MIDLANDS STATE UNIVERSITY

new fungicides for the management of *pythium* root rot in floatbed seedling production system

BY

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ABSTRACT

Pythium root rot possess major threats on float bed seedling productions. It is caused by oomycetes in the *Pythium* genus. It causes a huge of loss money as well as the seedlings. It is critically important to control this disease before it causes economic losses. A study was carried out from September 2013 to November 2013 at Kutsaga Research Station in the greenhouse. The purpose of the study was to evaluate the efficacy of metalaxyl + mancozeb from a new source (Citchem) for the control of *Pythium* root rot in the float bed seedling production system as well as to establish effective rates. *Pythium* is destructive plant pathogen that causes the damping off and root rot of seedlings in hydroponic seedling production. Two fungicides were used and these were Ridomil MZ 68% WG (metalaxyl) and metalaxyl + mancozeb (Metalaxyl-Citchem). Ridomil (metalaxyl) was used as the standard fungicide at a concentration of 05 parts per million per litre of water (ppm/L) (0.125 g/L of water). Metalaxyl + mancozeb was applied as two concentrations, 05 ppm/L (0.0625 g/L of water) and 10 ppm/L (0.125 g/L of water). One tobacco variety (KR K26) was used for seedling production, and the fungicides were applied at six weeks after seeding. *Pythiummyriotylum* was the test pathogen and was inoculated a week after fungicides had been applied. The tobacco seedlings were assessed for *Pythium* root damage at 9 and 22 days after inoculation (DAI). The assessments were done using a *Pythium*damage score (0-5) and the results were recorded on data sheets. The results revealed that all fungicides tested were effective, and were fungistatic against the test pathogen *P. myriotylum*. At 9 DAI Ridomil (metalaxyl) was the most effective and recorded an overall mean of 0.8 on the *Pythium* damage score. Metalaxyl + mancozeb (10 ppm/L) was found most effective at 22 DAI and recorded an overall mean of 1.9 on the *Pythium* damage score. In both assessments, the results showed that root damage varied among treatments ($p<0.05$). In both assessments the untreated seedlings were severely damaged by *Pythium* root rot.

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DEDICATION

This research is dedicated with love to my Mum and Dad, my two Brothers, Ernest and the late Tapiwa, and my four Sisters, Bridget, Precious, Enitah, and the late Agifa.

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CHAPTER 1

1.0 Introduction

1.1 Background

The backbone of Zimbabwe`s economy is agriculture. Tobacco (*Nicotiana tabaccum*) plays a pivotal role in the economy, a drop in tobacco earning means loss of revenue for the government (Mutsakani, 2004). Tobacco sales in the 2009-2010 season reached 100 million kilograms by the end of July 2010, beating the official target of 77 million which was set before the selling season opened (Zimbabwe Tobacco Production Records, 2010). Tobacco remains a key crop in Zimbabwe, earning the country over US$300 million in the 2010-11 farming season. This was by far the largest single contribution to the Gross Domestic Product from the agricultural sector (Karavina, and Mandumbu, 2012).

In Zimbabwe there are three types of tobacco which are morphologically different, and which also differ in the way in which they are cured. These types include Burley, Oriental and Flue-cured tobacco, of which flue-cured tobacco is most commonly grown in Zimbabwe. Increases in both planting areas and yields have contributed to a significant increase in output of tobacco over the past decades (Z.T.P Records, 2010). Large-scale commercial farmers used to dominate tobacco production but however this trend has changed; small scale farmers are dominating tobacco production (Z.T.P Records, 2010).

Despite the fact that tobacco is a vital crop in the economy of Zimbabwe, its generation is met with a great deal of restrictions. These stem from natural variables, plant pathogens, physiological disorders, and poor management (Moorman and Kim, 2004). Throughout the whole tobacco production cycle, diseases are the most common source of losses (Moorman and

Kim, 2004). The list of the most destructive plant pathogens includes *Pythium* species (Agrios, 1978), which causes post emergence damping off in the seed bed (Moorman and Kim, 2004). In addition to damping off, *Pythium* also causes crown and root rot of seedlings and feeder root necrosis of many agricultural crops, for example, cabbage, cucumber, maize, ginger (Plaats-Niterink, 1981) and tobacco (Gutierrez and Melton, 2001). *Pythium* causes major problems in the tobacco float bed seedling production system.

In Zimbabwe, tobacco seedling production is mostly done from June to September and *Pythium* had not been a problem with the conventional or traditional seedbed seedling production system (Sigobodhla, Dimbi, and Masuka, 2010). However, the introduction of the float tray seedling production system has seen the emergence of the pathogen (Sigobodhla *et al.*, 2010). Most tobacco agriculturists are presently utilizing the float tray seedling generation framework since it has demonstrated to deliver preferable seedlings over the conventional or traditional seedbed seedling production. Peedin, Pate and Smith (1997) stated that in the year 1996 in North Carolina, approximately 75% of the tobacco seedlings produced in the greenhouses were all grown using the float tray system.

1.2 Justification

Due to the sole use of the fungicide Ridomil MZ 68% WG (metalaxyl) in tobacco float beds, *Pythium* species have been observed to be developing resistance against this fungicide. The development of resistance within these pathogens has resulted in severe seedling losses (Commonwealth Mycological Institute, 2009). As a result of this, more fungicides have to be tested for efficacy for the control of *Pythium*. This needs to done in order to produce more fungicides with different ingredients or modes of action so that they can be used in rotation with Ridomil MZ 68% WG (metalaxyl), thus reducing incidences of pathogen resistance

(Commonwealth Mycological Institute, 2009). This project seeks to make more fungicides for the control of *Pythium* root rot available on the Zimbabwean market in order to prevent the development of resistance to the solely used fungicide Ridomil MZ 68% WG (metalaxyl).

Ridomil carries a high risk of inducing resistance amongst pathogen populations of the genus *Pythium.* Most *Pythium* species, specifically *Pythium myriotylum,* have been observed to be developing resistance against the sole use of Ridomil MZ 68% WG (metalaxyl) (Tobacco Research Board, 2006). Most tobacco farmers lack vital knowledge on the ideal application dates of fungicides. Since the fungicide Ridomil is being used solely by most tobacco growers, ideal application dates are well known (Tobacco Research Board, 2010). Zimbabwean tobacco farmers also lack knowledge on the implication of the ideal application dates and also the use of fungicides with different formulations. This has led to the ineffectiveness of some fungicides in Zimbabwe (Sigobodhla *et al*., 2010).

Pythium root rot is becoming one of the most vital limiting factors in seedling production as the pathogen is resident in irrigation water (Sigobodhla *et al.,* 2010). The Tobacco Research Board (T.R.B) particularly the Pathology Division conducted several trials which showed that better yields were obtained in fields from trays produced with metalaxyl as a preventive measure against *Pythium* (Sigobodhla *et al.,* 2010). If float bed seedlings were grown without the use of metalaxyl as a preventative measure, the yields were very low and the seedlings were greatly affected (Sigobodhla *et al.*, 2010).

Prior to the phase-out of methyl bromide, tobacco conventional seedbeds were either fumigated with methyl bromide or heat-treated with firewood (Karavina, and Mandumbu, 2012). The tobacco float tray system is an ideal alternative because it is environmentally friendly as it does

not emit greenhouse gases into the atmosphere which deplete the ozone layer (T.R.B, 2010). Methyl bromide, if released in the atmosphere depletes the ozone layer. Since the year 2005, a methyl bromide phase-out has been implemented in developed countries (Executive Summary, 2006).

Due to the ban of methyl bromide by 2015 worldwide and the transition from conventional seedbeds to the float tray system, this project seeks to lessen or reduce the incidence of *Pythium* in the hydroponic systems. This is done by either preventing or treating the most devastating fungal disease *Pythium* root rot.

Though float beds are an ideal environment for tobacco seedlings, they are also an ideal environment for the development of *Pythium* and other fungal species. If *Pythium* is left uncontrolled, *Pythium* root rot will cause severe damping off and stunting of seedlings if it is not controlled (Seebold, 2011). It also causes slow growth of transplants. The commercial seedbed production in Zimbabwe has reported a loss of up to 70% of the seedlings as a result of *Pythium* damage (Sigobodhla, *et al*., 2010). Zimbabwean float seedling production system is still under threat by *Pythium myriotylum* (Sigobodhla, *et al*., 2010). This project will provide ways of hindering fungal development so that tobacco farmers and other farmers, who also use the float bed system, obtain maximum returns.

1.3 Objectives:

1.3.1 Main objective:

- to evaluate the efficacy of metalaxyl + mancozeb from a new source (Citchem) for the control of *Pythium* root rot in the float bed seedling production system.

1.3.2 Specific objectives:

- to establish effective rates of metalaxyl + mancozeb from a new source (Metalaxyl-Citchem) in the control of *Pythium* root rot in the float bed seedling production system, and
- to compare the efficacy of the new fungicide Metalaxyl-Citchem (metalaxyl + mancozeb) against the standard Ridomil MZ 68% WG (metalaxyl).

CHAPTER 2

2.0 Literature Review

2.1 Tobacco Production in Zimbabwe

According to the Zimbabwe Tobacco Production Records (2010), tobacco production in Zimbabwe has increased drastically since the Land Reform Programme in the year 2000. Before the Land Reform Program over 70% of tobacco sold at the auction floors came from the large scale commercial farmers with the remainder being obtained from the small scale producers (Z.T.P Records, 2010). However, the trend began to change after the Land Reform Programme, at present over 60% of the tobacco leaf is being obtained from the small scale newly resettled farmers. This has been attributed to increase in tobacco prices and the realization of hard currency by farmers (Z.T.P Records, 2010).

The T.I.M.B (2011), on the other hand states that Zimbabwe` tobacco production may increase by 38% to 170 million kilograms in the growing season that time as more farmers started growing the crop. They also believed that the output may rise from 123 million kilograms which were obtained the previous season (T.I.M.B, 2011).

Three main types of tobacco are grown in Zimbabwe, namely flue-cured, oriental and burley tobacco (T.R.B, 2006). The most important and commonly grown type in Zimbabwe is the flue-cured tobacco and is generally produced in the better rainfall and warm areas such as the north and east of Harare. The northern regions produce a Virginia type of tobacco, whereas growers in the east produce a thicker, slower developing type used for blended cigarettes. Burley tobacco is grown mainly in the northeast and in the eastern highlands of Zimbabwe and is predominately a

smallholder crop. Oriental tobacco accounts for less than 1% of the total output by mass and is grown mainly by small-scale producers in Masvingo Province (T.I.M.B, 2011).

Tobacco is a cash crop which is also an important source of government revenue (Z.T.P Records, 2010). A levy system in which growers and buyers both pay a fixed percentage on the value of crop sales generates several millions of dollars annually. To encourage production, the tax rates have been reduced each year since 1999 (Z.T.P Records, 2010). Tobacco grown in different countries or different regions in a country varies in type and quality (Beghin and Chang, 1992). It is not a homogeneous product. The quality and quantity of nicotine content in different types of tobacco such as flue-cured, burley, or oriental tobaccos was investigated by Beghin and Chang (1992) and they found out that flue-cured tobacco provides the best leaf in terms of quality.

2.2 Tobacco botany

Tobacco is of the genus *Nicotiana* (Heiser, 1969). It is botanically grouped as a member of the Solanaceae family also known as the Nightshade family (Axton, 2009). The Solanaceae also includes other plants such as tomatoes, potatoes, peppers among others, and it is one of the largest families in the natural world (Heiser, 1969). Tobacco plants produce very tiny seeds. Approximately 300 000 seeds can be contained in 28 g of raw seed (Gleason, 2006). The leaves of the plant are very large and broad. Tobacco plants are cultivated for these leaves which store nicotine (Gleason, 2006). Nicotine is produced by the roots from as early as a few days after germination (Gleason, 2006).

2.3 Pests and Diseases of Tobacco

Pests of tobacco include insects such as white flies (*Bemisia tabaci*), aphids (*Myzus percicae*), budworms, cutworms, fungus gnat just to mention a few (Lucas, 1975). Some of these pests

spread diseases, for example the *Myzus percicae* spreads viruses such as the Tobacco mosaic virus (TMV) and the Bushy top virus (BT) (Lucas, 1975). Bacteria, fungi and viruses also attack the tobacco plants. Fungal diseases are the most common; examples are *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp. *Rhizoctonia solani* causes seedling damping off, root rot, soreshin and target spot. Soreshin causes seedling shortages if it heavily affects tobacco seedbeds (Lucas, 1975).

Pythium is common in the seedbeds and an important cause of crown rot, root rot and damping off of seedlings (Wick and Benton, 2006). The *Pythium* species' pathogenic capacity is determined largely by the possession of cellulolytic and pectolytic enzymes (Ali-Shtayeh, 1986).

2.4 The Genus *Pythium*

More than 120 species of the genus *Pythium* are well known for their pathogenic behaviours and are widely distributed throughout the world (Paul, 2001). They occupy wide range of ecological habitats in both aquatic and terrestrial environments (Dick, 2001). Their pathogenic effects are more pronounced on succulent plant tissues or plant juveniles. This restricts *Pythium* species to be main parasites of tender roots and root tips of older plants or seedlings, and in some cases stem tissues and watery fruits (Hendrix and Campbell, 1973).

Pythium belongs to the kingdom Straminopila (Paul, 2001), or Chromista (Kirk, Cannon, Minter and Staplers, 2008). Most fungi in this genus reproduce both sexually and asexually, and remain as diploids their entire life cycles (Paul, 2001). Asexual reproduction involves the formation of motile zoospores, whereas sexual reproduction is oogamous (Paul, 2001).

2.5 Scientific Classification of *Pythium*

Kingdom: Chromista

Phylum/Division: Oomycota

Class: Oomycetes

Order: Peronosporales

Family: Pythiaceae

Genus: *Pythium* (Nelson, 1994).

2.6 *Pythium* species

Several fungus-like organisms cause root and crown rots on tobacco seedlings (Seebold, 2011). A considerable number of *Phytophthora* species, *Fusarium*, *Pythium* and some other plant pathogens have been noticed to be present in the water used for irrigation (Bush, Hong and Stromberg, 2003). *Phytophthora* and *Pythium* generally favour wet conditions where there is rapid dispersal of the asexual flagellate zoospores (Pettitt, Wakeham, Wainwright and White, 2002).

Garret (1951) and Burges (1958) refer to *Pythium* species as 'sugar fungi' due to their inability to breakdown complex polymers of carbohydrates such as cellulose. The *Pythium* species, however, were removed from the substrate group 'sugar fungi' after it had been recently discovered that several *Pythium* species were capable of breaking down cellulose (Ali-shytayeh, 1986). Commonly encountered species of *Pythium* which cause *Pythium* root rots in the green houses are *Pythium ultimum*, *P. irregular* and *P. aphanidermatum* (Beckerman, 2007).

Pythium sporangia rapidly germinate approximately two and a half hours after the exposure to volatiles or exudates from plant roots or seeds (Osburn, Schroth, Hancock, and Hendson, 1989). This is followed by prompt infection and this makes the management of the pathogen exceptionally troublesome (Whipps and Lumsden, 1991). *Pythium* spp. are generalist and unspecific to their host range, which causes broad and devastating root rot and is frequently exceptionally hard to avoid or control (Jarvis, 1992).

2.7 Symptoms of *Pythium* root rot

The roots which usually protrude from the float tray into the water medium show the first *Pythium* root rot symptoms (Seebold, 2011). Above ground symptoms include stunted growth (Seebold, 2011). According to Garwe, Chirova, Muzhinji and Sengudzwa (2014), when the roots start protruding from the tray into the pond water (25 days or older), that's usually when the symptoms are observed. In the early stages of *Pythium* root rot infection, the roots become light brown to grey with a slimy texture (Gutierrez and Melton, 2001).

Contaminated roots will in the long run fall away and some re-development may be observed (Seebold, 2011). On the other hand the newly formed roots will probably get to be infected soon after regrowth (Seebold, 2011). As the disease advances, stunting and yellowing of seedlings is common and has a tendency to be restricted to all areas around well-defined ranges of a float bed (Seebold, 2011). If infected plants survive, their vigour is reduced and, as a result, poor quality seedlings are obtained (Gutierrez and Melton, 2001). Reduced seedling vigour may also lead to increased vulnerability to root diseases and seedling mortality (Schlub and Schmitthenner, 1978).

2.8 Control of *Pythium*

A successful growing season is determined by the quality of tobacco seedlings. With cautious monitoring, it is possible to effectively control *Pythium* root rot, resulting in the production of seedlings of good quality (Seebold, 2011). *Pythium* species control strategies may include biological or chemical control strategies. Natural enemies of *Pythium spp.* include *Streptomyces griseoviridis, Bacillis subtilis, Gliocladium virens* and *Trichoderma harzianum* which are produced commercially (Moorman and Kim, 2004). Wick and Benton (2006) state that *Pythium* is most efficiently controlled by systemic fungicides especially those that have metalaxyl as an active ingredient. Plants can be protected from *Pythium* spp. by using different classes of fungicides; some of these chemicals include azoxystrobin (strobilurin), mefenoxam and metalaxyl (acylanilide) and propamocarb (carbamate) (Moorman and Kim, 2004). Another crucial part for the management of *Pythium* root rot in the float beds is sanitation. Bleach may be used to disinfect trays (Seebold, 2011).

2.9 The Tobacco Float Tray Seedling Production

Tobacco seedling production began its transition in the 1990s (Hensely, 1999). The farmer's utilization of the float tray system expanded from under 1 % in 1990 to around 80 % in 1999. This increase in the rate of adoption of the float system was because of its advantages over the convectional seed bed (Hensely, 1999). The float bed system is a less labourious alternative compared to the convectional seedling production. Advantages of the float system include using nutrients and water effectively, foliar disease will be reduced since plant foliage stays dry, and also the leaching of nutrients is completely eliminated (Rideout, 2005). The labour of pulling transplants is eliminated, dry weather will not cause plant bed failure, weeds are also controlled using the float system and there is also the improvement of the seedling survival as well as early

season growth of seedlings (Hensely, 1999). The float bed system uses a manufactured growing medium, for example, pine bark is used for tobacco seedlings (Hensely, 1999).

2.9.1 Trays

Studies carried out at the University of Tennessee showed that seedling usability and survival among plants are not affected by the number of cells in a tray (Hensely, 1999). However, trays with large numbers of cells will produce smaller seedling which need to be clipped more often to obtain normal sized seedlings (Hensley, 1999).

2.9.2 Tray filling

Producing a lightly compressed column and uniform medium throughout each cell of the float tray is the main objective (Hensley, 1999). The medium should also not be too loose otherwise it will flow out of the cell into the water, therefore when manually filling the tray it should be gently dropped from a height of 30 cm onto a flat surface for the compaction of the medium. Dry cells are a result of the medium failing to get in contact with the water in the float bed. These can be prevented by wetting the trays just before filling in with the medium (Hensley, 1999).

CHAPTER 3

3.0 Materials and Methods

3.1 Experimental Site

The study site was at Tobacco Research Board (T.R.B) in Harare near the Harare International Airport. It is in the Agro ecological Region 2a, which usually receives an average annual rainfall between 820 - 1000 mm at an altitude (height above sea level) of 1496 meters above sea level. The area received average temperatures of 31˚C in summer and 17˚C in winter. The soil type mostly found at the institute is sandy loam.

3.2 Experimental Design

The Completely Randomised Design (CRD) was used in this experiment. The study had four treatments replicated four times. Assessments were done twice; 9 and 22 days after inoculation (DAI).

3.2.1 Treatments

Table 1: Treatments used in the experiment

Fungicide	Rate (ppm)	Rate g (product)
1. Untreated control	0	0
2. Metalaxyl (Ridomil MZ 68% WG)	05 ppm/L	0.125 g/L of water (standard)
3. Metalaxyl + mancozeb (Metalaxyl- Citchem)	05 ppm/L	0.0625 g/L of water
4. Metalaxyl + mancozeb (Metalaxyl- Citchem)	10 ppm/L	0.125 g/L of water

3.3 Experimental Procedure

3.3.1 Greenhouse Cleaning

The experiment was carried out in the Plant Pathology greenhouse. The greenhouse was first cleaned thoroughly and sterilized with a disinfectant (formalin), in order to destroy pathogens and insects which might spread diseases to the tobacco seedlings.

3.3.2 Pond Construction

Sixteen ponds were constructed in the greenhouse using bricks and a thick black polythene plastic. The ponds were constructed in such a way that only one float tray would fit into each pond. Four blocks containing four ponds of single trays were constructed. The pond spacing was maintained too close to the tray in order to prevent the growth of algae.

3.3.3 Media Mixing and Tray Filling

The media used in this experiment which supported the growth of the tobacco seedlings was pine bark media. Pine bark media (100%) was used because it is light and does not block the capillary action of the cells in the float tray. Pine bark media was mixed with water carefully to ensure that it was not too wet or too dry. Tray filling was done after mixing the pine bark. The float trays used in this experiment had 242 cells.

3.3.4 Dibbling and Sowing

Dibbling was done using a dibble board with 242 dibbles which aligned with cells of the float tray. This was done to ensure that the tobacco seed goes in the centre of the cell for proper germination to occur. After dibbling, pelleted Kutsaga root knot 26 (KR K26) seeds were sown by hand, placing one seed into each cell of the float trays. One tobacco variety was used in this

experiment (KR K26). After sowing, each tray was floated in each of the 16 constructed ponds. Sowing was done on the 25th of September 2013.

3.3.5 Fertilization

An additional fertilizer was added into the pond water to ensure that the pond water in the hydroponic systems contained enough nutrients to support normal plant growth. A liquid fertilizer known as the Kutsaga float fert was used in this experiment. The composition of the fertilizer was 20:10:20 for the nitrogen, phosphorus and potassium (T.R.B hand book, 2010). The first fertilizer application was done on the 3rd of October (one week after sowing) at the rate of 14 ml per tray. The same float fertilizer was also applied at three and five weeks after sowing at the rates of 28 ml and 42 ml per tray, respectively. Ammonium nitrate was also added in each pond at six weeks after sowing and the application rate was 6 g per tray.

3.3.6 Production of *Pythium* inoculum in the laboratory

Pythium oospores were preserved in the Plant pathology division for *Pythium* studies. The most virulent and most occurring strain in Zimbabwe, *Pythium myriotylum* was used in this experiment. *Pythium* isolate number 54 (*Pythium myriotylum*) was plated on Potato dextrose agar (PDA). In order to obtain adequate inoculum 30 petri dishes of PDA were used to culture the *Pythium* isolate. The plates were cultured in an incubator maintained at 28˚C until the fungus had completely covered the media in the petri dish. In order to produce large amounts of the inoculum, small pieces of the media together with the fungus were cut then sub-cultured in bottles containing fluid oat meal agar. Approximately 10 litres of the inoculum were produced.

3.3.7 Fungicide Application and *Pythium* Inoculation

The fungicides were applied six weeks after sowing on the 4th of November. The efficacy of the new fungicide metalaxyl + mancozeb (Metalaxyl-Citchem) against *Pythium* root rot was evaluated in two concentrations (05 ppm/L and 10 ppm/L). The concentration of Ridomil MZ 68% WG which was used in this experiment was 05 ppm/L. Seedlings in treatment 2 received 3 g of the fungicide (Ridomil) and those in treatment 3 and 4 received 1.5 g and 3 g of the new fungicide, respectively. *Pythium* was inoculated one week after the application of the fungicides. When inoculating the seedlings with *Pythium*, 1 ml of the inoculum was applied at the base of each seedling. The same amount of inoculum was also applied in empty cells of the float trays where seeds did not germinate.

3.3.8 Clipping and Pest Control

Clipping was done regularly in the green house to maintain uniform seedling growth in all treatments. Clipping also improves sunlight penetration and ventilation in the seedling canopy (Hensley, 1999). A chemical used to control insect pests (acephate) was sprayed once to control the aphids.

3.4 Data Collection

Pythium root rot disease assessments were done 9 and 22 days after inoculation (DAI). During the first *Pythium* assessment, 20 seedlings were uprooted on each tray. On the second assessment 20 seedlings were collected from each tray for assessment.

Pythium root rot damage assessment scale was used. The scale was as follows:

0 = No damage

1 = <25% of roots discoloured

2 = 25 – 50% of roots discoloured, <25% of root system rotted away, slight wilting of the plant

3 = 50 – 75% of roots discoloured, 25 – 50% of root system rotted away, moderate wilting of plant

4 = >75% of roots discoloured, >50% of root system rotted away, severe wilting of the plant

5 = Plant dead

3.5 Data Analysis

Data for the *Pythium* root damage were analysed using the one way analysis of variance (ANOVA). The factors were the fungicidal treatments and the response variable being the root damage. The tests for homogeneity of variances and normality were done. The statistical tool used for the analysis of data was the SPSS version 5.

CHAPTER 4

4.0 Results

The water level in the ponds with treatment 1 decreased at a slower rate than that in other ponds. Most of the younger seedlings in treatment 1 float trays succumbed to salt injury especially when the greenhouse temperatures got high. All the float trays with treatment 1 where no fungicide was added had fewer seedlings than the other float trays where fungicides were administered in the float water. These seedlings also showed stunted growth and they also had yellow lower leaves. The symptoms of the disease in this experiment included the rooting away of seedling roots. The uptake of nutrient from the float fertilizer added in the water was reduced in plants with damaged roots. This was shown by their pale green foliage even if excess fertilizer was added.

4.1 Damage caused by *Pythium*

4.1.1 Leaves

Some seedlings had yellow bottom leaves especially those in treatment 1 (Figure 4.1). Most of the yellow leaves were observed to be in contact with the water or the wet pine bark medium. A grey mould was observed on some leaves in contact with the wet float trays. Leaf chlorosis was observed in trays with the untreated control with the bottom leaves drying and rotting away (Figure 4.1). A few seedlings in treatment 1 float trays exhibited brown leaf spots.

4.1.2 Stems

Figure 4.4 shows the crowns of infected tobacco seedlings. The crowns of these seedlings were rotten and weak. Some seedlings were snapping at the bases of stems leading to the death of the

plant. Wire-stems of younger seedlings were observed in most cells of float trays with treatment 1.

4.1.3 Roots

Pythium infested roots were discoloured exhibiting a light to dark brown in colour. Plants with damaged roots showed wilting when the temperatures were high during the day (Figure 4.2). The roots of tobacco seedlings in untreated controls were greatly affected as compared to those that were treated with fungicides (treatment 2, 3 and 4). Infected roots had a slimy texture and would easily breakoff if touched. If the float tray was pulled out of the water, infect roots adhered to the bottom of the tray (Figure 4.3). By merely observing, the root densities of infected plants was reduced as compared to healthier plants. The roots of infected seedlings showed poor development and had few or no root hairs (Figure 4.4).

Figure 4.1 Yellowing and drying of lower leaves

Figure 4.2 Wilting seedlings in the greenhouse

Figure 4.3 Infected roots protruding from float tray

Figure 4.4 Diseased roots and stem rot

4.2 Effect of the treatments

Figure 4.5 below shows the mean scores of *Pythium* damage obtained from each treatment at nine days after inoculation (DAI). Treatment 1 which was the untreated control showed the highest root damage with an overall mean of the *Pythium* damage score at 3.4. The standard (Ridomil) which was treatment 2 scored the lowest on the overall mean of the *Pythium* damage score. Treatment 3 and 4 (the new fungicide) both scored an overall mean of 0.9 on the *Pythium* damage score, which was not very far from the 0.8 scored by treatment 2. The *Pythium* damage score for all the fungicides used in this study was lower as compared to the untreated control (Figure 4.5). The overall *Pythium* root damage score for all the fungicides tested here, did not

exceed one. After the first assessment at 9 DAI, the results also showed that root damage varied significantly across all treatments ($p < 0.05$ – appendix 1).

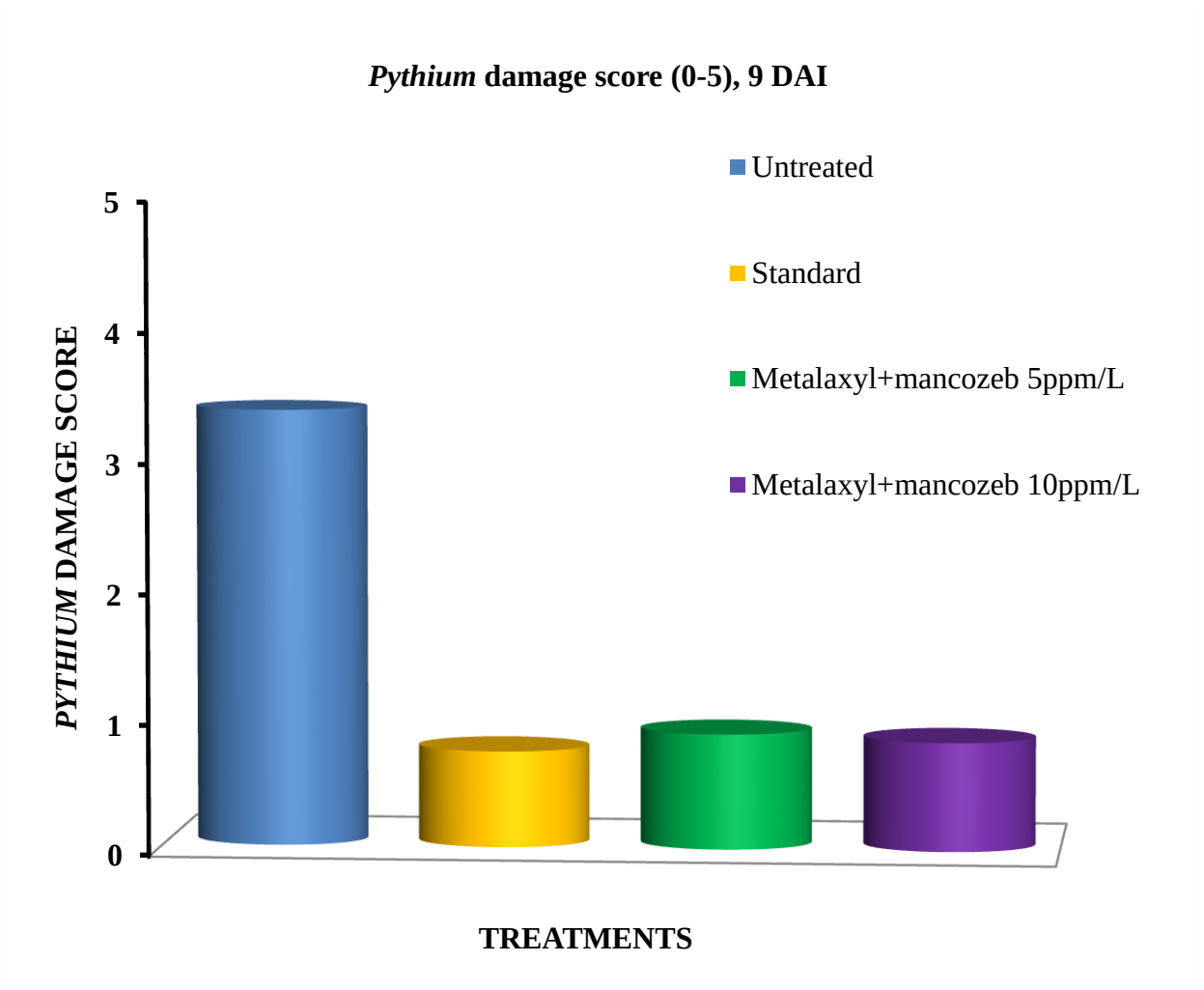

Figure 4.5 *Pythium* score overall means obtained on the first assessment (9 DAI)

Figure 4.6 shows the *Pythium* damage mean scores obtained at 22 DAI for each treatment. Treatment 1 again scored the highest *Pythium* root rot damage with an overall mean of 4.3. Most of the seedlings in treatment 1 were greatly damaged and some dead. Treatment 3 scored an overall mean of 2.4. Treatment 2 scored a low overall mean of 2.2 on the *Pythium* damage score, but however in this case treatment 4 scored the lowest overall mean of 1.9. There were no

significant differences on the *Pythium* damage score obtained from 05 ppm/L and 10 ppm/L of metalaxyl + mancozeb (Metalaxyl-Citchem). Again in the second assessment the fungicides had a lesser *Pythium* root damage score as compared to the untreated control. After the second assessment at 22 DAI, the results also showed that root damage varied across all treatments ($p < 0.05$ – appendix 2).

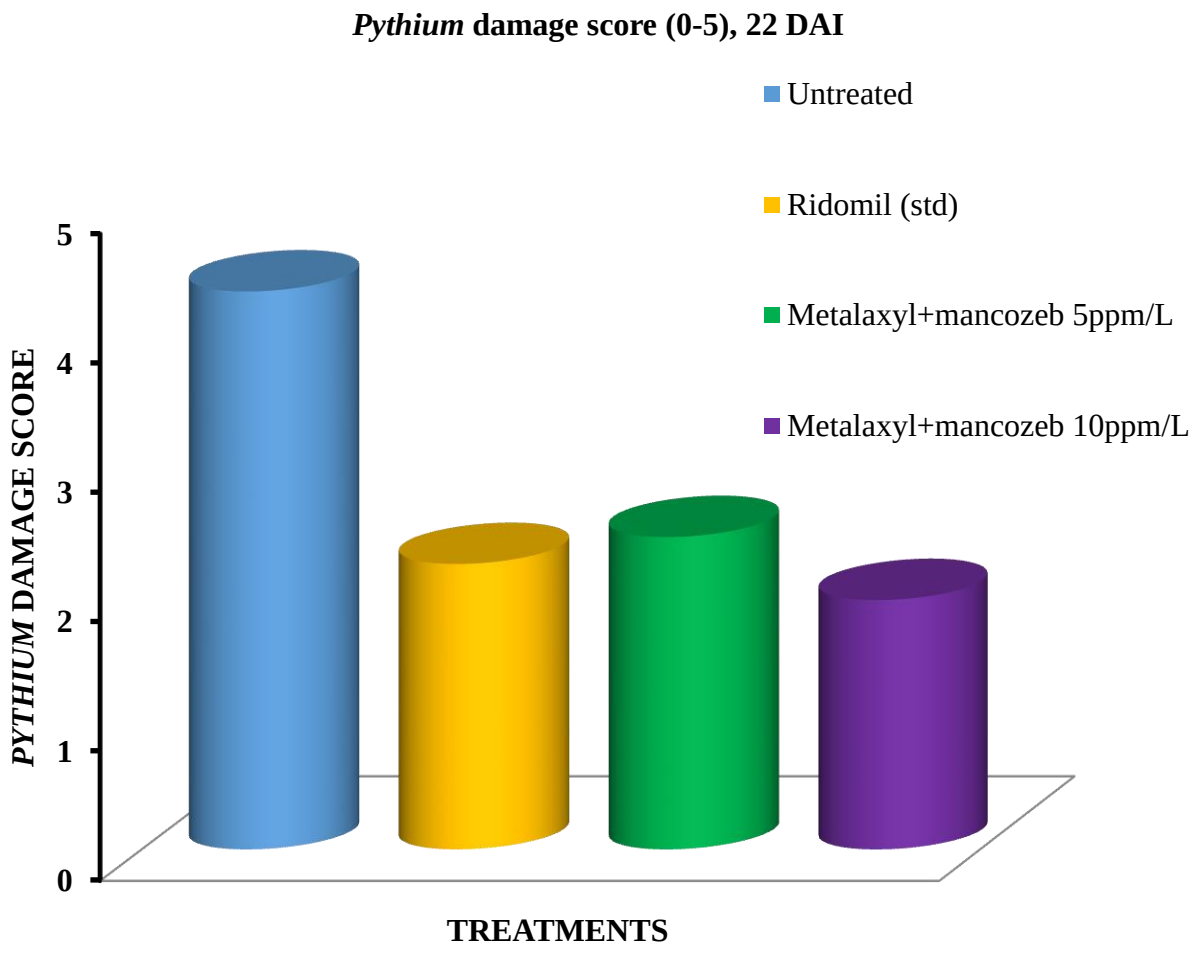

Figure 4.6 *Pythium* score overall means obtained on the second assessment (22 DAI)

After the results of the first and second assessment were collected and represented graphically (9 DAI and 22 DAI, respectively), the trends of the graphs were observed (Figure 4.7). The trends for both assessments were almost similar with treatment 1 scoring the highest overall mean

score, whilst treatments 2, 3 and 4 had very low *Pythium* damage scores compared to treatment 1. On the second assessment (22 DAI) the *Pythium* score damage for the fungicides was higher than that obtained during the first assessment (9 DAI) (Figure 4.7). There were no significant differences on the *Pythium* damage score obtained from 05 ppm/L, 10 ppm/L of metalaxyl + mancozeb (Metalaxyl-Citchem) and Ridomil MZ 68% WG (metalaxyl) ($p > 0.05$ – appendix 3).

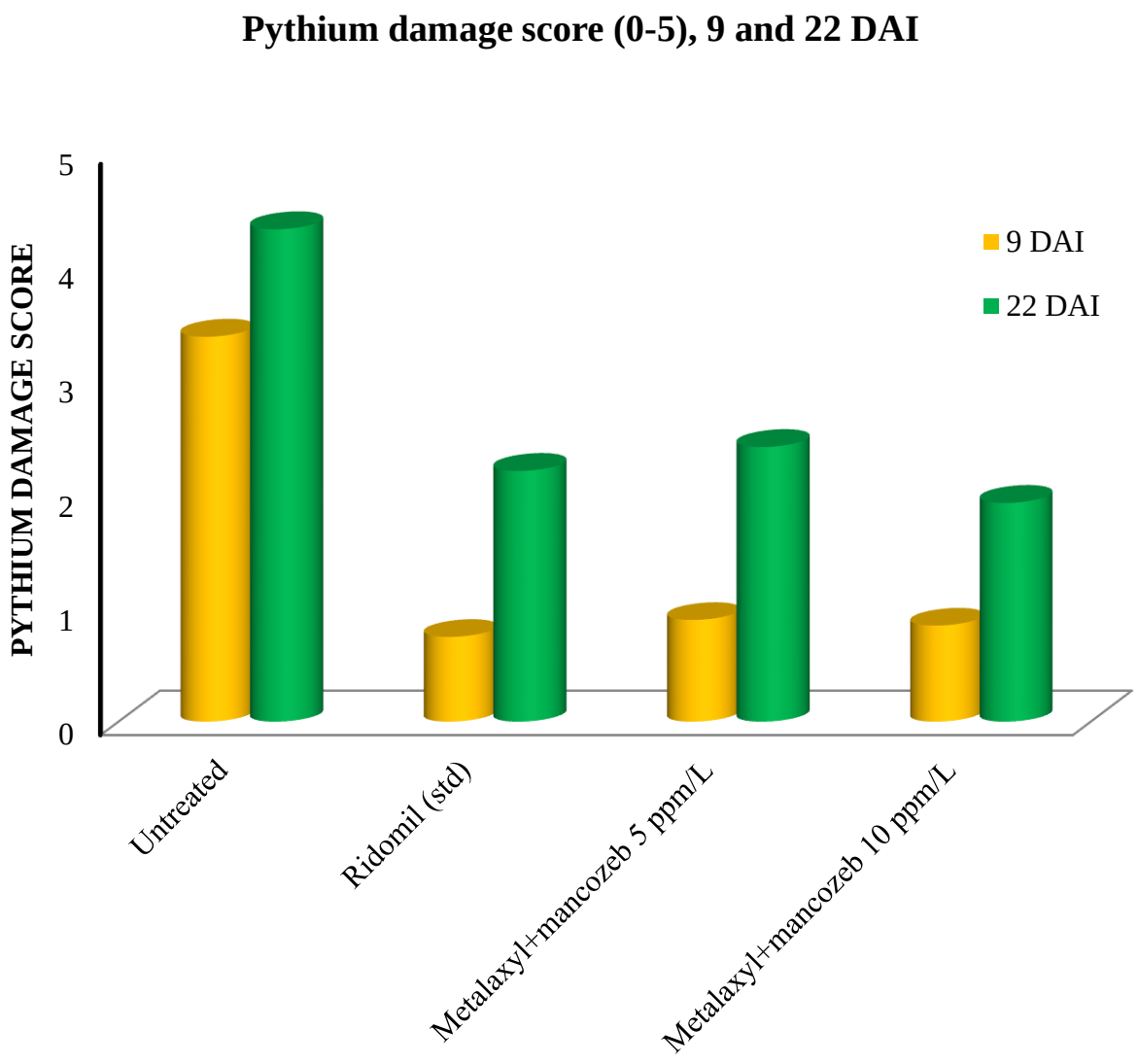

Figure 4.7 Comparisons of the results on 1st and the 2nd assessments

CHAPTER 5

5.0 Discussion

Pythium was a major plant pathogen in float tray seedling production of tobacco seedlings. Seebold (2011) stated that *Pythium* species require water for them to move, reproduce and complete their life cycles and this water is abundant in the float bed system. *Pythium* caused the root and crown rots of the seedlings in the floating tray systems. The *Pythium* root rot caused plants to have less functional root systems leading to the plants failing to take up water and mineral salts. Lower leaves of seedlings turned yellow due to the leaf chlorosis induced by the presence of the *Pythium* spp. Since the infected seedlings could not take up essential nutrients from the soil, older leaves transferred nitrogen to younger leaves and turned yellow. The plants lost their ability to absorb nutrients from the growing medium since their roots were damaged. These plants failed to respond to the addition of fertiliser, as a result, the growth of the plants was affected. Beckerman (2007) also found out that the first symptoms of *Pythium* infections included stunting; however, careful examination of root tips early in the infection showed only dead tips.

High temperatures in the greenhouse during the day resulted in the wilting of plants with damaged roots because they were unable to take up enough water to counter the water lost through transpiration. Tobacco seedlings in the untreated control were greatly affected by wilting as compared to those that were treated with fungicides because the roots of the untreated plants succumbed to severe *Pythium* root rot whilst those treated with fungicides were offered protection by the fungicides.

The water levels in untreated ponds decreased at a slower rate than others because most of the seedlings receiving treatment 1 had damaged roots and some of the seedlings were dead. The water in these ponds contained a lot of unabsorbed nutrients and these caused younger seedlings to succumb to salt injury when temperatures got high. High temperatures caused water from the pond to evaporate leaving salts on the surface of the pine bark thereby causing salt injury to younger seedlings.

Metalaxyl + mancozeb (Metalaxyl-Citchem) and Ridomil MZ 68% WG (metalaxyl) were effective in controlling *Pythium* root rot since there were no significant differences on the mean scores obtained after the *Pythium* root damage assessments. The fungicides produced almost similar results even though they had different active ingredients because they both had a common ingredient (metalaxyl). Metalaxyl is the standard fungicide used on the control of oomycetes.

A study carried out by Gholve, Tatikundalwar, Suryawanshi and Dey (2014) also confirms that metalaxyl is effective in the control of damping off caused by *Pythium ultimum*. In their study, metalaxyl was the most effective fungicide which inhibited the growth of *P. ultimum* with an overall mean of 84.22%. Metalaxyl was successful because of its mode of action. Metalaxyl is a systemic fungicide which disrupts nucleic acid synthesis of fungi especially oomycetes. The new fungicide however had metalaxyl a systemic ingredient plus mancozeb a contact ingredient. Mancozeb offered a protective layer around the roots of the seedlings inhibiting the germination of oospore. The combination of these two active ingredients provided an excellent *Pythium* control from within the plant and externally.

The 05 ppm/L of metalaxyl + mancozeb (Metalaxyl-Citchem) controlled *Pythium* root rot almost as good as 10 ppm/L of the same product and 05 ppm/L of Ridomil MZ 68% WG. This implies that it is more economical to use 05 ppm/L of metalaxyl + mancozeb than 10 ppm/L since they showed no significant differences in the control of *Pythium* root rot.

CHAPTER 6

6.0 Conclusions and Recommendations

6.1 Conclusion

Pythium control is a key process for successful float bed seedling production. Fungicides were effective in controlling *Pythium* in the tobacco float tray system. Following the right fungicidal application dates and the right amount of the fungicides controls *Pythium* root rot in the float beds before the pathogen causes severe seedling damage. Metalaxyl + mancozeb which is the new formulation from Citchem, controls *Pythium* in more or less the same way as Ridomil MZ 68% WG (metalaxyl) from Syngenta chemicals. Effective application rates of metalaxyl + mancozeb (Metalaxyl-Citchem) were also established in this experiment. The 05 ppm/L of can be used instead of 10 ppm/L of metalaxyl + mancozeb and still yield similar results.

6.2 Recommendations

Basing on the results obtained from this experiment, farmers are recommended to use the new fungicide formulation (metalaxyl + mancozeb) in rotation with Ridomil MZ 68% WG (metalaxyl) in the tobacco float beds. The use of Ridomil and the new fungicide for the control of *Pythium* root rot in rotation, will greatly aid in lowering the incidences of pathogen resistance to the one of the fungicides. Farmers are also recommended to apply the specified amounts of fungicides at the right time in order to protect their seedling from *Pythium* spp. as well as to hinder the pathogens from developing resistance.

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APPENDICES

Appendix 1: ANOVA results at 9 DAI

ANOVA

Root damage	Sum of Squares	df	Mean Square	F	Sig.
Between Treatments	19.427	3	6.476	72.794	.000
Within Treatments	1.068	12	.089		
Total	20.494	15			

Test of Homogeneity of Variances

rootdamage

Levene Statistic	df1	df2	Sig.
2.670	3	12	.095

Tests of Normality

	treatment	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
rootdamage	1	.243	4	.	.905	4	.457
	2	.364	4	.	.840	4	.195
	3	.307	4	.	.729	4	.024
	4	.329	4	.	.895	4	.406

Appendix 2: ANOVA results at 22 DAI

ANOVA

rootdamage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.111	3	4.704	16.386	.000
Within Groups	3.445	12	.287		
Total	17.555	15			

Levene Statistic	df1	df2	Sig.
2.868	3	12	.081

Tests of Normality

	treatment	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
rootdamage	1	.412	4	.	.725	4	.022
	2	.236	4	.	.941	4	.662
	3	.175	4	.	.985	4	.932
	4	.220	4	.	.948	4	.705

Appendix 3 ANOVA comparing the two fungicides

ANOVA

rootdamage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.675	1	14.675	12.024	0.72
Within Groups	36.613	30	1.220		
Total	51.287	31			