6.1.1.3 Correlations between spore densities with percentage mycorrhizal colonisation

In this study, no correlation was observed between spore densities and percentage mycorrhizal root colonisation. There may be a close relationship between colonisation and sporulation but according to Hetrick (1984) the two phenomena are not necessarily correlated. Factors such as the soil environment, host plant and fungal species may have had an influence on the colonisation and sporulation of AMF (Hetrick, 1984).

6.1.1.4 Soil factors and their relationships between spore densities and between percent mycorrhizal colonisation

In this study, negative correlation was observed between phosphorus (P) levels in soils and percentage root colonisation (Fig. 5.4). This suggests that infectivity of arbuscular mycorrhizal fungi is sensitive to increasing P concentrations under natural conditions. High levels of P decrease AMF colonisation. Previous studies have shown that increased P levels in the soil resulted in suppressed mycorrhizal root colonisation and hence reduced mycorrhizal effect on plants (Allison & Goldberg, 2002; Mohammad *et al.*, 2004). In this study total spore densities were negatively correlated with magnesium (Mg) concentrations in soil (Fig. 5.3). As the level of Mg increases in soil, it is likely that sporulation by some AMF decreases (Jarstfer *et al.*, 2004), hence the decrease in spore densities.

6.1.2 Identification of mycorrhizal spores

Most of the AMF spores (86.6%) that were obtained from the rhizosphere of *morogo* plants were small spores. In the Glomales, the small-spored species mainly fall into the genera *Acaulospora* and *Glomus*. The predominance of small-spored species may be explained by literature report

indicating that such types are the most common isolated AMF throughout the world (Shi *et al.*, 2006). A study by Picone (2000) has shown that small spores were more frequent at the tropical forest and pasture and had a low seasonal variation than larger spores. In this study species were identified from trap cultures of original soils. However, some species of AMF were reported not to sporulate in trap cultures (Miller *et al.*, 1985). This might be the reason why only few species were identified. A prolonged period was also not spent to try and isolate all the relevant species due to time constraints.

6.2 *Fusarium*

The present study investigated the occurrence of fusarial species associated with traditional *morogo* plants.

6.2.1 Identification of *Fusarium*

Twelve species of *Fusarium* were isolated from *morogo* leaves, air and soil of the four villages of the DDSS (Table 5.3). The most commonly occurring species were *F. proliferatum* and *F. verticillioides*, both recognised for their ability to produce fumonisin mycotoxins (Kpodo *et al.*, 2000; Bankole & Mabekoje, 2004; Lerda *et al.*, 2005).

6.2.2 Quantification of *Fusarium*

Results presented in Table 5.4 show that levels of *Fusarium* associated with *Cleome gynandra* plants growing away from maize at Moduane and Madiga were higher compared to those growing in close proximity of maize plants. This was not anticipated as it is well documented that *Fusarium* is a common genus of the field fungi that infect maize in the field (Kpodo *et al.*, 2000; Bankole & Mabekoje, 2004; Pietri, 2004). High levels of *Fusarium* were therefore expected on those *morogo* plants in close proximity of maize plants. The absence of significant differences in levels of *Fusarium* isolated from *morogo* leaves away from maize and in close proximity with maize might be an indication that *Fusarium* is a common genus of the field fungi that contaminate *morogo* plants.

6.3 Greenhouse trials

To investigate the effect of AMF on *morogo* plant growth and levels of *Fusarium proliferatum* in the rhizosphere, cowpea (*Vigna unguiculata*) was used as host plant.

6.3.1 Percentage root colonisation

Results shown in Table 5.6 seem to indicate interactions between AMF and *F. Proliferatum*. *F. proliferatum* affected mycorrhizal colonisation negatively and reduced it by 45.2% and 15.9% in the case of local and commercial AMF, respectively. These results seem to be in agreement with those reported by other researchers (Zambolin & Schenck, 1983; Caron *et al.*, 1986; Chakravarty & Mishra, 1986; Akköprü & Demir, 2005). The interaction between AMF and *F. proliferatum* is possibly due to competition *in planta*, but does not exclude extraradical interactions between the symbiont and *F. proliferatum* (Filion *et al.*, 1999). Some researchers reported that AMF root colonisation was not affected by fungi such as *Fusarium* spp. (Graham & Menge, 1982; Krishna & Bagyaraj, 1983; Kaye *et al.*, 1984).

6.3.2 Effect of AMF on *Fusarium* level

A significant reduction in the level of *F. proliferatum* was detected when roots were inoculated with commercial AMF (Fig. 5.8). However reduction in fusarial levels when roots were inoculated with local AMF was not significant. This might indicate that the ability of arbuscular mycorrhizal symbiosis to control potential pathogens such as *F. proliferatum* is not similar for different AMF and needs to be ascertained for each particular combination of arbuscular mycorrhizal fungus, host genotype, pathogen and environmental conditions (Aranda *et al.*, 2006). Results shown in Table 4.8 indicate that commercial AMF was also effective in reducing the level of fumonisin.

The results of this study demonstrates that the benefit of AMF to plants in controlling *F. proliferatum* in the rhizosphere environment was unrelated to P nutrition, since there was no correlation between percentage root colonisation and leaf P concentration. These results seem to be in agreement with results reported by Kasiamdari *et al.* (2002) that showed that P nutrition did not directly influence the resistance of plants to pathogen colonisation. Kasiamdari *et al.* (2002) suggested that other pathogen control mechanisms might be involved. Possible mechanisms could include: competition for nutrients between AMF and fusaria, induction of defence response (Volpin, 1994; Cordier *et al.*, 1996; Azcón-Aguilar & Barea, 1996), production of inhibitory

compounds by AMF or host plant, and stimulation of microorganisms that are known to have positive effects on plant growth might indirectly interfere with pathogens (Filion *et al.*, 1999). AMF can interact directly with other microorganisms in the mycosphere and directly or indirectly reduce the fusarial population.

6.3.3 The effect of AMF on plant growth

Cowpea growth was significantly improved by inoculation with local AMF in the present study (Table 5.8). Figure 5.6 illustrates that local AMF increased the stem height as well as both the root and shoot dry masses (Fig. 5.7) of cowpea plants. Improved growth of these plants may be attributed to improved P nutrition of local AMF plants. In terms of these growth parameters, no significant difference was observed between commercial AMF and control plants. It was reported by other studies that AMF might differ in their effectiveness to enhance plant growth (Ruiz-Lozano *et al.*, 2000, Linderman & Davies, 2004) probably because their ability to absorb nutrients from the soil vary. Efficiency of nutrient uptake by AMF might be influenced by the degree they can form extensive extraradical hyphae as well as the extensive colonisation of the host root system (Abbott & Robson, 1984).

6.3.4 The effect of *Fusarium* on plant growth

Results in Table 5.8 shows that inoculation of cowpea with *F. proliferatum* resulted in negligible reduction of shoot and roots weights. This might be an indication that *F. proliferatum* has no negative effect on the plant growth of cowpea. Contrary to these results, the study by Chakravarty and Mishra (1986) showed that *F. oxysporum* reduced growth of *Cassia tora* plants. This shows that host plant response to *Fusarium* species might also vary.

6.3.5 The relationship between mycorrhizal colonisation and plant dry mass

Figure 5.9 illustrates that a positive correlation existed between percentage root colonised by AMF and plant dry mass. Local AMF apparently contributed to improved growth of the host plant. The finding that root colonisation contributes to increased dry mass and growth of the host plant is supported by a study of Chaurasia and Khare (2005) that demonstrated that the intensive root colonisation of the host roots resulted in the better plant growth in terms of both wet and dry matter.

6.3.6 Leaf contents of nitrogen (N), phosphorus (P), potassium (K) and carbon (C).

High P mobility in the sand was not anticipated because the large pore sizes were expected to reduce water holding capacity and therefore any dissolved P (Menge, 1984). The higher P concentration (not analysed statistically) in plants inoculated with AMF only, compared to that of the control (not inoculated with any of the microorganisms), indicates that AMF improved P nutrition of host plants. Mechanisms proposed to explain improved P uptake by mycorrhizal fungi include: (i) a wider physical exploration beyond the root depletion zone of the soil by hyphae than by roots; (ii) roots and hyphae explore microsites differently; (iii) plant roots and mycorrhizal hyphae affect chemical changes and P solubility in the rhizosphere differently (Shibata & Yano, 2003). Plants in pots inoculated with *F. proliferatum*, without AMF had a notable higher P uptake compared to the control plants. An antagonistic effect between AMF and *F. proliferatum* is suspected based on the observation that plants inoculated with both AMF and *F. proliferatum* resulted in poor uptake of P (Fig. 5.10).

In this study, pasteurisation of the potting sand seemed not to have killed beneficial microorganisms (Bennett *et al.*, 2003) such as the nitrogen-fixing bacteria. Figure 5.12 shows that inoculation with commercial AMF notably increased N uptake by host plants which corresponded with the greater number of nodules that was observed on roots of plants. This supports the conclusion by Antunes *et al.* (2006) that root colonisation by AMF had a positive effect on nodulation by nitrogen-fixing bacteria. AMF could have indirectly affected N availability because enhanced uptake of P is important for nodulation in legumes (Cardoso & Kuyper, 2006). The physiological activities of both AMF and nitrogen-fixing bacteria in the host plant are regulated by genetic and biochemical interactions between the symbionts. AMF release the myc factor which activates the nodulation factor's inducible gene *mtENOD11*. The gene *mtENOD11* is involved in establishing symbiosis with the nitrogen-fixing bacteria, *Rhizobium* (Kosuta *et al.*, 2003).

Compared to control plants, plants inoculated with commercial AMF only showed lower percentage of leaf carbon (Fig. 5.10). However, the percentage leaf carbon of local AMF plants was higher than that of control plants. Species of AMF differ in their abilities to supply the plant with P and in some cases AMF provide little P while taking relatively high amounts of C (Bucking & Shacker-Hill, 2005). Some nitrogen-fixing bacteria live very close to the extraradical hyphae and spores of AMF. These bacteria, together with AMF obtain their carbon from photosynthesis by the plant (Hayman, 2005). This may be an explanation for the low percentage of leaf carbon in commercial AMF plants.

Leaf N, K and P for plants inoculated with both AMF and *F. proliferatum* were lower than those inoculated with AMF only. This might indicate interspecific competition for nutrients between AMF and *F. proliferatum*. Nutrient uptake by plants is related to the availability of nutrients in the soil; as such the impact of soil nutrients on mycorrhizal associations should be taken into consideration (Muthukumar *et al.*, 2003).

6.4 CONCLUSION

The results of the current study showed that arbuscular mycorrhizas are a common and important component in the DDSS soils and that AMF with small spores may be more adaptive to this area. This suggests that arbuscular mycorrhizas may play an important role in subsistence cultivation of *morogo* in this area.

It must be kept in mind that the information regarding host-fungus associations provided by spore analyses alone is not sufficient, because of the many factors that influence sporulation and spore germination. Root colonisation assessment is important to give some indication of degree of colonisation by AMF.

The genus *Fusarium* is one of the most economically important genera of the field fungi that contaminate *morogo* plants. In terms of colony forming units, fusarial populations seem to be evenly distributed among areas sampled in the villages of the DDSS.

Greenhouse results of this study demonstrate the positive effect of AMF on plant growth both under pathogen pressure and in the absence of the pathogen. Despite the positive effect of AMF on plant growth, colonisation by AMF failed to enhance P-uptake of plants inoculated with both AMF and *Fusarium proliferatum*. The observations imply that the control of *F. proliferatum* is not related to P nutrition.

Commercial AMF was more effective than local AMF in controlling the number of colonies of *F. proliferatum* in the soil and on root surfaces. Though in the case of *morogo* plants (cowpea) *F. proliferatum* did not seem to pathogenise the host plant, the effect of AMF in association with *morogo* could be important in rural subsistence agriculture where intercropping with maize is common. *F. proliferatum* is an important maize pathogen and has been reported to produce fumonisin in host plants (Nelson *et al.*, 1992; Bankole & Mabekoje, 2004). The results of this study suggest that commercial AMF can possibly act as a bioprotective agent against *F. proliferatum*.

Local AMF played a significant role in biofertilising since it was more effective in enhancing plant growth. Enhanced plant growth was probably a consequence of improved plant nutrition resulting from a mycorrhizal status, especially in P.

Different AMF behave differently in certain host-fungus relationships and variations in responsiveness of host plants can occur depending on arbuscular mycorrhizal fungal combination (Linderman & Davies, 2004). The physiology of AMF must be thoroughly understood and this is not completely the case in this study. More specially, the beneficial effects of AMF on nutrient absorption resulting in enhanced host plant growth and ultimately in higher crop production as well as the beneficial effects of AMF on reducing plant pathogens resulting in healthier plants should be observed.

Although mycorrhizal colonisation of plants is not the only factor that determines the plants' growth and survival, it can be concluded that plants with mycorrhizal colonisation are less susceptible to attack by pathogens; they show increased plant growth compared to plants without mycorrhizal colonisation.

The results of this study suggest that the local AMF at the DDSS should be supplemented with the commercial AMF in order to improve plant growth and control *F. proliferatum*. By managing *morogo* plants better using the AMF, subsistence farmers can cost effectively produce better yields of these valuable plants.

6.5 RECOMMENDATIONS

Based on the results obtained and conclusions drawn during this study and from related research by other authors, the following suggestions should be considered when further studies are conducted on the role of AMF as biofertilisers and as biological disease-control agents. We should:

- investigate the mechanisms by which AMF control pathogens, AMF species suitable to control particular pathogens and to better define the environmental conditions limiting growth of pathogens.
- investigate potential benefits of AMF to other types of *morogo* plants since the present study concentrated on cowpea only.

- test commercial and local AMF under field conditions for their effectiveness as biological disease-control agents, adaptability, survival and dispersal.
- create and maintain ecological conditions conducive to optimal growth and colonisation of local AMF in order to increase crop production.

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APPENDICES

Appendix A: Raw data Tables

Table 1- Arbuscular mycorrhizal spore number per 50 g dry matter of soil at different villages of the Dikgale Demographic Surveillance Site (DDSS)

Village	Spore size (μ)			Total/50g soil
	63-125	125-250	250+	
Mantheding	390	46	5	441
Mantheding	282	47	4	333
Mantheding	384	101	6	491
Moduane	136	7	1	144
Moduane	136	4	0	140
Moduane	165	11	2	178
Sefateng	202	33	2	237
Sefateng	275	34	2	311
Sefateng	206	25	2	233
Madiga	325	51	5	381
Madiga	341	30	3	374
Madiga	150	30	10	190

Table 2 - Arbuscular mycorrhizal spore number per 50 g dry matter of soil and percentage root colonisation of *Amaranthus* plants

Village	Spore densities	% Root colonisation
Mantheding	441	27.02
Mantheding	333	38.78
Mantheding	491	34.69
Moduane	144	32.35
Moduane	140	22.92
Moduane	178	30.18
Sefateng	237	25
Sefateng	311	20.67
Sefateng	233	27.78
Madiga	381	26.32
Madiga	374	48.89
Madiga	190	42.5

Table 3 - Soil factors

Village	Mg (mg/kg)	K (mg/kg)	Na (mg/kg)	P (mg/kg)	pH(H ₂ O)	EC (mS/m)
Mantheding	74.5	147.0	52.0	16.9	7.35	83
Moduane	143.0	254.0	56.0	84.0	8.18	47
Sefateng	141.0	209.0	71.0	99.8	8.17	61
Madiga	119.0	235.5	94.5	15.3	7.14	27

Table 4 - Levels of *Fusarium* on leaves of *Amaranthus thunbergii* and *Cleome gynandra* expressed in colony forming units (CFU)

Species	Mantheding	Moduane	Sefateng	Madiga
<i>A. thunbergii</i> MZ-	0	0	0	0
<i>A. thunbergii</i> MZ-	2000	0	0	0
<i>A. thunbergii</i> MZ-	0	1000	1000	2000
<i>A. thunbergii</i> MZ-	0	0	7000	0
<i>A. thunbergii</i> MZ-	0	1000	12000	1000
<i>A. thunbergii</i> MZ-	0	0	0	0
<i>A. thunbergii</i> MZ-	1000	0	0	0
<i>A. thunbergii</i> MZ-	0	0	100	0
<i>A. thunbergii</i> MZ-	1000	0	0	2000
<i>A. thunbergii</i> MZ-	20000	0	0	0
<i>A. thunbergii</i> MZ+	0	1000	0	0
<i>A. thunbergii</i> MZ+	0	0	0	0
<i>A. thunbergii</i> MZ+	0	1000	0	0
<i>A. thunbergii</i> MZ+	0	0	0	9000
<i>A. thunbergii</i> MZ+	200000	1000	10000	0
<i>A. thunbergii</i> MZ+	0	10000	1000	1000
<i>A. thunbergii</i> MZ+	0	0	2000	0
<i>A. thunbergii</i> MZ+	1000	1000	0	0
<i>A. thunbergii</i> MZ+	3000	0	20000	18000
<i>A. thunbergii</i> MZ+	0	0	0	18000
<i>C. gynandra</i> MZ-	1000	1000	8000	3000
<i>C. gynandra</i> MZ-	2000	1000	1000	10000
<i>C. gynandra</i> MZ-	1000	5000	1000	0
<i>C. gynandra</i> MZ-	1000	10000	1000	0
<i>C. gynandra</i> MZ-	1000	2000	3000	0
<i>C. gynandra</i> MZ-	20000	8000	0	10000
<i>C. gynandra</i> MZ-	3000	1000	2000	7000
<i>C. gynandra</i> MZ-	4000	6000	2000	10000
<i>C. gynandra</i> MZ-	2000	0	100	1200
<i>C. gynandra</i> MZ-	2000	11000	1000	23000
<i>C. gynandra</i> MZ+	1000	5000	1000	0
<i>C. gynandra</i> MZ+	4000	0	0	2000
<i>C. gynandra</i> MZ+	0	0	25000	1000
<i>C. gynandra</i> MZ+	0	0	0	0
<i>C. gynandra</i> MZ+	0	3000	1000	0
<i>C. gynandra</i> MZ+	0	0	16000	1000
<i>C. gynandra</i> MZ+	0	0	4000	4000
<i>C. gynandra</i> MZ+	0	0	1000	1000
<i>C. gynandra</i> MZ+	0	0	28000	2000
<i>C. gynandra</i> MZ+	0	3000	2000	0

Table 5 - Levels of *Fusarium* on leaves of *Amaranthus thunbergii* and *Cleome gynandra* expressed in colony forming units (CFU)

Village	<i>A. thunbergii</i> MZ-	<i>A. thunbergii</i> MZ+	<i>C. gynandra</i> MZ-	<i>C. gynandra</i> MZ+
Mantheding	0	0	1000	1000
Mantheding	2000	0	2000	4000
Mantheding	0	0	1000	0
Mantheding	0	0	1000	0
Mantheding	0	200000	1000	0
Mantheding	0	0	20000	0
Mantheding	1000	0	3000	0
Mantheding	0	1000	4000	0
Mantheding	1000	3000	2000	0
Mantheding	20000	0	2000	0
Moduane	0	1000	1000	5000
Moduane	0	0	1000	0
Moduane	1000	1000	5000	0
Moduane	0	0	10000	0
Moduane	1000	1000	2000	3000
Moduane	0	10000	8000	0
Moduane	0	0	1000	0
Moduane	0	1000	6000	0
Moduane	0	0	0	0
Moduane	0	0	11000	3000
Sefateng	0	0	8000	1000
Sefateng	0	0	1000	0
Sefateng	1000	0	1000	25000
Sefateng	7000	0	1000	0
Sefateng	12000	10000	3000	1000
Sefateng	0	1000	0	16000
Sefateng	0	2000	2000	4000
Sefateng	1000	0	2000	1000
Sefateng	0	20000	1000	28000
Sefateng	0	0	1000	2000
Madiga	0	0	3000	0
Madiga	0	0	10000	2000
Madiga	2000	0	0	1000
Madiga	0	9000	0	0
Madiga	1000	0	0	0
Madiga	0	1000	10000	1000
Madiga	0	0	7000	4000
Madiga	0	0	10000	1000
Madiga	2000	18000	12000	2000
Madiga	0	18000	23000	0

Table 6 - Colony forming units of *Fusarium* isolated from air and soil of the DDSS villages

Village	Air MZ-	Air MZ+	Soil MZ-	Soil MZ+
Mantheding	16	33	3000	3900
Mantheding	9	34	2900	4300
Mantheding	16	32	3700	3800
Moduane	10	26	3100	2500
Moduane	21	31	2800	2900
Moduane	11	26	3100	3000
Sefateng	3	30	6300	3200
Sefateng	1	29	6000	2600
Sefateng	3	28	5700	3200
Madiga	21	29	3000	2900
Madiga	14	90	2600	3400
Madiga	19	87	3100	3300

Table 7 - Stem height, shoot dry mass, root dry mass and percentage root colonisation of *Vigna unguiculata* plants

Treatment	Stem h (mm)	Shoot m (g/pot)	Root m (g/pot)	% root colonisation
Loc. AMF	142	1.87	0.59	79.3
Loc. AMF	140	1.798	0.561	80.1
Loc. AMF	136	1.912	0.604	78
Loc. AMF + Fus	146	1.921	0.389	30.8
Loc. AMF + Fus	148	2.011	0.463	37.5
Loc. AMF + Fus	150	2.153	0.513	33.4
Com. AMF	118	0.822	0.399	37.5
Com. AMF	121	0.799	0.458	36.8
Com. AMF	110	0.824	0.408	38
Com. AMF + Fus	120	0.831	0.401	20
Com. AMF + Fus	118	0.845	0.336	23.9
Com. AMF + Fus	123	0.819	0.228	20.7
Fus.	102	0.663	0.338	0
Fus.	104	0.715	0.319	0
Fus.	100	0.677	0.273	0
Control	117	0.777	0.297	2.7
Control	118	0.903	0.358	1.1
Control	103	0.62	0.49	2.3

Table 8 - Levels of *Fusarium Proliferatum* in soil and on root surface of *Vigna unguiculata*

Treatment	Soil (CFU/g)	Roots (CFU)
Loc. AMF	0	0
Loc. AMF	0	0
Loc. AMF	0	0
Loc. AMF + Fus	5000	2
Loc. AMF + Fus	6000	4
Loc. AMF + Fus	6100	3
Com. AMF	0	0
Com. AMF	0	0
Com. AMF	0	0
Com. AMF + Fus	4300	2
Com. AMF + Fus	3900	1
Com. AMF + Fus	3800	1
Fus.	20000	3
Fus.	13000	7
Fus.	15000	5
Control	0	0
Control	0	0
Control	0	0

Table 9 - Concentration of fumonisin from roots of *Vigna unguiculata*

Treatment	[Fumonisin] (µg/g)
Loc. AMF	0
Loc. AMF + Fus.	0.684
Com. AMF	0
Com. AMF + Fus.	0.155
Fus.	0.191
Control	0

Table 10 - Elemental composition of *Vigna unguiculata* leaves

Treatment	Percent			
	N	C	P	K
Loc. AMF	2.08	41.3	0.104	1.85
Loc. AMF + Fus.	1.92	41.2	0.080	1.62
Com. AMF	2.21	39.5	0.099	1.87
Com. AMF + Fus.	1.95	40.4	0.084	1.62
Fus.	1.95	40.9	0.100	1.69
Control	2.14	40.3	0.095	2.16

Appendix B: Recipes

Polyvinyl-Lacto-Glycerol (PVLG) (INVAM, 2006)

Ingredients

100 ml distilled water (dH₂O)

100 ml lactic acid

10 ml glycerol

16.6 g polyvinyl alcohol (PVA)

- Mix the liquid ingredients in the dark bottle. Add PVA to the liquid ingredients and place in water bath (70 – 80 °C) for 4 – 6 hours.

Meizer' Reagent (INVAM, 2006)

Ingredients

100 g chloral hydrate

100 ml dH₂O

1.5 g iodine

5.0 g potassium iodide

- Mix together all the ingredients.

Lactoglycerol (Smith & Dickson, 1997)

Lactic acid : glycerol : water; 1 : 2 : 1 (by volume)

Modified Nash-Snyder PCNB agar medium (Nelson *et al.*, 1983)

Ingredients

15 g Difco Peptone

1 g KH₂PO₄

0.5 g MgSO₄·7H₂O

20 g agar

1 g Pentachloronitrobenzene

1 l Water

0.2 ml Benzyl penicillin

0.6 ml Pendistrep

0.28 ml Chloramphenicol pure

- Autoclave the medium and allow to cool. Add the antibiotics just before pouring into Petri dishes.

Carnation Leaf Agar (CLA) (Seifert, 1996)

Cut about 1 cm² carnation leaf pieces, air dry and sterilise by gamma irradiation or propylene oxide fumigation. Place a few sterile pieces on 2 % water agar as it is solidifying in Petri dishes.

Synthetic Nutrient Agar (SNA) (Seifert, 1996)Ingredients

1 g KH₂PO₄

1 g KNO₃

0.5 g MgSO₄·7H₂O

0.5 g KCl

0.2 g glucose

0.2 g Saccharose

20 g agar

1 l dH₂O

- Place pieces of sterile filter paper (about 1 cm²) on the surface of the agar after it has solidified.

Appendix C: Plants for identification (SANBI)

PLANTE VIR IDENTIFIKASIE

Direkteur: Navorsing
Nasionale Botaniese Instituut
Privaatsak X101
0001 PRETORIA

'N HANTERINGSFOOI SAL VIR ELKE EKSEMPLAAR WAT INGEHANDIG WORD, GEHEF WORD.

Wanneer u plante vir identifikasie stuur, moet die volgende nagekom word:

1. VERSAMELING VAN PLANTEKSEMPLARE

Volledigheid en grootte van eksimplare

- Waar moontlik moet die hele plant, volledig met wortels, stingels, blare, blomme en vrugte en voldoende om op 'n herbariumvel (380 x 250 mm) te pas, versamel word. Steriele materiaal kan selds akkuraat benaam word en is van weinig of geen wetenskaplike waarde nie.
- In die geval van houtagtige plante, soos bome, waar dit onmoontlik is om die hele plant te versamel, moet 'n verteenwoordigende deel gekies word om 'n herbariumvel (380 x 250 mm) te kan volmaak.
- Groot kruidagtige plante en grasse (wat wortels en wortelstokke moet insluit) kan gebuig word of verdeel word om op meer as een vel te pas.
- In die geval van klein plantjies, moet genoeg individuele plante versamel word om min of meer 'n herbariumvel te vul.
- Moenie slegs sade en vrugte vir identifikasie instuur nie, maar stuur altyd 'n volledige eksimplaar soos beskryf in a-d hierbo, saam.
- Boonop, skryfies of tekeninge van plante moet, waar moontlik, versamel wees van 'n eksimplaar van die plant om te help met identifikasie.

Duplikaadmateriaal

- Wanneer plantmateriaal vir identifikasie gestuur word, hou altyd 'n duplikaatversameling terug omdat planteksemplare wat vir benaming ontvang word, slegs in uitsonderlike omstandighede na die versamelaar teruggestuur sal word.
- Indien moontlik, stuur asseblief een of meer duplikaat-eksemplare saam met die oorspronklike eksimplaar aangeien die na ander herbariums in Suid-Afrika en oorsee versprei word.

Pers en draging van eksimplare

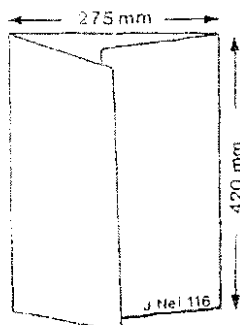
- Plante moet so gou moontlik nadat hulle versamel is in 'n plantpers geplaas word om verwelking te voorkom. Druk moet toegepas word om die eksimplare plat te stryk.
- Indien 'n permanente pers nie beskikbaar is nie, kan koerantpapier, blaas van tydskrifte of vloeiop papier, plek-plek versterk deur stywe kartonblare of soortgelyke materiaal, gebruik word. Matige druk moet toegepas word om die eksimplare plat te druk.
- Plaas pers in die son of naby 'n verwarmmer om draging te bespoedig.
- By plante met 'n hoë waterinhoud, bv. sukkulentie, moet die droogpapier daagliks verander word tot taamlik droog. Dit is nodig om aanval deur fungi te voorkom.

2. VOORBEREIDING VAN GEDROOGDE MATERIAAL

Omslag

Plaas elke eksimplaar in 'n skoon en netjiese beskermende omslag. Indien moontlik gebruik onbedrukte of bedrukte koerantpapier. Moenie die eksimplare op 'n karton monteer nie. Elke omslag moet soos volg voorberei word:

- Vou 'n vel soos op skets.
- Skrif versamelaar se naam en nommer regs onder.



- Moenie toekram of bo- en onderkant toevoel nie—plante en stukke val nie maklik uit nie.

- Indien u nie duplikaadmateriaal kon versamel nie en die eksimplaar graag wil terughê, merk die omslag met 'n duidelike stertjie (*) na die versamelaar se nommer.

Etiket

- 'n Etiket waarop die volgende inligting in drukskrif aangegee word, moet elke genommerde eksimplaar vergeesel:

- Naam van versamelaar.
- Versamelnommer (nommer eksimplare opeenvolgend beginnende by 1—'n nommer moet nooit meer as een keer gebruik word nie en moenie letters of simbole as deel van 'n nommer gebruik nie).
- Datum versamel.
- Ruitverwysing (bv. 2527 AC).
- Presiese vindplek (lokaliteit).
- Habitat (omgewing)—bv. rivieroewer, hang van kopas.
- Grondtipe.
- Groei vorm en grootte van plant.
- Blomkleur.
- Volksnaam, indien bekend.
- Gebruike, ongewenste dienskapijens.

Verpakking

- Plante moet nooit in plastieksakkies of ander lugdigte hoesse gestuur word nie.
- Om te verhoed dat plante raaf, moet hulle nie verpakk word tensy hulle heeltemal droog is nie.
- Droë eksimplare word maklik in die pos beseig. Daar word aanbeveel dat u ongeveer twintig eksimplare (elk in sy eie omslag) in 'n dubbelvei koerantpapier te vou en vaspak; die aantal pakkeles moet dan in 'n stewige kartondoos verpak word.
- Plantidentifikasie-versoek vorms (verkrygbaar van die NBI) moet elke beseiding wat gestuur word, vergeesel.
- Elke doos moet die versamelaar se naam en adres in drukskrif daarop hê, tesame met die verklaring "Planteksemplare vir identifikasie."
- 'n Brief om die versending van 'n versameling om te verklaar en afsonderlik geplaas word en nie in 'n pakkie ingestuur word nie. Dit moet ook die nodige inligting oor die aantal dose en eksimplare wat afgestuur word, bevat.
- Personne wat groot versamelings van meer as 100 eksimplare wettig stuur, moet eers die nodige toestemming van die NBI verkry alvorens die eksimplare afgestuur word.

3. VOLKSNAMIE

- Moenie inligting omtrent plante vra deur slegs na die volksnaam te verwys nie, aangesien dieselfde volksnaam dikwels vir verskillende plantspesies gebruik word.
- Stuur altyd 'n eksimplaar van die plant waarop die volksnaam betrekking het.

4. ERKENNINGSVORM

Nadat u planteksemplare geïdentifiseer is, ontvang u saam met 'n finale lys van name 'n erkenningssvorm. Vul asseblief hierdie vorm in en stuur d't aan die NBI terug.

5. ADRES

Pakkies en navrae moet aan bogenoemde adres gestuur word.

Appendix D: Questionnaire

Household information

Sampling point no.: -----

House no.: -----

Name: -----

Occupation of bread winner(s): -----

No. of household members of specific age group:

0 – 10: ---

11 – 20: ---

21 – 30: ---

31 – 40: ---

41 – 50: ---

50+ : ---

Dietary information

1. What is the staple diet of the household?

Pap & *morogo* ☐ Wheat ☐ Legumes ☐ Sorghum ☐

2. What is the consumption frequency of the staple per day?

Once ☐ Twice ☐ Three times ☐ Other ☐ specify -----

3. What other foods do you cook and eat?

Meat ☐ Vegetables ☐ Beans ☐ Other ☐ specify -----

4. What is the consumption frequency of these foods per day?

Once ☐ Twice ☐ Three times ☐ Other ☐ specify -----

5. What is the consumption frequency of *morogo* per day?

Once ☐ Twice ☐ Three times ☐ Other ☐ specify -----

6. What is the average weight of *morogo* eaten by members of the household per meal?

(estimate in grams per meal)

Children ☐ Teenagers ☐ Adults ☐

7. Do some members of the household eat foods from the field that are not cooked?

Yes ☐ no ☐

8. If yes, who are they?

Children ☐ Adults ☐

Morogo information

9. Give the names of *morogo* that you use for consumption in this area

10. Do you eat *morogo* because you
 like it? ☐ have no choice? ☐

11. Do the children like to eat *morogo*?

yes ☐ no ☐

12. Do teenagers like *morogo*?

yes ☐ no ☐

Cultivation

13. Where do you usually get your *morogo*?

At home ☐ schemes ☐ relatives ☐ other ☐ specify -----

14. Do you plough?

yes ☐ no ☐

15. If yes, where do you plough?

At home ☐ Fields ☐

16. Do you rely on rain for water?

yes ☐ no ☐

17. Did you plough this season (2005 – 2006)?

yes ☐ no ☐

18. Does the lack of rain affect ploughing and plant growth?

yes ☐ no ☐

19. When do you get the best yield of *morogo*?

In rainy seasons ☐ In dry seasons ☐ other ☐ specify -----

20. How did you cultivate your *morogo*?

From seedlings ☐ From seeds ☐ Grow on its own ☐ other ☐ specify -----

21. How long after planting is *morogo* collected?

Less than a month ☐ 1 month ☐ 2 months ☐ 3 months ☐ other ☐ specify -----

22. Which parts of the *morogo* plants do you collect for consumption?

Young leaves ☐ Older leaves ☐ Flowers ☐ Seeds ☐ Roots ☐

23. Where do you get the seeds from?

Previous years crop ☐ Store ☐ Relatives ☐ other ☐ specify -----

24. Do you use anything for soil improvement?

yes ☐ no ☐

25. If yes, what do you use?

Cow droppings ☐ Chemical fertilizers ☐ Chicken droppings ☐ Compost ☐

26. Do you sometimes find any mouldy leaves?

yes ☐ no ☐

27. On which leaves do you find mould?

Old leaves ☐ Young leaves ☐ On almost all leaves ☐ Flowers ☐ Seeds ☐

28. Do you apply pesticides on the infected leaves?

yes ☐ no ☐

29. If yes, which type of pesticide?

Chemical ☐ Organic ☐ other ☐ specify -----

30. What do you do with those mouldy leaves?

Throw them away ☐ Use them with other leaves as food ☐ other ☐ specify -----

31. Where do you throw the mouldy leaves?

In the compost ☐ In the field ☐ other ☐ specify -----

Methods of preparation

32. Do you store these *morogo* for the winter season?

yes ☐ no ☐

33. How do you preserve these *morogo* during storage?

Dry before cooking ☐ Dry after cooking ☐ None of the above ☐

34. Where do you dry these *morogo*?

In the sun ☐ In the shadow ☐

35. Where do you store these *morogo*?

In closed containers ☐ Open containers ☐ other ☐ specify -----

36. How long do you cook the fresh *morogo*?

Less than 10 min ☐ More than 10 but less than 20 min ☐ More than 20 min ☐

37. How long do you cook the dried *morogo*?

Less than 10 min ☐ More than 10 but less than 20 min ☐ More than 20 min ☐

38. What other ingredients do you add to the *morogo*?

Tomatoes ☐ Salt ☐ Onions ☐ other ☐ specify -----

39. Do you get *morogo* in the form of weeds amongst the maize?

yes ☐ no ☐

40. Do you also collect *morogo* amongst maize for consumption?

yes ☐ no ☐

Health related risk

41. Have you noticed any difference between the *morogo* amongst maize and the one cultivated separately? Explain

42. Can you associate any symptoms like diarrhea, sore throat or other with the consumption of the *morogo*?

43. Are you aware of any known myths of the healing effect caused by these *morogo*?

Interviewer

Date completed