MANAGEMENT OF POTATO LATE BLIGHT (*Phytopthora infestans*) USING ARBUSCULAR MYCORRHIZAL FUNGI

BY

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DECLARATION

DECLARATION BY STUDENT

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DEDICATION

This is a great gift of knowledge and intellectual ability granted to me by God. I wish to dedicate it to my beloved mother Ms. Irene Kibiro who, through great efforts and perseverance, guided me to this point.

ABSTRACT

Potato (*Solanum tuberosum*) is a key food as well as cash crop in the highlands of Kenya, and is extensively produced by small-scale farmers. It is the second most significant food crop in Kenya after maize employing over 2.5 million persons. It plays a key role in Kenyan food security and contributes to alleviation of poverty. The potato sector is plagued by many problems, among them potato blight whose management still poses a great challenge, particularly among small-scale farmers in the Kenyan highlands. About 30 – 60% of the potato crop is lost to the late blight yearly in Kenya. Because of development of more virulent and fungicide-resistant strains and the poor ability of resource-strained farmers to control late blight, the optimum management of the disease in Kenya is likely to be achieved through biological control and in this case, by use of abuscular mycorrhiza fungi. Potato tubers were planted in the greenhouse and the effectiveness of arbuscular mycorrhizal fungi, *Glomus intraradices* and *Glomus mosseae* in conferring resistance to *Phytopthora infestans* and in promoting growth and yield was tested. Some plants were inoculated with the isolates of arbuscular mycorrhiza fungi while others were not inoculated to serve as controls. All the plants were then inoculated with *P. infestans* thirty days after planting. The effects of the mycorrhizal treatments on the pathogen progression were evaluated throughout the season. The mixture of *Glomus mosseae* and *Glomus intraradices* recorded the highest mean weight of the tubers per plant (103.20±5.56g), with the control recording the lowest (63.20±5.17g). There was a significant difference between the control and all the treatments ($p>0.05$). In terms of number of tubers, *G. mosseae* showed the highest mean (7.60±0.60). The number of tubers in all the treatments were significantly higher than those of the control ($p>0.05$). This study demonstrated that inoculation of potato plants with abuscular mycorrhiza fungi confers significant degree of reduction in severity of potato blight. Arbuscular mycorrhizal fungi inoculation in potato plants was also found to significantly promote growth and increase potato yield. *Glomus mosseae* showed the highest reduction of potato blight severity while control had the least. This research study indicated that inoculation of potato plants with abuscular mycorrhiza fungi conferred some degree of resistance to potato blight, promoted growth and increased yield. Further investigation under field conditions and using mixtures of different abuscular mycorrhiza fungi isolates needs to be carried out.

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ABBREVIATIONS

AMF	Arbuscular mycorrhiza fungi
VAM	Vesicular arbuscular mycorrhiza
CIP	International Potato Center
ECAPAPA	Eastern and Central Africa's Rural Development Challenge
P	Phosphorous
PREPACE	French acronym for Regional Potato and Sweet potato Improvement Network in Eastern and Central Africa
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
MD	Mycorrhizal Dependency
AM	Arbuscular Mycorrhiza
RXLR	Arg-X-Leu-Arg

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Phytophthora infestans is a hemibiotrophic pathogen that has a biotrophic behaviour in the course of the early infection and necrotrophic behaviour during the later colonization stage. The pathogen causes late blight and is regarded among the most annihilating microorganisms, which induce disease in potato crops (Erwin and Ribeiro, 1996; Kamoun, 2003). It is ill-famed as the causal agent of the Irish Potato Famine over 150 years ago, and the losses of crop because of *P. infestans* remain astounding (Erwin and Ribeiro, 1996). Global losses in production of potatoes alone contributed by late blight surpass $5 billion yearly, rendering *P. infestans* the single most significant biotic danger to food security in the world (Duncan, 1999). In Europe, annual losses, arising from costs of control and damage, are approximated to be more than 1 billion ₤ (Haverkort *et al.*, 2008).

In Kenya, yield as well as economic losses due to infection by late blight computed using fungicide evaluation trials data in on-farm and on-station trials for a period of 17 year between 1991 and 2007 were 22.6 to 80.9% and Kshs 37,500 to I19,500 per hectare respectively (Lung'aho *et al.*, 2008). Several years ago, epidemics of severe late blight took place after the migration to Europe, North America as well as other areas of aggressive strains of mating type (Fry and Goodwin, 1997a; Fry and Goodwin, 1997b). Schemes for management of late blight remain costly and unsustainable and the majority of populations have turned resistant to earlier effective fungicides (Smart and Fry, 2001; Ristaino, 2002; Shattock, 2002).

The control of this disease is also carried out through integrated management practices including utilization of certified seeds, sanitation, breeding programs for resistant cultivars (Vleeshouwers *et al.*, 2008) and fertilization management (Ros *et al.*, 2008). In the recent years, natural compounds like plant extracts and microorganisms have been examined in the context of production of organic potato (Dorn *et al.*, 2007). Agents of biological control like *Pseudomonas* species, fungal antagonists as well as arbuscular mycorrhizal fungi (O'Herlihy *et al.*, 2003; Andreote *et al.*, 2009; Bae *et al.*, 2011) have been used against *Phytophthora* disease.

Arbuscular mycorrhizal fungi are soil microbes that develop obligate symbiotic associations with the roots of over 80% of higher plants including the large majority of agricultural crops (Smith and Read, 1997). They belong to the monophyletic phylum *Glomeromycota* that contains three orders *Archaeosporales*, *Paraglomerales* and *Diversisporales* (Schüβler *et al.*, 2001). The fungal symbiont gets carbohydrates from the plant, needed for completion of its life cycle, in exchange for nutrients like phosphate as well as water transferred to the plant (Smith and Read, 1997). This bi-directional exchange leads to enhanced growth of the plant as well as profuse development of the AM fungus. In parallel, AM fungi have been broadly reported to confer biological protection to plants, particularly against soil-borne pathogens. Several studies have reported decrease in occurrence and/or severity caused by fungi like *Rhizoctonia solani, Fusarium oxysporum or, Verticillium wiltand* by fungal-like oomycetes such as *Phytophthora* sp., *Pythium spp*. or *Aphanomyces euteiches* (Whipps, 2004; Pozo *et al.*, 2009). The protective effect of AM fungi against soil-borne pathogenic fungi, which has been extensively investigated and demonstrated, makes AM a potential tool for biological control of pathogens (Wehner *et al.*, 2010). Arbuscular mycorrhiza influences the activity of root apices in some plants causing

their precocious senescence with loss of mitotic activity and differentiation of the meristematic cells which become parenchymatic. Arbuscular mycorrhizal fungi, then, could increase the resistance to *Phytophthora nicotianae var parasitica* by reducing the percentage of active apices, possibly a preferential site of pathogen penetration, or they could determine, by modulating apex activity, modifications of the root architecture which, in turn, could interfere with the spread of pathogenic fungi (Fusconi *et al.*, 1999; Wehner *et al.*, 2010).

1.2 Statement of the problem

Late blight of potato is one of the most devastating potato crop diseases in the tropical highlands of Sub-Saharan Africa (Sengooba and Hakiza, 1999). Efforts applied to manage the disease are mainly through application of fungicides on varieties with low to moderate resistance. For most poor farmers, there is insufficient control of the disease, leading to heavy losses and, in some cases, complete crop loss. In addition, these fungicides have hazardous effects to the environment as well as human beings. There is estimation that approximately 30 – 60% of the crop is lost to late blight annually in Kenya (Njuguna *et al.*, 1998; Lung'aho *et al.*, 2008). Management of potato late blight thus remains a priority and a significant challenge, particularly among the small-scale farmers in the tropical highlands of Kenya (Lung'aho *et al.*, 2008. The emergence of new strains of *P. infestans* with deviant sexuality and the fungicide resistance by some isolates (Erselius *et al.*, 1998) raises concern for effective disease management. In Kenya, 86% of *P. infestans* isolates collected from potato fields have been reported to have a high level of metalaxyl resistance (Ordoñez *et al.*, 2000). With the current existence of more virulent and fungicide-resistant strains and the poor ability of resource-strained farmers to manage late blight, the

optimal management of the disease in Kenya is likely to be achieved through biological control and thus the need for the study.

1.3 Justification

Management of late blight has been heavily based on application of fungicide whose overuse has increased over the last two decades. This has been exacerbated due to emergence of new and more virulent strains of the pathogen. There are societal pressure for reduction of use of pesticides on crops and acreage of organically grown food crops, potatoes included. There is also need for innovative as well as effective control measures in order to reduce use of fungicide. For many years organic production of potatoes has relied on the use of copper-based fungicides for management of late blight. Nevertheless, there has been rising pressure to look for substitutes for these products due to the environmental contamination caused by residues of copper. In order to completely abandon use of copper-based fungicides, there is need to find non-chemical alternatives such as arbuscular mycorrhizal fungi to protect potato fields against *Phytophthora infestans*.

1.4 Objectives

1.4.1 Main objective

To determine the efficacy of arbuscular mycorrhiza fungal isolates in reducing severity of potato late blight as well as promoting growth and yield of potato plants.

1.4.2 Specific objectives

i. To assess the efficacy of arbuscular mycorrhiza fungal isolates in reducing the severity of *Phytopthora infestans*, promoting growth and yield of the potato plant

ii. To compare the efficacy of arbuscular mycorrhiza fungal isolates in reducing potato blight severity, and promote growth and yield.

1.5 Study hypotheses

i. The Arbuscular mycorrhiza fungal isolates have no ability in reducing severity of *Phytopthora infestans* and promoting growth and yield in potato plants

ii. The arbuscular mycorrhiza fungal isolates are not different in their efficacy in reducing potato blight severity, and promoting growth and yield.

CHAPTER TWO

LITERATURE REVIEW

2.1 Late blight of potato

Late blight (LB) of potato (*Solanum tuberosum*), caused by the oomycete pathogen *Phytophthora infestans*, is considered to be the most important disease of potato worldwide (Hijmans *et al.*, 2000). The late blight regularly causes serious crop losses in solanaceous crops worldwide. This is especially important in areas, where potatoes are the main staple crop or a prominent source for the rural population. Due to the severity of the disease preventive chemical pest management measures are almost always the first option for the control of the disease, often resulting in over and misuse of fungicides (Apel *et al.*, 2003).

The late blight pathogen still causes considerable harvest losses in a number of regions globally. Field studies results show that epidemics typically lead to yield losses of between 40% and 70%, based on varietal susceptibility as well as environmental conditions (Hans and München, 2006). If infection occurs early in the season, the entire harvest can be lost. The financial loss caused by this disease has been estimated at more than US$ 2.7 thousand million in developing countries alone (CIP1) as well as reducing yield. The pathogen also causes reductions in quality, which can bring considerable economic penalties.

As a consequence of the damage caused by *P. infestans*, in the year 2003 The American Phytopathological Society rated it as one of the immediate priority fungal as well as oomycete targets of genome sequencing. In the year 2002 the same group

had rated *P. infestans* second among 26 species of fungi and oomycete. There has been a rising trade in potatoes so as to supply the quick growing cities and towns with the cheap staple food, and to meet the demand of the fast growing industry. Out of the produced potatoes supplied by urban traders in Kenya, 60-65% is utilized in hotels and street stalls (Kirumba *et al.*, 2004; ECAPAPA *et al.*, 2005). Among the plants grown worldwide, potato (*Solanum tuberosum L.*) experiences the major losses from attack by diseases that translate to $3.4 billion (Olanya *et al.*, 2001).

Potato late blight is among the most significant diseases of potatoes, being particularly annihilating in the key Sub-Saharan African potato producing tropical highlands. Key economic effects frequently arise from total or partial damage of infected fields (Sengooba and Hakiza, 1999). The appearance of A1 *Phytophthora* strains, with an abnormal sexuality as well as resistance to fungicide in a number of isolates in potato growing areas of tropical Africa (Erselius *et al.*, 1998) have increased concern for effective management of the disease. Because of the quick development of late blight, infections occurring at various levels of crop development stand for large economic threat. Management of potato late blight still poses a great challenge, particularly among small-scale farmers in the Kenyan tropical highlands.

Late blight is mostly controlled through repeated applications of pesticide, which is time-consuming, harmful for the environment and costly (Song *et al.*, 2003). For many poor farmers, there is inadequate control of the disease, resulting in great losses and, in some instances, total crop loss. It is estimated that approximately 30 – 60% of the potato crop is lost to the late blight yearly in Kenya (Njuguna *et al.*, 1998). There has been a major change in the population of *P. infestans* worldwide with the emergence of more resistant strains (Fry *et al.*, 1993; Fry and Goodwin, 1997a;

Griffin *et al.*, 2002). The new strains are resistant to metalaxyl, one of the most effective fungicides for controlling the disease (Goodwin *et al.*, 1996; Nærstad, 2000), and they are more aggressive than the old strains (Kato *et al.*, 1997; Lambert and Currier, 1997; Miller *et al.*, 1998).

2.2 Causal agent and host range of potato blight

Phytophthora infestans belongs to the kingdom *Chromista*, Phylum *Oomycota*, order *Peronosporales*, family *Peronosporaceae* genus *Phytophthora*. It is the causal agent of potato and tomato late blight, and one of the most damaging fungi of potatoes (Hohl and Iselin, 1984). It is a heterothallic oomycete that produces motile zoospores (biflagellate) in lemon-shaped sporangia that form at the top of sporangiophore branches. It can reproduce asexually and sexually. Sexual reproduction in this heterothallic fungus only occurs when thallium of opposite mating types (A1 and A2) mate. Pairings result in the production of oospores, which can survive in the absence of a host (Drenth *et al.*, 1995). Prior to the 1980s, the A1 mating type was distributed throughout the world, whereas the A2 mating type was detected only in central Mexico (Hohl and Iselin, 1984). In the absence of the sexual cycle, P. *infestans* survives between seasons in infected potato tubers either used as seed potatoes or discarded, often in refuse piles. The late blight pathogen *Phytophthora infestans* originated in the central highlands of Mexico. The spread of the pathogen to the rest of the world occurred in two major waves of migration. The first migration occurred about 160 years ago. The fungus was inadvertently transported across the Atlantic on ships, after which it established itself quite rapidly throughout Europe, Asia and Africa. For a long time, populations in Central Europe remained very uniform: only mating type A1 was present in Europe and Asia (Hohl and Iselin, 1984).

Any adaptive changes by the pathogen that occurred would have been through mutation or mitotic crossing-over. Exchange of genes through sexual recombination would not have been possible, because only one mating type (A1) was present (sexual recombination depends on two mating-types {A1 and A2} being present). This remained the situation until about 1975 (Hausladen, 2006). The pathogen's second great wave of migration occurred in the mid-nineteen seventies. In 1976 and 1977, large numbers of Mexican ware potatoes were imported into Europe: among them were tubers with latent infections, including new A1-strains, and for the first time, the A2-mating type. These new strains quickly spread throughout Europe. *Phytophthora* populations deriving from the first wave of migration are referred to as "old", whereas those from the second migration wave are referred to as "new" populations (Hausladen, 2006).

Phytophthora infestans infects a wide range of solanaceous species. It can infect foliage, stem, potato tubers as well as tomato fruits during all development stages. A number of wild solanaceous plants can as well be infected by *P. infestans* (Forbes and Landeo, 2006). Other economically important hosts include pepper, eggplant on which it causes late blight (Eduardo *et al.*, 2007).

2.3 Economic importance of potato late blight

Late blight caused by the fungus *P. infestans* can devastate a potato crop (Stewart *et al.*, 2003). Global estimates of economic damage as a result of yield losses and management costs of late blight in developing countries are estimated at $3 billion annually (Baker *et al.*, 2004). The magnitude of losses of potato caused by late blight in African countries can range from 30 to 75% on susceptible varieties (Njuguna *et al.*, 1998; Olanya *et al.*, 2001). According to Nyakanga *et al.* (2003) farmers lose up

to 30% of potatoes due to late blight, with Meru Central farmers encountering even higher loss.

Due to high disease pressure in the highland tropics, some farmers apply fungicides more than ten times per growing season (Namanda *et al.*, 2004). Nyankanga *et al.* (2004) estimated that 98% of potato farmers in Meru Central, Mount Elgon and Njabini division rely on fungicides to protect their potato crop against late blight, with an average of 5 sprays per season. The most used fungicides are Ridomil, containing the systemic fungicide Metalaxyl as well as the contact fungicide, Mancozeb and different brands of Mancozeb alone, of which Dithane M45 is most used (Nyankanga *et al.*, 2004).

2.4 Life cycle of *Phytophthora infestans*

Phytophthora infestans is a host-specific as well as an obligate parasite. It, thus, initially grows as a biotroph and when the infected tissues die, the fungus then spreads out through the leaf as a necrotroph. *Phytophthora infestans* reproduces asexually. Its life cycle usually begins as the sporangia are carried by the wind, land on plant tissue, and release their zoospores (Judelson, 1997). This happens when the conditions are wet and cool and is called indirect germination. These spores are biflagellated, having both a tinsel and whiplash flagellum which is characteristic of oomycota and swims until they encyst in the host. When conditions are warmer, *P. infestans* infects the plant by direct germination, that is, a germ tube forms from the sporangium and penetrates the host tissue allowing it to gain nutrients from its host (Schumann and D'Arcy, 2000).

Sexual reproduction can and does take place when both mating types are present. The antheridium and oogonium, the only haploid parts in the life cycle of *P. infestans*, nuclei will fuse together (karyogamy) when the antheridium enters the oogonium. They will form a diploid oospore, which will develop into a sporangium and the cycle continues as it would asexually (Schumann and D'Arcy, 2000). Infected plants show signs of small brown or black lesions on the leaves or stems, but soon spread and kill the plant. The growth of sporangia on the surface of the leaves or stems makes is white, which is another warning sign of infection. Tubers can also become infected when the sporangium are washed into the soil. The tubers show signs by copper brown, reddish, or purplish color in the cracks, eyes, or lenticels. Soft rot bacteria then invade causing the tuber to become smelly and rotten. Similar signs exist for the tomato plants (Schumann and D'Arcy, 2000). The life cycle of *P. infestans* is summarized in Figure 1.

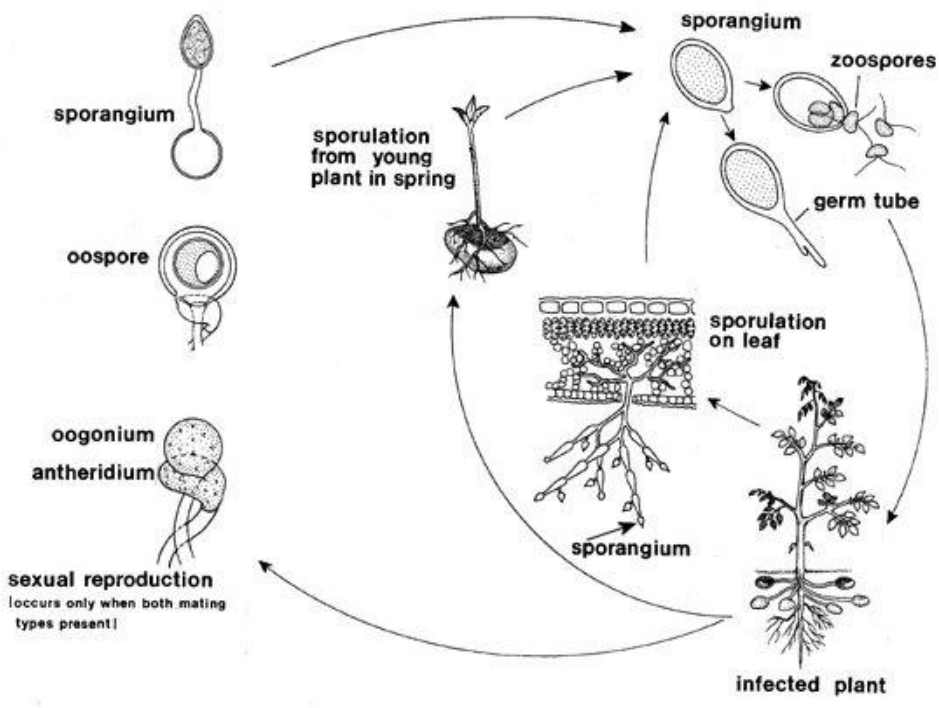

Figure 1: Life cycle of *Phytophthora infestans* (Paola *et al.*, 2008)

2.5 Symptoms of late blight of potato

Initially lesions on tomato or potato foliage appear as irregular, water-soaked spots that may become bigger quickly into pale green to brown, covering extensive surface of large leaves as well as stems (Fry *et al.*, 2001). Tomato fruit lesions appear as dark olivaceous spots that may become big and cover the entire surface of the fruit. In potato tubers, lesions are reddish brown, dry and granular and can develop in inner tissue (Fry *et al.*, 2001). When there is high humidity, lesions can be covered with a gray to white moldy growth that comprises of specialized hyphae known as sporangiophores that produce the asexual propagules called sporangia (Fry *et al.*, 2001).

2.6 Genetic makeup of *Phytophthora infestans*

Phytophthora infestans is diploid, with about 11-13 chromosomes, and in 2009 scientists completed the sequencing of its genome (Haas *et al.*, 2009). The scientists found that the genome was considerably larger (240 Mbp) than that of most other *Phytophthora* species whose genomes have been sequenced, *Phytophthora sojae* has a 95 Mbp genome and *Phytophthora ramorum* had a 65 Mbp genome. About 18,000 genes were detected within the *P. infestans* genome. It also contained a diverse variety of transposons and many gene families encoding for effector proteins that are involved in causing pathogenicity. These proteins are split into two main groups depending on whether they are produced by the water mould in the symplast (inside plant cells) or in the apoplast (between plant cells). Proteins produced in the symplast included RXLR proteins, which contain an arginine-X-leucine-arginine (where X can be any amino acid) sequence at the amino terminus of the protein. Some RXLR proteins are avirulence proteins, meaning that they can be detected by the plant and

lead to a hypersensitive response which restricts the growth of the pathogen (Haas *et al.*, 2009).

Phytophthora infestans was found to encode around 60% more of these proteins than most other P*hytophthora* species. Those found in the apoplast include hydrolytic enzymes such as proteases, lipases and glycosylases that act to degrade plant tissue, enzyme inhibitors to protect against host defense enzymes and necrotizing toxins. Overall the genome was found to have an extremely high repeat content (around 74%) and to have an unusual gene distribution in that some areas contain many genes whereas others contain very few (Haas *et al.*, 2009).

2.7 Development and spread of potato blight

For late blight to occur, three conditions ought to be present namely, abundant inoculum (late blight spores), a susceptible host (potatoes, tomatoes, or other hosts), as well as the environmental conditions favorable for late blight. The pathogen overwinters on infected tubers (Zwankhuizen *et al.*, 1998). These can be cull tubers discarded as storage sheds are emptied for packing or processing, tubers left as volunteers in the field, chips from seed cutting operations or infected tubers planted as seed. All serve as sources of inoculum. Wind and air currents will move the spores over a wide area. Late blight spores have been known to travel over 40 miles under the right conditions. A small amount of inoculum can contaminate a large area very quickly (Zwankhuizen *et al.*, 1998).

The process of pathogenesis starts with the oospores, zoospores or sporangial germination. Sporangia are the most abundantly dispersed propagules and germination can be either direct through germ tube development or indirect through

the release of zoospores. Mode of sporangial germination is regulated by temperature. Direct germination of sporangia takes place at 18 to 24°C, while indirect germination takes place at temperatures below 18°C (Crosier, 1934). The pathogen can penetrate plant tissue through the epidermal and cuticle cells and develops specialized structures, which can extract nutrients from the cells of the host (Zwankhuizen *et al.*, 1998).

2.8 Prevention and control of potato blight

A number of approaches have been developed to control late blight of potato. *Phytophthora infestans* remains a difficult disease to manage today through ordinary methods. Many options exist in agriculture for the management of both infections of the tuber and damage to the foliage. These include fungicidal control, haulm destruction, and resistance breeding.

2.8.1 Fungicidal control

Control of potato blight traditionally relied on copper-based fungicides such as Bordeaux mixture (consisting of copper sulphate and calcium oxide). However, copper is potentially phytotoxic (Yang *et al.*, 2002), so disease forecasting and modeling was developed to enable growers to predict when the environmental conditions were highly conducive for the spread of the pathogen and thus when the growers needed to spray to protect their crops (Jim, 2011). Forecasting methods for blight epidemics differ in different countries but in Britain they are based on the "temperature-humidity rule" devised by Beaumont (1947) who determined that after a certain date, depending on locality, blight developed within 15-22 days following a period when the temperature was not less than 10^{o}C and the relative humidity was

over 75% for 2 consecutive days. There are now warnings of the Beaumont periods or updated versions of these in the early-morning farming programmes.

Copper is a broad-spectrum fungicide which is applied as a protectant. It has been superseded by modern systemic fungicides, which move within the plant and can both protect and eradicate existing infections. These fungicides are much more specific in their mode of action. Chief among these for control of potato blight are the acylalanine fungicides such as metalaxyl and furalaxyl. They act specifically on the RNA polymerase of *Phytophthora* and closely related fungi. However, resistance to them can develop quickly in the pathogen population – it requires only a single gene mutation leading to a minor change in the RNA polymerase molecule. In many parts of the world, *P. infestans* is now resistant to these fungicides (Jim, 2011).

2.7.2 Haulm destruction

If *P. infestans* gets established on the potato foliage then sporangia can be washed down into the soil to infect the tubers, or the tubers can be contaminated with sporangia during crop harvesting. This can lead to rotting of the tubers during storage, and carry-over of inoculum from one season to the next. In order to minimize these problems it is common practice to destroy the foliage (the haulm) with sprays of sulphuric acid or herbicide 2-3 weeks before the tubers are lifted (Nick, 2006).

2.7.3 Resistance breeding

The cultivated potato (*Solanum tuberosum*) originates from the Andean region of South America, where there are several other species of the genus *Solanum*. The potato blight fungus also is thought to have its centre of origin in this region, so it is advisable to seek sources of genetic resistance to the pathogen in the wild potato

plants of this region. The species *Solanum demissum* proved to be an important source of resistance, and by conventional plant breeding (crossing and back-crossing) in the 1940s and 1950s this resistance was bred into commercial potato cultivars. Four major resistant genes (termed R genes) were discovered and were introduced successively into commercial cultivars. However, within a few years of each R gene being introduced widely into potato cultivars, the fungus was found to be able to attack these plants – the resistance was overcome by new strains, physiologic races, of the pathogen that developed in response to the selection pressure imposed by the specific R genes (Asakaviciute *et al.*, 2008).

Thus, race 1 of the pathogen could cause disease of potato cultivars carrying the R1 gene, and so on. With four R genes there are a possible 16 combinations –potatoes can be bred with, for example, R1 and R2, or R1 and R4, or R1, R2, R3 and R4, etc. But eventually a pathogen race would emerge that had the corresponding virulence genes to overcome all these. For long-term control, this form of resistance breeding based on a few major resistance genes seems destined to fail. So, many plant breeders now prefer to develop cultivars that have polygenic or field resistance to the pathogen. Such plants have combinations of a number of minor genes, none of which gives absolute resistance, but together they slow the rate of development of the fungus and enable the plant to tolerate infection (Jim, 2011).

2.9 Arbuscular mycorrhiza fungi

Mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant (Kirk *et al.*, 2001). In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF), or extracellularly as in ectomycorrhizal fungi. They are an important component of soil

life and soil chemistry. Mycorrhizas form a mutualistic relationship with the roots of most plant species, and while only a small proportion of all species has been examined, 95% of these plant families are predominantly mycorrhizal (Trappe, 1987). They are typically found in the rhizosphere of a root system.

2.9.1 Basics of arbuscular mycorrhizal fungal biology and taxonomy

Arbuscular mycorrhizal fungi get their name from their characteristic formation of branching structures called arbuscules within the cortical cells of roots. Arbuscules increase the contact area between plant and fungus and are thought to be the primary sites of exchange of the plant's carbon for the fungus's phosphorus. One suborder of these fungi, Glomineae, also forms vesicles, or sack-like reservoirs, within plant cortical cells. Consequently, AMF are also known as vesicular–arbuscular mycorrhizal (VAM) fungi (Morton and Bentivenga, 1994). Arbuscular mycorrhizal fungi are believed to propagate via infective hyphae, hyphal fragments, or asexual spores. A generalized life history begins with colonization of a root and the development of arbuscules from branch hyphae within the root. Hyphae may extend from one infected root to another or from an infected root to the root of another plant.

Spores form in the root cortex or in the soil. These spores may be dormant for a period, but they will eventually germinate and colonize another root. Spores may be dispersed away from the site in which they were formed. Viable spores are generally ephemeral (some spores of *Acaulospora* species are exceptions), and viability is limited by dormancy, susceptibility to pathogens, and other factors. Although the morphology and architecture of external hyphae and internal mycorrhizal structures can differ between families of AMF, there are few differences between species within each genus. Therefore, taxonomy of these fungi is based on the discrete characters of

the spore sub cellular structure, which can vary from simple to very complex for a single multinucleate cell (Morton and Bentivenga, 1994). On the basis of spore wall characters and spore ontogeny, AM fungi are grouped into genera that encompass approximately 145 species described to date. Undoubtedly the majority of AM fungal species remains un-described.

2.10 Evolution of mycorrhizal symbiosis

2.10.1 Paleobiology

Paleobiological as well as molecular evidence show that AM is an ancient symbiosis, which originated at least 460 million years ago. AM symbiosis is ubiquitous among land plants, which suoposes that mycorrhizas were there in the early ancestors of extant land plants. This positive association with plants may have facilitated the development of land plants (Simon *et al.*, 1993).

The Rhynie chert of the lower Devonian has yielded fossils of the earliest land plants in which AMF have been observed (Remy *et al.*, 1994). The fossilized plants containing mycorrhizal fungi were preserved in silica. The Early Devonian saw the development of terrestrial flora. Plants of the Rhynie chert from the Lower Devonian (400 million years ago) were found to contain structures resembling vesicles and spores of present *Glomus* species. Colonized root fossils have been observed in Aglaophyton major and Rhynia, which are ancient plants possessing characteristics of vascular plants and bryophytes with primitive protostelic rhizomes (Remy *et al.*, 1994). Intraradical mycelium was observed in intracellular spaces of roots, and arbuscules were observed in the layer thin wall cells similar to palisade parenchyma. The fossil arbuscules appear very similar to those of existing AMF. The cells

containing arbuscules have thickened walls, which are also observed in existing colonized cells.

Mycorrhizas from the Miocene show a vesicular morphology closely similar to that of present Glomerales. The need for further evolution may have been lost due to the readily available food source provided by the plant host (Kar *et al.*, 2005). However, it can be argued that the efficacy of signaling process is likely to have evolved, which could not be easily detected in the fossil record. A fine tuning of the signaling processes would improve coordination and nutrient exchange between symbionts while increasing the fitness of both the fungi and the plant symbionts.

The nature of the relationship between plants and the ancestors of AMF is contentious. There are two hypotheses stating that mycorrhizal symbiosis evolved from a parasitic interaction that developed into a mutually beneficial relationship, and that mycorrhizal fungi developed from saprobic fungi that became endosymbiotic (Remy *et al.*, 1994).

Both saprotrophs and biotrophs were found in the Rhynie Chert, but there is little evidence to support either hypothesis. There is some fossil evidence that suggests that the parasitic fungi did not kill the host cells immediately upon invasion, although a response to the invasion was observed in the host cells. This response may have evolved into the chemical signaling processes required for symbiosis (Remy *et al.*, 1994).

In both cases, the symbiotic plant-fungi interaction is thought to have evolved from a relationship in which the fungi was taking nutrients from the plant into a symbiotic relationship where the plant and fungi exchange nutrients.

2.10.2 Molecular evidence

Increased interest in mycorrhizal symbiosis and the rapid development of sophisticated molecular techniques has led to the rapid development of genetic evidence. Wang *et al.* (2010) carried out an intensive investigation of three broadly occurring plant genes, which encode for a signal transduction cascade vital for communication with order *Glomales* fungal partners (DMI1, DMI3, IPD3) (Wang *et al.*, 2010). Sequences of these three genes were obtained from all major clades of modern land plants (including liverworts, the most basal group), and the maximum probability phylogeny of the three genes was in complete agreement with the current land plant phylogenies. These findings imply that the mycorrhizal genes must have been present in the common ancestor of land plants, and that these genes must have been vertically inherited since the colonization of land by plants (Wang *et al.*, 2010).

2.11 Physiology of arbuscular mycorrhiza fungi

2.11.1 Presymbiosis and symbiosis

The development of AMF prior to root colonization, known as presymbiosis, consists of three stages: spore germination, hyphal growth, host recognition and appressorium formation. Spores of the AMF are thick-walled multi-nucleate resting structures (Wright, 2005). The germination of the spore does not depend on the plant, as spores have been germinated under experimental conditions in the absence of plants both *in vitro* and in soil. However, the rate of germination can be increased by host root exudates (Douds and Nagahashi, 2000). Arbuscular mycorrhizal fungal spores germinate given suitable conditions of the soil matrix, temperature, carbon dioxide concentration, pH, and phosphorus concentration (Wright, 2005).

The growth of AM hyphae through the soil is controlled by host root exudates known as strigolactones, and the soil phosphorus concentration (Akiyama *et al.*, 2005). Low-phosphorus concentrations in the soil increase hyphal growth and branching as well as induce plant exudation of compounds that control hyphal branching intensity (Nagahashi *et al.*, 1996).

The branching of AM fungal hyphae grown in phosphorus media of 1 mM is significantly reduced, but the length of the germ tube and total hyphal growth were not affected (Nagahashi *et al.*, 1996). A concentration of 10 mM phosphorus inhibited both hyphal growth and branching. This phosphorus concentration occurs in natural soil conditions and could thus contribute to reduced mycorrhizal colonization (Nagahashi *et al.*, 1996).

Root exudates from AMF host plants grown in a liquid medium with and without phosphorus have been shown to affect hyphal growth (Nagahashi *et al.*, 1996). Pre-germinated surface-sterilized spores of *Gigaspora magarita* were grown in host plant exudates. The fungi grow in the exudates from roots starved of phosphorus had increased hyphal growth and produced tertiary branches compared to those grown in exudates from plants given adequate phosphorus (Douds and Nagahashi, 2000). When the growth-promoting root exudates were added in low concentration, the AM fungi produced scattered long branches (Douds and Nagahashi, 2000). As the concentration of exudates was increased, the fungi produced more tightly clustered branches. At the highest-concentration of arbuscules, the AMF structures of phosphorus exchange were formed.

This chemotaxic fungal response to the host plants exudates is thought to increase the efficacy of host root colonization in low-phosphorus soils (Douds and Nagahashi,

2000). It is an adaptation for fungi to efficiently explore the soil in search of a suitable plant host (Nagahashi *et al.*, 1996).

Further evidence that AMF exhibit host-specific chemotaxis: Spores of *G. mosseae* were separated from the roots of a host plant, nonhost plants, and dead host plant by a membrane permeable only to hyphae. In the treatment with the host plant, the fungi crossed the membrane and always emerged within 800 μm of the root. Whereas in the treatments with non-host plants and dead plants the hyphae did not cross the membrane to reach the roots (Sbrana and Giovannetti, 2005). This demonstrates that AMF have chemotaxic abilities that enable hyphal growth toward the roots of a potential host plant.

Molecular techniques have been used to further understand the signaling pathways that occur between arbuscular mycorrhizae and the plant roots. In the presence of exudates from potential host plant roots, the AM undergoes physiological changes that allow it to colonize its host. AM fungal genes required for the respiration of spore carbon compounds are triggered and turned on by host plant root exudates. In experiments, there was an increase in the transcription rate of 10 genes half-hour after exposure and an even greater rate after 1 hour. A morphological growth response was observed 4 hours after exposure. The genes were isolated and found to be involved in mitochondrial activity and enzyme production. The fungal respiration rate was measured by oxygen consumption rate and increased by 30%, three hours after exposure to root exudates. This indicates that AMF spore mitochondrial activity is positively stimulated by host plant root exudates. This may be part of a fungal regulatory mechanism that conserves spore energy for efficient growth and the hyphal

branching upon receiving signals from a potential host plant (Tamasloukht *et al.*, 2003).

When arbuscular mycorrhizal fungal hyphae encounter the root of a host plant, an appressorium, an infection structure, is formed on the root epidermis. The appressorium is the structure from which the hyphae can penetrate into the host's parenchyma cortex (Gianinazzi-Pearson, 1996). The formation of appressoria does not require chemical signals from the plant. AM fungi could form appressoria on the cell walls of "ghost" cells in which the protoplast had been removed to eliminate signaling between the fungi and the plant host. However, the hyphae did not further penetrate the cells and grow in toward the root cortex, which indicates that signaling between symbionts is required for further growth once appressoria are formed (Douds and Nagahashi, 2000).

Once inside the parenchyma, the fungus forms highly branched structures for nutrient exchange with the plant called "arbuscules" (Gianinazzi-Pearson, 1996). These are the distinguishing structures of AMF. Arbuscules are the sites of exchange for phosphorus, carbon, water, and other nutrients (Wright, 2005). There are two forms: Paris type is characterized by the growth of hyphae from one cell to the next; and Arum type is characterized by the growth of hyphae in the space between plant cells (Lara and Larry, 2002). The choice between Paris type and Arum type is primarily determined by the host plant family, although some families or species are capable of either type (Matekwor *et al.*, 2005; Yamato, 2005). The host plant exerts a control over the intercellular hyphal proliferation and arbuscule formation. There is a de-condensation of the plant's chromatin, which indicates increased transcription of the plant's DNA in arbuscule-containing cells (Gianinazzi-Pearson, 1996). Major

modifications are required in the plant host cell to accommodate the arbuscules. The vacuoles shrink and other cellular organelles proliferate. The plant cell cytoskeleton is reorganized around the arbuscules.

There are two other types of hyphae that originate from the colonized host plant root. Once colonization has occurred, short-lived runner hyphae grow from the plant root into the soil. These are the hyphae that take up phosphorus and micronutrients, which are conferred to the plant. Arbuscular mycorrhizal fungal hyphae have a high surface-to-volume ratio, making their absorptive ability greater than that of plant roots (Tuomi *et al.*, 2001). Arbuscular mycorrhizal fungus hyphae are also finer than roots and can enter into pores of the soil, which are inaccessible to roots (Bolan, 1991). The third type of AMF hyphae grows from the roots and colonizes other host plant roots. The three types of hyphae are morphologically distinct (Wright, 2005).

2.12 Nutrient uptake and exchange by arbuscular mycorrhiza fungi

Arbuscular mycorrhizal fungi are obligate symbionts. They have limited saprobic ability and are dependent on the plant for their carbon nutrition (Harley and Smith, 1983). AM fungi take up the products of the plant host's photosynthesis as hexoses. The transfer of carbon from the plant to the fungi may occur through the arbuscules or intraradical hyphae (Pfeffer *et al.*, 1999). Secondary synthesis from the hexoses by AM occurs in the intraradical mycelium. Inside the mycelium, hexose is converted to trehalose and glycogen. Trehalose and glycogen are carbon storage forms that can be rapidly synthesized and degraded and may buffer the intracellular sugar concentrations (Pfeffer *et al.*, 1999). The intraradical hexose enters the oxidative pentose phosphate pathway, which produces pentose for nucleic acids.

Lipid biosynthesis also occurs in the intraradical mycelium. Lipids are then stored or exported to extraradical hyphae where they may be stored or metabolized. The breakdown of lipids into hexoses, known as gluconeogenesis, occurs in the extraradical mycelium (Pfeffer *et al.*, 1999). Approximately 25% of the carbon translocated from the plant to the fungi is stored in the extraradical hyphae (Hamel, 2004). Up to 20% of the host plant's photosynthate carbon may be transferred to the AMF (Pfeffer *et al.*, 1999). This represents a considerable carbon investment in mycorrhizal network by the host plant and contribution to the below-ground organic carbon pool.

An increase in the carbon supplied by the plant to the AMF increases the uptake of phosphorus and the transfer of phosphorus from fungi to plant (Bücking and Shachar-Hill, 2005). Phosphorus uptake and transfer is also lowered when the photosynthate supplied to the fungi is decreased. Species of AMF differ in their abilities to supply the plant with phosphorus (Smith *et al.*, 2003). In some cases, arbuscular mycorrhizae are poor symbionts, providing little phosphorus while taking relatively high amounts of carbon (Smith *et al.*, 2003).

2.13 Beneficial attributes of arbuscular mycorrhizal fungi

The benefit of mycorrhizas to plants is mainly attributed to increased uptake of nutrients, especially phosphorus. This increase in uptake may be due to increase surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment, and increased storage (Bolan, 1991). Mycorrhizas can be much more efficient than plant roots at taking up phosphorus. Phosphorus travels to the root or via diffusion and hyphae reduce the distance required for diffusion, thus increasing uptake. The rate of inflow of phosphorus into

mycorrhizae can be up to six times that of the root hairs (Bolan, 1991). In some cases, the role of phosphorus uptake can be completely taken over by the mycorrhizal network, and all of the plant's phosphorus may be of hyphal origin (Smith *et al.*, 2003). Less is known about the role of nitrogen nutrition in the arbuscular mycorrhizal system and its impact on the symbiosis and community. While significant advances have been made in elucidating the mechanisms of this complex interaction, much investigation remains to be done.

The available phosphorus concentration in the root zone can be increased by mycorrhizal activity. Mycorrhizae lower the rhizosphere pH due to selective uptake of NH4+ (ammonium-ions) and release of H+ ions. Decreased soil pH increases the solubility of phosphorus precipitates. The hyphal uptake of NH4+ also increases the flow of nitrogen to the plant as NH4+ is adsorbed to the soil's inner surfaces and must be taken up by diffusion (Hamel, 2004).

2.14 Arbuscular mycorrhizal fungi and plant ecology

Associations with arbuscular mycorrhizal fungi increase plant access to scarce or immobile soil minerals, particularly phosphorus, and thereby increase plant growth rates. In vegetation as different as the prairies, the dry shrub lands and the rich rainforests, the presence of these fungi has been shown to be essential for the sustained growth and competitive ability of plants (Koide *et al.*, 1994). Moreover, the presence of these fungi has been shown to alter plant community structure, productivity (Klironomos *et al.*, 2000), and the course of succession (Gange *et al.*, 1990); provide resistance to pathogens (Newsham *et al.*, 1995); and stabilize soil aggregates (Miller and Jastrow, 2000). Although evidence of the ecological importance of AMF in general is abundant, understanding of the distinct roles of

individual AM fungal species is relatively limited. Many studies have shown that individual species of AMF differ in their ability to promote plant growth, and promotion of plant growth can depend on the particular matching of plant and fungal species (van der Heijden *et al.*, 1998a).

Individual fungal species also differ in their growth response to plant species (Sanders and Fitter, 1992; Bever *et al.*, 1996), in their response to agricultural disturbance (Schreiner and Bethlenfalvay, 1997; Douds and Millner, 1999), and even in their ability to bind soil particles (Wright and Upadhyaya, 1998). However, scientists are just beginning to incorporate this evidence of the unique ecologies of AM fungal species into a framework that makes the biology of individual fungi important to plant ecology as a whole. As a result, ecologists are starting to appreciate the importance of the diversity of mycorrhizal fungi per se. For example, two recent studies of grasslands in Europe and North America demonstrate that increasing the diversity of AMF may directly increase the diversity of plants (Van der Heijden *et al.*, 1998b).

Investigations of the importance of mycorrhizal fungal diversity to plant ecology are understandably rare for two reasons. First, until recently ecologists have assumed that AM fungal species are functionally redundant. This belief has been supported both by the observations that these fungi have low specificities of association (individual species can associate with a broad range of host plants) and by the perception that AM fungal communities are imperfectly developed relative to plant communities (Law and Lewis, 1983; Allen *et al.*, 1995). Second, investigations of AM fungal diversity in plant ecology have been hampered by limitations in researchers' ability to monitor and manipulate the identity and diversity of the AM fungal community. Indeed, even measuring the species richness of the AM fungal community is fraught with

difficulties. Not only can distinguishing soil-borne spores of one species from those of an-other be difficult, but our limited knowledge of the population ecology of individual fungal species may itself constrain the measurement of AM fungal community composition.

2.15 Environmental variables on which arbuscular mycorrhiza fungi differentiate

2.15.1 Host specificity

Arbuscular mycorrhiza fungi are considered to have low specificities of association with plant host species, but these conclusions are based almost exclusively on experiments in which individual isolates of species are grown separately, apart from competitive interactions. When fungi are examined as a community, there is abundant evidence that AM fungal growth rates are highly host specific (Bever *et al.*, 2001). In an experiment by Bever *et al* (1996) AMF were trapped on different plant hosts, isolates of different fungal species sporulated differentially, with the relative dominance of fungal species being reversed, depending on the plant species with which they were associated. For instance, *Acaulospora colossica* has been observed to dominate in association with *Allium vineale*, field garlic, but this fungus is a minor component of the community associated with *Plantago lanceolata* (Bever *et al.*, 2001).

Alternatively, *Scutellospora calospora* sporulates profusely with Plantago, but is a minor component in association with *Allium* (Bever *et al.*, 2001). Distribution of fungi in the field is similarly host specific (Bever *et al.*, 1996). As this pattern of host specificity of growth rates in this nonspecific association has been observed in many other systems, including tall grass (Johnson *et al.*, 1992), sand dunes (Koske, 1981),

California grasslands (Nelson and Allen, 1993), chalk grasslands (Sanders and Fitter, 1992), and agricultural fields (Douds and Millner, 1999), this appears to be a general property of this interaction. This specificity of fungal response could contribute to the maintenance of diversity within the AM fungal community.

2.15.2 Seasonality as a factor of arbuscular mycorrhiza fungi differentiation

Arbuscular mycorrhiza fungi differ in their seasonality, with some fungi sporulating in late spring and others sporulating at the end of summer. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. Therefore, *Gigaspora gigantea*, which sporulates most abundantly in the fall and appears to overwinter as spores, is likely to be physiologically active during the warm season (Bever *et al.*, 2001). Similar patterns have been seen for *G. gigantea* in a sand dune on the coast of Rhode Island (Gemma *et al.*, 1989; Lee and Koske, 1994). Alternatively, *A colossica*, which sporulates most profusely at the beginning of summer and over summers as spores, is physiologically active with the cool season plant community (e.g., *A. vineale*).

2.15.3 Abiotic factors

Arbuscular mycorrhizal fungi are also known to vary in their response to the mineral environment of the soil. Individual fungi show opposite associations with certain soil parameters. For example, the distribution and abundance of *A. colossica* negatively associates with soil phosphorus concentration, while the reverse is true for *G. gigantea* (Bever *et al.*, 1996). This dependence of fungal spatial distributions on edaphic factors is consistent with observations in other communities, including tall grass prairie (Johnson *et al.*, 1992) and sand dunes (Koske, 1981).

2.16 Arbuscular mycorrhizal fungi and plant–pathogen relations

Arbuscular mycorrhizal fungi are essential parts of soil communities in a number of temperate as well as tropical ecosystems, and relate with a majority of species of terrestrial plants (Allen *et al.*, 1995). Growth-chamber and greenhouse experiments have showed repeatedly that these root symbionts can benefit nutrition of the host, fundamentally through facilitation of uptake of phosphorus (P). Hyphae capture phosphorus in areas of the soil that are less accessible to plant roots, and exchange the P for carbohydrates in specialized structures referred to as arbuscules (Hayman, 1983; Smith and Gianinazzi-Pearson, 1988). In spite of convincing support from laboratory experiments, field studies propose that the role of AM fungi in P facilitation is not universal (Newsham *et al.*, 1995a).

Since the association between AMF and terrestrial plants is more common than can be explained by P facilitation alone, other hypotheses have been advanced for how this association may be mutually advantageous (Newsham *et al.*, 1995b). A leading hypothesis suggests that AMF mitigate the effects of plant pathogens (Smith and Gianinazzi-Pearson, 1988). The AM fungal effects on pathogens are most likely indirect, and are a result of improved nutrition or modified physiology of the host (Smith and Gianinazzi-Pearson, 1988).

Most commonly AMF appear to enhance host tolerance, ability to sustain infection without reduced performance, through improvement of root growth as well as function (Smith and Gianinazzi-Pearson, 1988). Mycorrhizal plants may even sustain greater attack by pathogens yet grow better than nonmycorrhizal counterparts (Vaast *et al.*, 1998). Arbuscular mycorrhiza fungi may also raise host resistance (reduce

pathogen performance) by stimulating a defense response (Volpin *et al.*, 1994; Morandi, 1996) or altering root exudations used by pathogens.

Arbuscular mycorrhiza fungi are also hypothesized to inhibit pathogen growth through competition with pathogens for sites of infection or photosynthate (Smith and Gianinazzi-Pearson, 1988; Muchovej *et al.*, 1991; Traquair, 1995), or promoting growth of soil microbes that are antagonistic to pathogens (Thomas *et al.*, 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The field site for the soil sampling was Kinangop. It is located in central Kenya, 84Km North (338º) of Nairobi and has a latitude of -0.58(0º, -35' 0S) and a longitude of 36.53 (36º 31' 60E). This experimental site is one of the highest potato production areas in Kenya and it is also one of the areas that experiences great economic losses due to potato blight. Soil samples from two sites were collected from ten places (1kg per place) per site using a soil sampler at a depth of approximately 30 cm. Prior to the experiments, 20 soil samples were thoroughly mixed to give one composite sample. The soil samples were sterilized twice at 80°C for 24 h at an interval of 24 h and left for one week for stabilization before use (Bharadwaj, *et al.*, 2007). This soil was used as the growth substrate for the potato plants. Eight isolates of *Phytopthora infestans* were collected from leaves that were showing symptoms of potato blight in different farms.

3.2 Planting materials

Certified tubers of *Solanum tuberosum* were obtained from Kenya Agricultural Research Institute (KARI) Tigoni. Kenya Mpya variety was used in the experiment. It was subjected to a preliminary experiment to confirm that they were susceptible to *Phytophthora* infestans, as well as AMF. The mycorrhizal isolates that were used, as biological control, in the experiment were obtained from Dudutech Naivasha.

3.3 Experimental design

The experimental design was complete randomization with four treatments replicated five times. Plots consisted of 20 plants per block.

3.4 Culturing of *Phytophthora infestans* and pots inoculations

Cultures of *P. infestans* isolates were selected based on the aggressiveness criteria following a pathogenicity test (Haas *et al.*, 2009). Each of the eight isolates of *Phytophthora infestans* collected from the field site were was placed on the underside of a healthy leaf in a Petri dish containing a piece of moist cotton wool (Plate 1). They were then assessed for potato blight symptoms. The isolate that showed potato blight symptoms first was identified as the most aggressive. These isolates were acquired from field infections from 2012 on foliage and tubers of potatoes commonly grown in Kenya. Cleaned and sterilized potato tubers were cut into small slices. On the underside of every slice, a piece of infected leaf with the most aggressive isolate was placed and the slice was put in a moist Petri dish and incubated at 15 –18ºC. After 3 days, a colony of the pathogen was formed on tubers slices.

These colonies were then transferred to corn meal agar for 14 days at room temperature. Sporangia and mycelium were harvested by flooding with cold sterile water (4ºC) and gentle scraping of the surface of the culture. The mycelium/sporangia suspension was stirred with a magnetic stirrer for 1 h. The suspension was strained through four layers of cheesecloth and sporangia concentration was estimated using a hemocytometer and adjusted to about 1 x 10^6 spores ml^{-1} (discharged and non-discharged). The sporangial suspensions were stored for 6 h at 4ºC to encourage zoospore release from the sporangia before inoculating into the plants.

Plate 1: Pathogenicity test set up to determine the most aggressive *P. infestans* strain (Author, 2013)

3.5 Inoculation with *Phytophthora infestans*

Twenty days after planting, a sporangial suspension was foliar sprayed on the plants with a hand sprayer. All the plants were inoculated with the *P. infestans* spore suspension. To enhance potato blight symptoms, there was maintenance of humid conditions by watering the floor as shown in Plate 4.

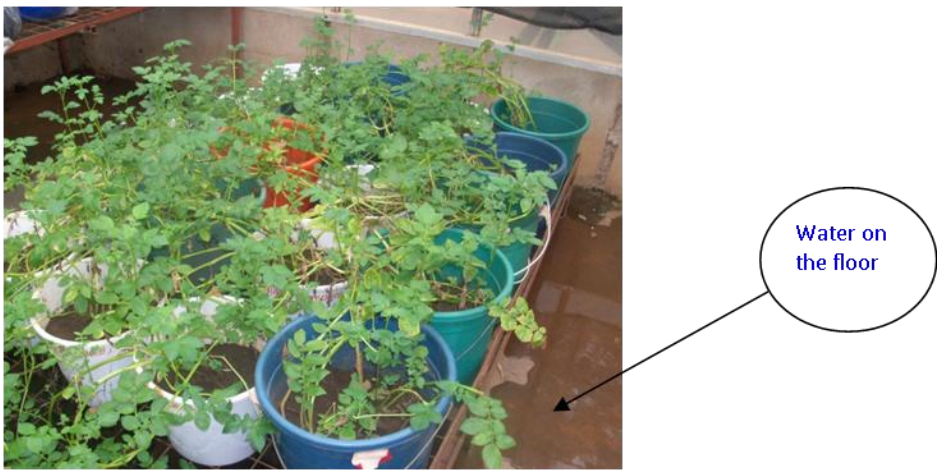

Plate 2: Maintenance of humidity in the greenhouse to enhance the growth conditions for *P. infestans* by flooding the greenhouse floor with water (Author, 2013).

3.6 Evaluation of blight

The effects of the treatments, set up in the greenhouse, with mycorrhiza on the progress of the pathogen were monitored throughout the season. Symptoms of potato

blight were observed every day and the numbers of infected leaves per plant were counted every two weeks after inoculation with the pathogen. In addition, plant heights were measured and number of leaves counted every two weeks from the time of inoculation with the spore suspension until the plants begun to flower. Different plants flowered at different periods depending on the time of sprouting. Mycorrhizal colonization and persistence were assessed by examination of the roots for presence of AM structures at the end of the experiment (Duffy *et al.*, 1999). In the roots examination, potato roots were cut into 30 cm segments and autoclaved at 90 ^{o}C in 10 % KOH for 30 minutes, a process known as clearing. The cleared segments were rinsed in tap water after which they were placed in HCL for 1 hour. After this, the root segments were stained in trypan blue. Thirty segments were mounted on a glass slide and covered with a cover slip then examined under a compound microscope for mycorrhizal structures.

3.7 Greenhouse experiment

The pots, five per treatment, were arranged in a completely randomized design. The efficacy of two (*Glomus intraradices, Glomus mosseae*) isolates of AMF and a mixture of the two were studied using potato, (*S. tuberosum*) as host plant. Prior to the actual experiment, a preliminary experiment was carried out to determine whether the two mycorrhiza isolates had the ability to infect the roots of the potato plant. The isolates were selected on the basis of their ability to infect potato plant roots. Plants were cultivated in pots (30 cm in diameter.) containing the sterilized field soil and sand mixture (1:1, v/v) as growth substrate. Each pot was inoculated with mycorrhiza except the control pots that contained the growth substrate alone. Potato tubers were planted in the pots (1 tuber per pot). A small hole was dug at the center of each

planting pot, containing sterilized soil, in which a potato tuber was put together with 50g of the relevant mycorrhizal isolate and then covered. There were five pots per treatment and control. The experiment was repeated twice making it a total of 40 pots. Twenty days after planting, each was inoculated with 50 ml of 1 x 10^6 ml^{-1} sporangial suspension of *Phytophthora infestans*. Pots were irrigated regularly.

3.8 Estimation of arbuscular mycorrhiza fungal colonization and growth dependency of potatoes

To estimate the colonization potential of the two AMF isolates potato roots were harvested once before final harvesting. The first harvesting was done at 20 days after planting to confirm that the plants had been colonized by the AMF and whether the soil sterilization was effective. The final harvesting was to assess the colonization of roots by the AMF. Small portion of the harvested roots were carefully processed by repeated washing in aerated tap water, cut into 1-cm segments and kept in 10% KOH at +60^{o}C in a water bath for approximately 7 min (the time depending on the size/structure of the roots and their pigmentation). Roots were stained using 0.05% trypan blue after washing with tap water according to Phillips and Hayman (1970) except that lacto glycerol was used instead of lacto-phenol.

Thirty root segments per replicate were examined under a light microscope and AM colonization percentage was determined as per procedure of Biermann and Linderman (1981). Plants harvested were used to determine the mycorrhizal dependency of the plants within each treatment. After removing a small amount of root tissue for analysis (approximately 5 g per plant), the remaining roots were washed free of adhering soil. Plants were placed in brown paper bags and dried in a drying oven at 53°C until a constant weight results. Growth dependency (Vestberg, 1992) of potatoes

on mycorrhiza was determined as the dry weight (g) of the mycorrhizal plant minus the dry weight (g) of the non-mycorrhizal plant divided by the dry weight (g) of the mycorrhizal plant multiplied by 100 summarized as follows

$$(\mathrm{MD}) = \frac{\text{dry weight (g) of the mycorrhizal plant} - \text{dry weight (g) of the non-mycorrhizal plant}}{\text{dry weight (g) of the mycorrhizal plant}}$$

(Damodaran *et al.*, 2012).

3.9 Final Harvesting

Approximately 16 weeks after planting at the trial site, the final tuber harvest was done. Tubers from the plants in each treatment were randomly sampled. Records of the number of tubers per plant and weight (g) of tubers per plant were made.

3.10 Statistical analysis

Data obtained from the greenhouse experiment, for number of tubers, weight of the tubers, plant heights, number of leaves and number of stems, was managed in Microsoft Excel and analyzed using SPSS version 20. Significant difference between control and the treated groups on the various parameters measured was assessed using students t test. One factor analysis of variance (ANOVA) was used to assess any significant difference between the treated groups and homologous subsets were classified using Tukey's pair wise comparisons. Significant correlation was determined using Pearson correlation analysis. The level of significance was set at p= 0.05. The percentage leaf infection was determined as the number of infected leaves per plant in every treatment compared with the total number of leaves expressed in percentage using the formulae;

$$\%\ leaf\ infection = \frac{number\ of\ infected\ leaves\ per\ plant per\ treatment}{number\ of\ leaves\ per\ plant\ per\ treatment\ total} \times 100.$$

The data for number of tubers, weight of the tubers, plant heights, number of leaves and number of stems was expressed as a mean of two independent experiments ± standard error of the mean (SEM).

CHAPTER FOUR

RESULTS

4.1 Culturing of *Phytophthora infestans*

Out of the eight isolates of *Phytophthora infestans* subjected to pathogenicity tests, one isolate was found to be the most aggressive strain and it took three days to show potato blight symptoms. From the preliminary experiment, it was confirmed that the Kenya Mpya potato variety was susceptible to *Phytophthora infestans* and AMF. The *Phytophthora infestans* colonies were fluffy and white in colour during the first few days of growth and they produced a pink pigment upon maturity (Plate 2 A-D). Full growth was exhibited in between seven and fourteen days after the most aggressive strain was successfully cultured in cornmeal agar as illustrated in Plate 3.

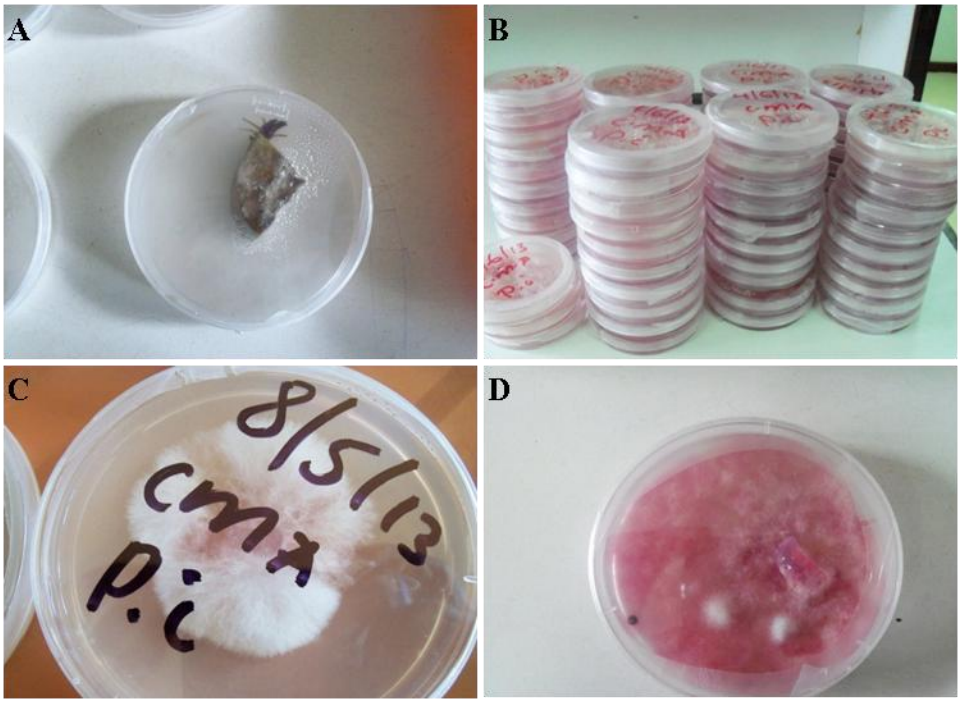

Plate 3: Images of different culture setups for P. infestans, A. *Phytophthora infestans* cultured in a potato tuber, B. *Phytophthora infestans* cultures in corn meal agar, C. *Phytophthora infestans* three days old cultures and D. seven days old fully grown culture (Author, 2013)

4.2 Inoculation with *Phytophthora infestans* and evaluation of blight

Potato blight symptoms were observed five days after inoculation of the pathogen (Plate 4).

Plate 4: Potato plants showing symptoms of potato blight five days after inoculation with the pathogen in different treatments, A. *G. mosseae, B.* Mixture of *G. mosseae* and *G. intraradices,* C. Control, D. *G. intraradices* (Author, 2013)

The mycorrhiza treated plants showed few potato blight symptoms with *Glomus mosseae* showing the least number of infected leaves (29.54±2.45%) (Figure 2). A mixture of *Glomus mosseae* and *Glomus intraradices* followed with 31.60±0.79%. *Glomus intraradices* and control had 33.76±3.50% and 35.66±2.41% leaves with potato blight symptoms respectively. Even though there was no significant difference

between the controls and the treatments, the controls had a higher number of infected leaves than all the treatments (Table 1).

Table 1: Percentage number of infected potato leaves treated with *Glomus mosseae, G. intraradices*, and a mixture of *G. mosseae* and *G. intraradices*

Treatment	Percentage of mean number of infected leaves	
	Mean±SE	%Reduction
Control	35.660±2.4145	6.12
Glomus Moseae	31.600±0.7918	2.06
Glomus intraradices	33.760±3.4981	4.22
Glomus Moseae and *Glomus intraradices*	29.540±2.4490	

4.3 Arbuscular mycorrhiza fungal colonization and growth dependency of potatoes

Mycorrhizal colonization was confirmed in all the treated plants on the basis of the presence of arbuscules in the infected roots. Vesicles, intraradicle spores and hyphae were observed in the mycorrhiza treated plants (Plate 5). These structures were not present in the controls. There was minimum root colonization 30 days after initial inoculation for all the treatments, and colonization reached maximum 16 weeks after the inoculation. The potato roots hyphal colonization, however, did not vary among treatments.

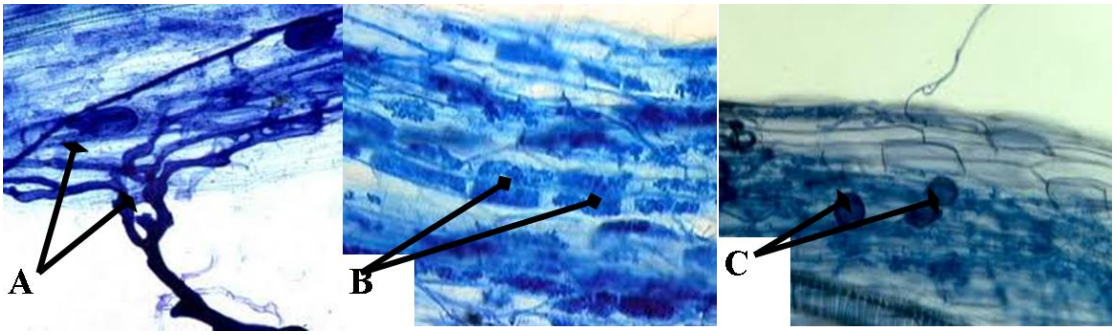

Plate 5: Structures that confirmed potato root colonization by arbuscular mycorrhiza fungi, A. Hyphae, B. Arbuscules and C. Vesicles (Author, 2013)

The highest MD (46.49%) was exhibited by plants treated with a mixture of *G. mosseae and G. intraradices*, followed by those treated with *G. intraradices*, which had 41.36%, dependency (Table 2). The plants treated with *G. mosseae* had the lowest MD (28.85%).

Table 2: Plants mycorrhizal dependency on each treatment

Arbuscular mycorrhiza fungal isolate	Mycorrhizal dependency (%)
G. mosseae	28.85
G. intraradices	41.36
G. mosseae and G. intraradices	46.49

4.4 Final harvesting

The average of the two experiments, for all the parameters, was used for analysis in which significance was set at p=0.05 (Appendix 1). The mean of number of tubers, leaves, stems and tuber weight per plant in every treatment are shown in Table 3 where homogenous subsets are presented with similar letters (Appendix 3-5). In terms of number of tubers, *G. mosseae* showed the highest mean (7.60±0.60) followed by the mixture of *G. mosseae* and *G. intraradices* (5.60±0.25) then *G. intraradices* (5.40±0.40) all of which were higher than that of the control (2.40±0.40). The number

of tubers in all the treatments were significantly higher than those of the control ($\rho<0.05$) (Appendix 2).

Table 3: Means of different parameters (number of tubers, number of leaves, number of stems, tuber weight, and plant heights) per plant in each treatment.

Treatment	No. of tubers	Leaves	Stems	Tuber weight	Plant heights
Control	2.40±0.40[a]	77.00±5.99[a]	5.20±0.58[a]	63.20±5.17[a]	77.08±6.06[a]
G. mosseae	7.60±0.60[c]	85.40±10.06[a]	6.00±1.00[a]	101.20±5.10[b]	76.34±8.43[a]
G. intraradices	5.40±0.40[b]	99.20±14.02[a]	6.40±1.29[a]	94.60±8.90[b]	80.52±6.38[a]
G. mosseae and *G. intraradices*	5.60±0.25[b]	97.20±12.27[a]	5.80±0.80[a]	103.20±5.56[b]	85.12±1.95[a]

Means with the same letters within the same column are not significantly different $\rho \geq 0.05$

Glomus intraradices had the highest mean of the number of leaves per plant (99.20±14.02), while control had the lowest (77.00±5.99). There was no significant difference between the control and the treatments (Appendix 2).

Glomus intraradices showed the highest mean of the number of stems per plant (6.40±1.29) with control showing the lowest (5.20±0.58). There was no significant difference in the number of stems between the control and the treatments (Appendix 2).

The mixture of *Glomus mosseae* and *Glomus intraradices* recorded the highest mean weight of the tubers per plant (103.20±5.56g), with the control recording the lowest (63.20±5.17g). There was a significant difference between the control and all the treatments ($p<0.05$) (Appendix 2).

The mixture of *Glomus mosseae* and *Glomus intraradices* had the highest mean (85.12±1.94) of plant heights, while *Glomus mosseae* had the lowest (76.34±8.43). There was no significant different between the control and all the treatments ($\rho<0.05$) (Appendix 2).

4.5 General observations

One week after planting, it was observed that 100% percent of the mycorrhiza treated plants had sprouted while only 60% of the controls had sprouted. It was also observed that the mycorrhiza treated plants had dark green foliage, were strong and vigorous as compared to the controls.

CHAPTER FIVE

DISCUSSION

Reduction in the severity of potato blight was exhibited in the current study. A number of studies have also reported the decrease in severity of diseases caused by soil-borne pathogens in plants that have been colonized by AMF (Whipps, 2004; Pozo *et al.*, 2009). In the current study, commercial AMF were tested on their efficacy to reduce severity of potato blight in potato plants. The percentage of leaves in each plant showing symptoms of potato blight indicated that the treated plants had lower percentage of leaves with symptoms of potato blight thus reduced severity of the disease. Even though there was no significant difference in the percentages of infected leaves, the controls had a higher number of infected leaves as compared to the treated groups. Mycorrhizal infection promotes lignifications in root tissues, which is responsible for preventing penetration of the plants by root pathogens (Dehne *et al.*, 1978). Mycorrhiza treated plants also develop more mechanical strength and lower the damaging effects of pathogens (Strobel *et al.*, 1982) which could have been the case in the current study.

In a previous study (Adrien *et al.*, 2011), there was indication of a reduction in the symptoms of late blight in the plantlets treated with AMF. An analysis of Real-Time Quantitative PCR disclosed that this decrease in symptoms was linked to a higher induction of PR1 (Jasmonic Acid (JA)-dependent pathway) and PR2 genes (salicylic acid (SA)-dependent pathway) in the AM fungal potato plantlets. SA, JA as well as ET, are known to play key function in a number of plant defence aspects. These include defence against abiotic stresses, like exposure to ozone and wounding, as well

as defense against attack by pathogenic microorganisms (Ecker, 1995; Creelman and Mullet, 1997) For instance, levels of salicylic acid rise in plant tissue after infection by a pathogen, and exogenous salicylic acid application leads to enhanced resistance to a wide range of pathogenic microorganisms (Ryals *et al.*, 1996).

The reduction in severity of potato blight observed in the current study could also be linked to an expression of systemic resistance in the leaves of potato plants treated with AMF in the early stages of development of *P. infestans* as was reported on tomato plants infected with *B. cinerea* (Jung *et al.*, 2009). In the current study, *G. mosseae* appeared to offer more reduction in severity of potato blight than any other treatment. This agreed with the recent demonstration that the AMF, *G. mosseae* had the ability to stimulate an induced systemic resistance against the necrotrophic fungus *B. cinerea* linked to a JA plant defence priming (Jung *et al.*, 2009).

A certain level of MD by the potato plants was clearly demonstrated by the results. This could have been significantly influenced by the concentration of soil solution P as well as moisture content in the soil. Species of plant vary in their mycorrhizal dependency at different levels of available P (Kahiluoto, et al., 2000). High moisture and P levels reduce potential for a growth benefit that is mycorrhiza-mediated (Allison and Goldberg, 2002, Stampe and Daehler, 2003) and, therefore, as the available moisture and P in the soil increases, the dependency of plants on the mycorrhizal association reduces (Beatriz and Walter, 2009). Kahiluoto *et al*. (2001) reported higher mycorrhizal colonization, as well as greater benefit of mycorrhizal association in flax grown on soils that had a low content of P. The pots treated with a mixture of *G. mosseae* and *G. intraradices*, which had the highest MD, may have had lower P concentrations than the other treatments. The moisture content, however, was

the same for all the treatments and thus this could not have affected the dependency of the potato plants on mycorrhiza.

Yield was enhanced with inoculation of AMF regardless of the species used. This is an indication that mycorrhiza played an important role in absorption of P by the plants, which is a major contributor to high yield. This was not the case in the controls, which had significantly lower yield than the treatments. These results are in agreement with a number of previous studies (Ryan *et al.*, 2003; Davies *et al.*, 2005). For instance, potato micro plants inoculation with a commercially available AMF comprising of three species raised the tuber yield when grown in the greenhouse in sand with slow release fertilizer (Ryan *et al.*, 2003). In another experiment in a greenhouse (Davies *et al.*, 2005), Peruvian potato prenuclear minitubers inoculation with AMF increased yield at an average of 85% over controls at low available P. The effect of the mixture of *G. mosseae* and *G. intraradices*, and *G. intraradices* on potato yield was not significantly different in the current study.

Glomus intraradices gave the highest mean of number of leaves per plant than the control and the other treatments. This shows the important role played by mycorrhiza in the generative growth in the potato plants and, thus, making a significant rise in the number of leaves per plant. This could be related to the highest mean number of stems that *G. intraradices* showed. This, though, was not significant and the number of stems could have been as a result of the number of the eyes the potato seeds had. The highest mean weight of the tubers per plant was recorded in the mixture of *G. mosseae* and *G. intraradices*. It indicated that the two AMF species have different type of benefits they confer on plants growth as well as development. It also indicated that the two species would give better results combined.

One week after planting, 100% percent of the mycorrhiza treated plants had sprouted while only 60% of the controls had sprouted. However, this difference cannot be associated with the mycorrhizal treatments; rather it could have been due to the degree to which the potato tuber seeds had germinated prior to planting. The mycorrhiza treated plants generally exhibited strength and vigour as compared to the plants that were not treated with mycorrhiza. They also exhibited a dark green foliage. This suggested the benefits that inoculation with mycorrhiza gave to the plants. These may include raised supply of plant nutrient (Kahiluoto and Vestberg, 1998), breakdown phenolic compounds in soils that may interfere with uptake of nutrients (Bending and Read, 1997), protection from nematodes and parasitic fungi, among others (Morin and Dessureault, 1999). Mycorrhizae adhere to the root structure of plant and utilize the sugars produced by the plant and extend the roots of the plant deep into the soil helping it to take up nutrients that the plant can absorb (Morin and Dessureault, 1999). This was evidenced in the current study by the strength as well as the vigour exhibited by mycorrhiza treated plants.

It could as well be attributed to increase in chlorophyll level which gives plants the green colour (Bayrami *et al.*, 2012). Moreover, AMF have been known to increase levels of chlorophyll when combined with different percentages of P in different plants (Wright *et al.*, 1998). The increase in chlorophyll content may also have been due to the interaction between AMF and P level (Siamak *et al.,* 2012). Increase in content of chlorophyll because of inoculation with AMF in plants has been earlier reported (Bian, *et al*., 2001; Feng, *et al*., 2002; Sannazzaro, *et al*., 2006; Elahi, *et al*., 2010). In a study carried out by Siamak and colleagues (2012) to test the reaction of potato to inoculation of seed with strains of mycorrhiza in different phosphorus fertilization, potato tubers inoculation with *Glomus mosseae* together with 67% of

superphosphate application of recommended dose led to increase in content of chlorophyll up to 78.2. In another experiment carried out by Wright *et al.* (1998) using clover (*Trifolium repens*) seeds inoculation with mycorrhiza had a large leaf area that the authors attributed to an increase in chlorophyll content. In another study, cowpea plants inoculated with *G. mosseae* led to significantly higher content of chlorophyll compared to control plants (Boby, *et al*., 2008). The rise in chlorophyll content in plants inoculated with AMF may also be as a result of increased phosphorus uptake, which increases the activity of photosynthesis and ultimately the chlorophyll content. The current results are in agreement with previous studies. For instance, total chlorophyll content of leaves inoculated with AMF showed significant increase at different phosphate levels as compared to control plants (Mathur and Vyas, 2000; Morte, et *al*., 2000; Ayoob, *et al*., 2011). In the current study the potato plants that were inoculated with *Glomus intraradices*, *G. mosseae* or a mixture of the two had dark greener foliage. Even though the content of chlorophyll was not quantified, the colour could phenotypically be distinguished between the treatments and the controls. The level of P was also not quantified in the current study but it was expected that P was present in all the pots. Therefore, the increase in chlorophyll level in the mycorrhiza treated plants was attributed to the uptake of P that was enhanced by AMF, which were absent in the controls.

This study demonstrated that inoculation of potato plants with AMF confers significant degree of reduction in severity of potato blight. This was evidenced by the percentages of infected leaves per pot showing symptoms of potato blight. Arbuscular mycorrhizal fungi inoculation in potato plants was also found to significantly enhance growth of the potato plant. Inoculation with AMF also demonstrated increase in potato yield. Individual species conferred varying benefits to the potato plants and it

was apparent that more than one species could give better outcomes combined. Mycorrhization, therefore, ought to be regarded as a strategy for enhancing economic production of potatoes as well as a biological control of potato blight.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. The study demonstrated that the two mycorrhizal isolates, *G. mosseae* and *G. intraradices*, and their mixture are effective in conferring reduction in severity of *Phytopthora infestans*
2. The study demonstrated that the two mycorrhizal isolates and their mixture are effective in promoting growth of the potato plant
3. It was also demonstrated, from the study, that the two mycorrhizal isolates and their mixture are effective enhancing potato plant yield
4. The two mycorrhizal isolates and their mixture differ in their efficacy in reducing potato blight severity and promoting growth and yield

6.2 Recommendations

From this study, it was recommended that,

1. Potato farmers were recommended to consider the use of AMF as a means to reduce the severity of potato blight and increase potato yields.
2. Further investigations on the efficacy of AMF on the control of potato blight should be carried out under field conditions.
3. Further investigations on the efficacy of AMF on the control of potato blight should be carried out using mixtures of different AMF isolates.

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APPENDICES

Appendix I; Results for Analysis of Variance

		Sum of Squares	df	Mean Square	F
Tubers	Between Groups	68.950	3	22.983	24.847
	Within Groups	14.800	16	.925	
	Total	83.750	19		
Leaves	Between Groups	1631.400	3	543.800	.898
	Within Groups	9686.800	16	605.425	
	Total	11318.200	19		
Number of Stems	Between Groups	3.750	3	1.250	.275
	Within Groups	72.800	16	4.550	
	Total	76.550	19		
Tuber Weight (g)	Between Groups	5189.350	3	1729.783	8.491
	Within Groups	3259.600	16	203.725	
	Total	8448.950	19		
Total Tuber Weight (g)	Between Groups	263970.200	3	87990.067	2.812
	Within Groups	500606.800	16	31287.925	
	Total	764577.000	19		

The experiment had five replicates and repeated twice. The average of the two experiments was used for analysis in which significance was set at p=0.05

Appendix II: Multiple comparison between treatments using Tukey's test

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Tubers	Control	GM	-5.200*	0.608	0.000
		GI	-3.000*	0.608	0.001
		GM and GI	-3.200*	0.608	0.000
	GM	Control	5.200*	0.608	0.000
		GI	2.200*	0.608	0.011
		GM and GI	2.000*	0.608	0.022
	GI	Control	3.000*	0.608	0.001
		GM	-2.200*	0.608	0.011
	GM and GI	GM and GI	-0.200	0.608	0.987
		Control	3.200*	0.608	0.000
		GM	-2.000*	0.608	0.022
		GI	0.200	0.608	0.987
Leaves	Control	GM	-8.400	15.562	0.948
		GI	-22.200	15.562	0.502
		GM and GI	-20.200	15.562	0.577
	GM	Control	8.400	15.562	0.948
		GI	-13.800	15.562	0.812
		GM and GI	-11.800	15.562	0.872
	GI	Control	22.200	15.562	0.502
		GM	13.800	15.562	0.812
	GM and GI	GM and GI	2.000	15.562	0.999
		Control	20.200	15.562	0.577
		GM	11.800	15.562	0.872
		GI	-2.000	15.562	0.999
Number of Stems	Control	GM	-0.800	1.349	0.933
		GI	-1.200	1.349	0.810
		GM and GI	-0.600	1.349	0.970
	GM	Control	0.800	1.349	0.933
		GI	-0.400	1.349	0.991
		GM and GI	0.200	1.349	0.999
	GI	Control	1.200	1.349	0.810
		GM	0.400	1.349	0.991
	GM and GI	GM and GI	0.600	1.349	0.970
		Control	0.600	1.349	0.970
		GM	-.200	1.349	0.999
		GI	-.600	1.349	0.970
Tuber Weight (g)	Control	GM	-38.000*	9.027	0.003
		GI	-31.400*	9.027	0.015
		GM and GI	-40.000*	9.027	0.002
	GM	Control	38.000*	9.027	0.003

		GI	6.600	9.027	0.883
		GM and GI	-2.000	9.027	0.996
		Control	31.400*	9.027	0.015
	GI	GM	-6.600	9.027	0.883
		GM and GI	-8.600	9.027	0.777
		Control	40.000*	9.027	0.002
	GM and GI	GM	2.000	9.027	0.996
		GI	8.600	9.027	0.777
		GM	4.0600	3.5136	0.662
	Control	GI	1.9000	3.5136	0.948
		GM and GI	6.1200	3.5136	0.336
		Control	-4.0600	3.5136	0.662
	GM	GI	-2.1600	3.5136	0.926
Percentage Infestation (%)		GM and GI	2.0600	3.5136	0.935
		Control	-1.9000	3.5136	0.948
	GI	GM	2.1600	3.5136	0.926
		GM and GI	4.2200	3.5136	0.635
		Control	-6.1200	3.5136	0.336
	GM and GI	GM	-2.0600	3.5136	0.935
		GI	-4.2200	3.5136	0.635

. The mean difference is significant at the 0.05 level. GM= *Glomus mosseae* and GI= *Glomus intraradices

Appendix III: Homogenous subsets for the average number of tubers for different treatments

Treatment	N	Subset for alpha = 0.05		
		a	B	c
Control	5	2.40		
GI	5		5.40	
GM and GI	5		5.60	
GM	5			7.60
Sig.		1.000	.987	1.000

The table displays means for groups in homogeneous subsets.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix IV: Homogenous subsets for average weight of tubers for all treatments

Treatment	N	Subset for alpha = 0.05	
		a	B
Control	5	63.20[a]	
GI	5		94.60[b]
GM	5		101.20 [b]
GM and GI	5		103.20 [b]
Sig.		1.000	.777

Appendix V: Homogenous subsets for average number of infected leaves for all treatments

Treatment	N	Subset for alpha = 0.05
		1
GM and GI	5	29.540
GM	5	31.600
GI	5	33.760
Control	5	35.660
Sig.		.336

Means for groups in homogeneous subsets are displayed.