OCCURRENCE OF STREPTOMYCES ANTAGONISTIC TO PLANT PATHOGENS IN PATHOGEN-INFESTED SOILS OF MOROGORO, TANZANIA

BY

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (SOIL SCIENCE AND LAND MANAGEMENT) OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

This study was carried out to investigate the occurrence of *Streptomyces* antagonistic to plant pathogens in pathogen-infested soils. Soils were sampled from the Horticultural Unit of Sokoine University of Agriculture (SUA) and from Mlali in Morogoro district, Tanzania. Isolation of *Streptomyces* was done using starch-casein agar. Fungi were isolated using Potato Dextrose Agar (PDA) amended with Rose Bengal. Plant pathogenic fungi were identified using culture and microscopic characteristics. There were significant ($p=0.05$) differences in populations of *Streptomyces* in soils obtained from rhizosphere of different tomato varieties/lines with different disease severity levels. The populations were lowest in soils associated with tomato line 2 from Horticultural Unit and highest in soils of the other tomato varieties/lines. There were significant ($p=0.05$) differences in populations obtained from soils sampled under different disease severity. The populations of fungi showed significant ($p=0.05$) differences between the two locations, being lower at Mlali and higher at SUA Horticultural Unit. There were significant ($p=0.05$) differences in populations of fungi isolated under rhizospheres of tomato plants with varying levels of disease. Numbers of pathogenic fungi identified varied not only from one site to another but also one tomato variety/line to another, and also among different disease severity levels. About 73% of *Streptomyces* isolates produced antibiotic to various levels against plant pathogenic fungi which were identified as *Phytopthora sp.* 1 and 2, *Aspergillus sp.* 1 and 2, *Phoma sp.* 1 and 2, *Pythium sp.*, *Penicillium sp.* and *Botrytis sp.* Other isolates (27.0% of all isolates) did not produce antibiotic against any of the tested plant pathogenic fungi.

DECLARATION

I, DEUSDEDIT PETER, do declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been submitted for a higher degree award in any other university.

Signature: ____________

Date: 10 · 09 . 2002

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DEDICATION

This dissertation is dedicated to my late father P.N. Mlay who made me reach up to this education level. During his lifetime he declared that: "As long as I am living, my ambition is to make sure that all my children will be educated at least up to Masters level." I have tried to fulfil his lifetime ambition. May God rest his soul in peace. Amen.

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CHAPTER ONE

1.0 INTRODUCTION

Soils have long been recognised as a reservoir for micro-organisms capable of producing a variety of antimicrobial substances. Some of these compounds are of interest for their potential therapeutic value, and some have stimulated extensive studies for their possible ecological role in soil-plant systems. Some attention has been directed toward the modifying influences of antagonistic micro-organisms on phytopathogens (Johnson and Curl, 1972).

The pioneering work of Walksman in the 1940s' unequivocally established soil actinomycetes as prominent producers of antibiotics (Walksman, 1967). Actinomycetes, in which the genus *Streptomyces* belongs, are numerous and widely distributed not only in soils but in a variety of other habitats including composts, river muds, and lake sediments (Alexander, 1977; 1983). They are present on the soil surface and also in the lower horizons to a considerable depth. In general, fewer organisms are found with increasing depth but in some virgin soils number of bacteria and actinomycetes increase with increasing depth to the upper limits of the B horizon, then decrease with further depth (Johnson and Curl, 1972). Actinomycetes rank second to simple bacteria in abundance, and sometimes their viable counts are equal to that of simple bacteria.

Streptomyces are gram negative bacteria that grow as branching filaments. They form about 75% of all colonies in dilution plates during microbial count in the laboratory (Alexander, 1961b; 1977; 1983; Lachevalier and Lachevalier, 1967).

Actinomycetes, especially the streptomycetes, have been observed *in vitro* to produce antibiotics which are capable of inhibiting the growth and development of some fungi and bacteria (Alexander, 1961a). *Streptomyces,* despite being famous for production of antibiotics, few species are known to cause diseases in plants (Brock *et al.,* 1994).

Over the last decades, hundreds of antibiotic substances have been extracted from laboratory cultures of soil micro-organisms, including bacteria, but mostly from actinomycetes of the genus *Streptomyces* (Brock, *et al.,* 1994 as cited by Ndonde, 1998). Among the major antibiotic substances produced by *Streptomyces* are streptomycin, chloramphenicol, oxytetracycline, chlorotetracycline and cycloheximide. Despite the great industrial and therapeutic value of these chemicals, there is still no clear picture of the function of these antibiotics neither in the metabolism of the organism nor the significance of such compounds in natural processes (Johnson and Curl, 1972). Some are remarkably effective against plant pathogens *in vitro* and few have been proven to be of practical value in the field e.g. *Streptomyces griseus,* which controls potato scab in potatoes (Wheeler, 1969). Some species are effective against pathogenic bacteria such as *Erwinia amylovora* causing fire blight in wheat, while others are effective against fungal plant pathogens such as *Sclerotium rolfsii* and *Fusarium oxysporum*, which are pathogens of *Lens culinaris* (Mehrotra and Claudius, 1974).

In a recent study carried out in Tanzania by Mvungi (2000), most of the isolates of *Streptomyces,* about 83.3%, showed antibiosis against all test plant pathogens

(*Clavibacter michiganensis sub sp michiganensis, Acidovorax avenae, Xanthomonas vascatoria* and *X. phaseoli*). Capacity of streptomycetes to produce antibiotics is now of interest to many scientists in the world, including their potential use in controlling soil borne plant pathogens in crops such as vegetables (Johnson and Curl, 1972).

Most crops are affected by soil borne pathogens. However, vegetable crops, like cabbage, tomatoes, potatoes, and onions, are more susceptible due to frequent irrigation, which favours disease development. It is a common practice to spray crops with systemic or protectant fungicides in the field and/or to coat crop products like grain/seed material with a fungicide solution prior to storage in order to prevent fungal and/or bacterial diseases (Lefert *et al.*, 1993). However, many fungicides or their residues have recently been proven to have adverse effects on human health, and some persist for long periods in the environment (Huffaker *et al.*, 1976; Lefert *et al.*, 1993). Continued use of such fungicides is now of growing concern. Therefore, alternatives to conventional fungicides need to be explored and microbial metabolites may be one of the alternatives.

Tomatoes are highly susceptible to attack by several soilborne pathogenic fungi (e.g. *Phytopthora infestans, Fusarium oxysporum var. lycoperisicon*) and bacteria (e.g. *Xanthomonas campestris, Clavibacter michiganensis*). Tomato growers are forced to use heavy doses of fungicides to effect control or eradication of these pathogens (El-Raheem *et al.*, 1995). Since *Streptomyces* have been shown to have antibiotic activity against several fungi and bacteria (El-Banna and Winklemann, 1998; El-Raheem *et al.*, 1995) research is needed to investigate the use of these antibiotics to control of tomato diseases caused by the above listed micro-organisms. This would eliminate health risks and the environmental pollution posed by the conventional fungicides. The general objective of this study was to explore *Streptomyces,* from pathogen-infested soils, which have ability to inhibit plant pathogens.

The specific objectives of the study were:

(i) to establish the abundance of pathogens in soils of the study area,

(ii) to establish the abundance of *Streptomyces*,

(iii) to determine the proportion of *Streptomyces* which are able to inhibit identified pathogens,

(iv) to explore the relationship between abundance of pathogens and abundance of *Streptomyces* capable of inhibiting plant pathogens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ecology of *Streptomyces*

The genus *Streptomyces* represents 90% of all soil actinomycetes (Xu *et al.*, 1996). The free-living forms are saprophytes, but a few species can cause diseases to plants, domestic animals, and even humans (Alexander, 1983). Plating methods have been used for ecological investigations and have been found to be suitable for quantitative estimation of *Streptomyces* (Xu *et al.*, 1996). It has been reported that the counts do not seem to be greatly affected by the composition of the medium, an indication that the organism can utilise a variety of organic materials (Krasiln'kov, 1958). *Streptomyces* populations are high in grassland and pasture soils than in cultivated land and the abundance in cultivated fields exceeds that in adjacent virgin sites (Vionis *et al.*, 1998). In addition, the cool climate, dry and poor soils lead to low numbers of actinomyces but lead to high percentage of *Streptomyces* (Brian, 1960). The limit of occurrence of thermophilic and psychrophilic actinomycetes are reported to be about 3500 metres above sea level (Xu *et al.*, 1996).

2.2 Ecological factors influencing population of *Streptomyces*

The primary ecological factors influencing populations of *Streptomyces* are the organic matter, status of the soil, pH, moisture, and temperature (Krasilni'kov, 1958).

2.2.1 Organic matter

Streptomycetes are affected by the presence of carbon and their numbers are especially high in soils rich in organic matter. Sites high in carbonaceous materials and humus have high numbers of *Streptomyces* than habitats poor in organic matter (Hadegon, 1997). Amendments with organic materials such as protein derivatives, crop residues, and animal manure increase the abundance of streptomycetes (Baker and Cook, 1974). The high growth rates and biochemical versatility of certain bacteria and fungi make them the initial agents of destruction whereas the streptomycetes only appear when the more readily available compounds have been metabolised and competitive stress has diminished (Mitchell, 1974).

2.2.2 pH

It has generally been accepted for many years by scientists that most soil actinomycetes cease to grow at about pH 5.0 and therefore comprise a very small component of the microbial populations of acid soils (Walksman, 1922). *Streptomyces* are not tolerant to low pH and the population size is inversely related to the hydrogen ion concentration (Krasilni'kov, 1958). According to Krasilnikov (1958), most strains of *Streptomyces* and related forms fail to proliferate, or have negligible activity below pH 5.0, and the actinomycetes in highly acid environments frequently make up less than 1% of total viable numbers (Mitchel, 1974). However, the early work of Jensen (1930) demonstrated that some actinomycetes, termed *Actinomyces* (*Streptomyces*) *acidophilus*, required acidity for their growth. Furthermore, several acidophilic *Streptomyces* stains were isolated by Williams and Davies (1964), who suggested that acidophilic actinomycetes may be widespread in acid soils. In another study by Khan and Williams (1975) revealed that out of 174 acidophilic strains of actinomycetes tested most strains (143) were of the genus *Streptomyces*, one *Norcadia* and the rest were unidentified. It seems that acidophilic actinomycetes have an important role in such environments which have been presumed to be unimportant. They contribute to decomposition of acid litter above the soil and organic matter below ground. They may also play an important part in antagonistic interactions in acid soils. *In vitro* tests of antibiotic production by acidophiles have shown antifungal activity as a common phenomenon (Khan and Williams, 1975). It is likely that fungi are their major competitors in acidic environments.

Some acid tolerant strains have also been demonstrated, but their scarcity suggests their minor biochemical significance. Lowering the pH of a medium selective for streptomycetes influences the numbers of the streptomycetes that are to be isolated (Hadegon, 1976). The limiting pH for *Streptomyces* is in the vicinity of pH 5.0 has found practical application in control of certain plant diseases caused by *Streptomyces* e.g. potato scab and soil rot of sweet potato, which are caused by *Streptomyces scabies and S. ipomoea,* respectively (Baker and Cook 1974). Therefore, the acidification of soil may be is one of the strategy to suppress pplant diseases (Snyder *et al.*, 1976).

2.2.3 Moisture

Moisture is a critical environmental determinant for the survival of *Streptomyces*. At 85 to 100% of the water holding capacity, *Streptomyces* are rarely observed. This is the consequence of reduced aerobic metabolism of all common soil actinomycetes and their inability to develop and spread when free oxygen is lacking under such high moisture conditions (Alexander, 1977; 1983). Streptomycetes are not greatly influenced by semi-dry conditions as are the simple bacteria, but the filamentous groups tend to be favoured by low moisture levels at vegetative development (Xu *et al.*, 1996). The numbers of actinomycetes remain high as soil dries out while the relative incidence of bacteria diminishes because of their lack of tolerance to arid conditions.

2.2.4 Temperature

There is little growth of mesophilic streptomycetes at 5°C and essentially none at 39°C. Increasing temperature from 5 to 27°C leads to increased development of Streptomycetes, and the optimum range is generally from 28 to 37°C (Krasilni'kov, 1958).

2.3 Activity and functions of *Streptomyces* in the soil

As much as 75% of the actinomycetes isolated from soils may produce antimicrobial agents known as antibiotics (Tuite, 1969; Alexander, 1977; 1983). The antibiotic substances produced in culture by streptomycetes have been reported to inhibit growth or cause the elimination of populations of bacteria, yeast and fungi of different taxonomic categories (Snyder *et al.,* 1976). However, the percentage of actinomycetes producing antibiotics varies with soil type and season of the year. Some test organisms are sensitive to compounds produced by several species of actinomycetes but some are inhibited by metabolites excreted by only a few species (Vionis *et al.,* 1998). In addition to production of antimicrobial metabolites, many species of *Streptomyces* produce extracellular enzymes which lyse bacteria. Production of such enzymes may be important in the microbiological equilibrium in soils (Krasilni'kov, 1958).

Among the activities of the streptomycetes in the soil is the microbial antagonism effect, which helps in regulating the composition of the soil microbial community (Gray, and Williams 1971; Song *et al.*, 1997). This role in the ecosystem is attributed by the ability of streptomycetes to excrete antibiotics and enzymes which are responsible for lysis of fungi and bacteria. In this relationship, it has been observed that amendment of soils with substances, such as chitin, that favours hyphal development of streptomycetes, sometimes leads to a marked suppression of fungi that cause diseases of higher plants (Alexander, 1977; 1983; Tate, 1995). The formation of antibiotics by streptomycetes is widespread, with approximately 50-70% of all isolated streptomycetes being able to produce antibiotics of one kind or another.

2.4 In-vitro production of antibiotis by *Streptomyces*

As it was stipulated earlier in the previous sections that 75% of the streptomycetes produce antimicrobial agents known as antibiotics (Alexander, 1977, 1983). In Brazil, for example, *Streptomyces* isolated from soybean rhizosphere soils were capable of producing streptomycin (Huddlestone *et al.*, 1997). In Egypt, inoculation of tomato seedlings with *Streptomyces corchorusii* and *Streptomyces mutabilis* induced production of tomatine, which reduced growth and sporulation of *Fusarium oxysporum* (El-Raheem, 1995) and the reduction was proportional to the concentration of tomatine; the largest amount had the higher effect. The antimicrobial activity of a *Streptomyces prunicolar* culture filtrate was reported by Fatar (1996) to be effective against different test organisms (*Aspergillus niger, A. parasiticus, Fusarium oxysporum, Candida albicans, Sacchoromyces cerevisiae*). Saadoun and Al-Momani (1997) reported 73% of streptomycetes isolated from Jordan soils to have antibiotic activity against plant pathogenic bacteria, and 25% of these were also effective against *Agrobacterium tumefaciens*.

In Tanzania, Ndonde (1998) isolated *Streptomyces,* which inhibited plant pathogens, namely *Acidovorax avenae, CMM IPO 543, Xanthomonas oryzae pv. Oryzae, X. vascatoria, X. campestris, and X. phaseoli var. fuscoris,* of which more than 90% of the streptomycetes isolates were capable of producing antibiotics. These antibiotics are thought to be responsible for inhibiting the growth of the above mentioned plant pathogens.

Some streptomycetes have been reported to have lower antimicrobial activity *in vivo* than *in vitro,* e.g. *Streptomyces antibioticus*. The low *in vivo* activity was believed to be due to presence of microorganisms which compete with antibiotic producing *Streptomyces,* e.g. presence of *Pseudomonas* species (El-Banna *et al.,* 1998).

2.5 Biocontrol of plant diseases

Debach (1972) defined biocontrol as the regulation by natural enemies of another organism's population density at a lower average than otherwise would be. Control of plant diseases using biological agents has been studied extensively by many workers. Studies conducted by Papavizas and Lumsden (1980) revealed that there was three to four-fold increase in streptomycetes population antagonistic to *Rhizoctonia solani* in soils amended with corn or oat tissue, compared to non-amended soils. These amendments also reduced *Rhizoctonia* root rot of beans and increased the populations of antagonistic *Streptomyces spp.* and of *Bacillus subtilis* which were 50% higher in amended than in non-amended soils. The increase in antagonist populations was correlated with a reduction in disease severity. Decomposing organic matter also stimulated natural antagonists of pathogenic actinomycetes and fungi (Baker and Cook, 1974; Papavizas and Lewis, 1981). In field trials, Dicklow *et al.* (1993) found in field trials that some strains of *Streptomyces* caused significant reduction of tomato root galling induced by the nematode *Meloidogyne incognita,* contributing to a significant increase in yields of tomatoes as compared to the control.

Some physical treatment can also upset the soil ecosystem to an extent that allows proliferation of microflora antagonistic to a particular pathogen (Sewel, 1965). For example, an air stream at 60-70°C passed over a soil for 30 minutes was used to kill or weaken soilborne plant pathogens and leaving a residual saprophytic flora with high thermal tolerance. Populations of these saprophytes, which may be antagonistic to certain pathogens, rapidly increased due to lack of competition.

Absence of a disease, therefore, may mean that the pathogen is absent or that antagonists are inhibiting the growth or there is no infection of plants by the pathogen (Baker and Sciaron, 1952; Mai, 1978). Biological control may, thus, contribute to establishing a disease inhibiting balance in nature.

2.6 Antibiotic production by *Streptomyces* in soils as a factor in biocontrol

Streptomyces that produce antibiotics capable of inhibiting plant pathogens *in vitro* have been isolated from many soils. Much of the early work dealt with antibiotics of actinomycetes culture filtrates on petri plates was that reported by Snyder *et al.* (1976). Such antibiotics were demonstrated against *Streptomyces scabies, Fusarium oxysporum, Pythium arrhenomanes* and other pathogens (Xu *et al.*, 1996).

Factors which limit production of antibiotics is the shortage of carbon sources; fast degradation of the antibiotics after production and high affinity of adsorption by the soil colloidal couples as it has been reported by Siminoff and Gottlieb (1951) of streptomycin.

In attempting to introduce antagonists into nontreated soil, microorganisms isolated from soil have usually been screened for antagonistic properties, especially antibiotic production, and selected isolates have been grown in mass culture. When they were reintroduced into the same ecosystem from which they came, they declined in numbers because the prevailing biotic and abiotic environment did not support the natural increase of the introduced antagonist populations to high levels (Baker and Cook, 1974). Moreover, nutrients carried over with the organisms from culture medium (medium itself should not be added to the soil), and those released upon the death of the cells, may result in changes in the resident flora that may even hasten the return of the introduced species to its original place (in terms of abundance) in the community.

2.7 Manipulation of antagonists in biocontrol

Biological control rarely eliminates a pathogen from the site, but rather reduces its numbers or its ability to induce and produce disease. Such control may, at times, be achieved with little or no reduction in the population of the pathogen, or perhaps without preventing infection (Huffaker, 1972).

According to Baker and Cook (1974), when suitable antagonists are already present in the soil but do not provide satisfactory level of disease control, it is desirable to intensify their activity, at least during the period when such control is needed and this can be through:

1. Crop rotation. This lowers inoculum density of the pathogen, but in some cases it may have a long term effect of prolonging disease loss by delaying return to a natural balance, as in the take-all disease of wheat.
2. Adding amendments that selectively stimulate the antagonists.
3. Altering pH of the soil to one favourable to antagonists, unfavourable to pathogen, by adding sulphur, or lime or by selection of proper fertiliser.
4. Selecting a method of culture that favours the antagonists at the soil depth where infection by the pathogen occurs. For example, *Sclerotium rolfsii* and some strains of *Rhizoctonia solani* infect very near the soil surface, while *Phymatotrichum omnivorum, Pythium* and *Phytopthora* are active at depths of 1-3m.

2.8 Suppressive soils and their effect in biological control

Studies of suppressive soils led to a basic concept of soil "receptivity" to diseases (Alabourette, 1986). Suppressive soils are those soils where natural biological control occurs (Baker and Cook, 1974). The soil is not a neutral milieu where the pathogen interacts freely with the roots of the host-plant: on contrary, the soil interferes with relationships between micro-organisms, pathogens and plants. In fact, every natural soil process, and soil receptivity, is considered a continuum from strongly suppressive to highly conducive (Linderman *et al.*, 1983). This ability to limit disease is thought to be due to antagonistic micro-organisms present in those soils. Since suppressiveness can be transferred by mixing a small amount of suppressive soil with a previously disinfected soil or with peat, the possibility of using natural suppressiveness in biological control is possible, although it does not seem practical to base biological control on the transfer of suppressiveness from suppressive soil to conducive soil (Vionis *et al.*, 1998).

Physical and chemical soil properties play a major role in determining the microbiological nature of the suppressive soils, but from the present state of knowledge does not allow an experimental evaluation of these abiotic properties. There are two main abiotic factors suspected to be involved in suppression and these are a high pH and high clay content (Linderman, 1983). The higher the magnitudes of these factors the higher the micro-organism populations (Ndonde, 1998; Linderman *et al.*, 1983).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted on soils sampled in the Horticultural Unit of the Sokoine University of the Agriculture (SUA) and in Vitonga village, 5 km South-west of Mlali ward. Both sites are located in Morogoro district and are known to be infested by plant pathogens *Fusarium oxysporum, Phytopthora infestans, Pseudomonas solanacerum and Clavibacter michiganensis* (Swai, 1995). These areas have been under tomato cultivation for a long time and among others farmers have been facing disease problems caused by these pathogens.

3.2 Soil sampling

Soils were sampled from the two locations, Horticultural Unit (SUA) and Mlali. At each location, two tomato varieties/lines were chosen for the studies. Soils in the root zones were sampled from three varying levels of the disease severity (i.e. healthy, slightly and heavily infected plants), in each test variety. This sampling was replicated four times. The depth of sampling was 0 - 10cm as recommended by Johnson and Curl (1972). The samples were packed in polyethene bags, and taken to the laboratory. They were stored in a fridge at -4 °C, for up to one week, to arrest further microbial activities, before use.

Composite soil samples for routine analysis were sampled from each study area in a zigzag pattern, and a small sample of 1kg was obtained through quartering. These samples were packed, labelled, transported to the laboratory where they were dried and sieved to pass through a 2mm sieve.

3.3 Soil analysis

3.3.1 pH

Ten g soil sample was weighed and transferred to a 100ml broad-necked plastic bottle followed by addition of 25mls of water by a dispenser (Moberg, 2000). Bottles were then cappped and shaken for 30 minutes using a mechanical shaker. After settlement of the large particles, pH was measured using the glass electrode.

3.3.2 Organic carbon

The organic carbon content of the soil was determined using the method described by Walkley and Black (Nelson and Sommers, 1982). One g of finely ground soil (sieved to pass through 0.5mm sieve) was weighed in a 500ml conical flask. 10mls of the potassium dichromate solution were added into the soil, followed by 20mls of 98% H_2SO_4 to oxidize the organic carbon, then 20mls of 85% H_3PO_4 were added. The flask was carefully swirled and allowed to stand for 30 minutes. After that 200mls of distilled water were added and the flask was left to cool. Two mls of diphenylamine indicator were added and the excess dichromate titrated with 0.5M ferrous ammonium sulphate solution to the end point. The colour changed from brown to purple, then to blue, and finally to green, indicating the end point. Beside the soil samples, two blanks were also titrated. The amount of dichromate reduced was used to calculate the organic content in the soil, which was multiplied by 1.33 as recovery factor for unoxidized carbon (Nelson and Sommers, 1982).

3.3.3 Total Nitrogen

The total nitrogen content of the soils was determined by the micro-Kjeldahl method (National Soil Service, 1990). One g of soil was digested using conc. H_2SO_4 in a Kjeldahl flask, and distilled into boric acid in the presence of 40% NaOH. Distillation was continued until about 200ml distillate were collected. A blank, containing no soil but only the various chemicals, was similarly treated. Soil samples and blanks were titrated with 0.5M sulphuric acid to the end point (the colour changing from green to light red), and total N calculated from the titration reading (National Soil Service, 1990).

3.3.4 Extractable Phosphorous

The extractable P content of the soils was determined by the Bray and Kurtz 1 method (Page *et al.*, 1982; Murphy and Riley, 1982). Five g of soil were weighed into a 50 ml plastic bottle, and 35 ml of the Bray 1 extracting solution (dilute HCl-NH_4F, which was a mixture of 0.025M HCL and 0.03M NH_4F) was added into the bottle, shaken for one minute by hand, and immediately filtered through a Whatman No. 40 filter paper.

Colour development was obtained by pipetting 5 ml of the filtrate into a 50-ml volumetric flask and adding 15 ml of distilled water. Ten mls of 1.65% ascorbic acid were added into the volumetric flask containing the extracted soil P, and the contents made to volume with distilled water and thoroughly mixed. The mixture was left to stand for 30 minutes for the blue colour to develop. Absorbance was measured in a 10mm cuvette at 882nm, with appropriate standards (containing only reagents) included.

3.3.5 Particle size distribution

The particle size distribution of the soil samples was determined using the Bouyoucos hydrometer method as described by National Soil Service (1990) and Day (1965). Fifty g air-dried soil were weighed into 250 ml polythene bottles and mixed with 50 ml of the dispersing agent (5% sodium hexametaphosphate solution). Distilled water was added to about 200ml, and the samples shaken overnight, with the bottles held in a horizontal position, on a reciprocating shaker at 150 resprocations per minute. The suspension was quantitatively transferred to a sedimentation cylinder and the volume of suspension made to one litre with distilled water. Hydrometer readings were taken after 40 seconds, 4 minutes and 4 hours and the relative size proportions calculated.

3.4 Microbiological studies

3.4.1 Enumeration of *Streptomyces*

Enumeration of *Streptomyces* was carried out in starch-casein agar using the plate count method (Kuster and Williams 1964; Shirling and Gottlieb, 1966; Johnson and Curl, 1972). K_2HPO_4 and KH_2PO_4 were added in the solutions used to simulate the pH of the two soils at pH 6.75 and 7.79 for the Horticultural Unit and Mlali, respectively, as follows: K_2HPO_4 was used to prepare a solution litres of pH 9 solution by weighing 17.418g/l, and KH_2PO_4 to prepare a solution of pH 4 by weighing 13.60g/l. These solutions were mixed in proportions which gave pH 6.75 or 7.79 which were used to prepare all the media used in this study. In this way, the pH levels of the soils were simulated. The medium was sterilized in an autoclave at 15 pounds per square inch and 121°C for 15 minutes (Johnson and Curl, 1972). The medium was further fortified prior to plating by aseptically adding penicilin G and actidione to suppress the growth of bacteria and fungi, respectively (Porter *et al.*, 1960; Davies and Williams, 1970;Johnson and Curl, 1972). Petri dishes and pipettes were sterilized in an oven at 170°C for 2 hours.

Ten g of soil (oven-dry equivalent) were weighed and transferred into bottles containing 90 mls of sterile water, and shaken at 95 revolutions per minute for 30 minutes using a mechanical shaker to detach microbial cells from soil particles. This made the dilution of 10^1. Then ten-fold dilutions were made to obtain the dilutions required for plating. One ml portion from two dilutions, i.e. 10^3 and 10^4, were plated and incubated at 25°C to 28°C for 14 days and *Streptomyces* colonies were counted from the dilution.

The number of microorganisms per gram of soil were calculated using the following formula:

$$\text{No./g soil} = \frac{\text{plate count}}{\text{Volume plate}} \times \frac{\text{Total vol. of suspension}}{\text{weight of soil}}$$

Colony counts were converted to *Streptomyces* populations per gram of oven-dry soil. These data were transformed to the logarithmic (base 10) scale. The transformed data was subjected to analysis of variance to evaluate the influences of location and pH changes on the *Streptomyces* populations. For the 48 samples, the *Streptomyces* populations were regressed on soil pH, organic carbon, total N, extractable P, and % clay to assess the influence of these parameters on the populations of *Streptomyce*.

3.4.2 Isolation of *Streptomyces*

Isolation of *Streptomyces* was done in a laminar flow chamber. This chamber was first sterilized for 12 hrs using U.V. radiation from an in-built ultraviolet lamp. Portions of *Streptomyces* colonies from starch-casein agar plates were picked using sterile inoculating loop and transferred to the plates with oatmeal agar (Johnson and Curl, 1972; Kuster and Williams 1964). The oatmeal agar was prepared at different two pH levels (i.e. pH 6.75 and 7.79), the same it was done for the starch-casein agar (section 3.5.1). The plates were incubated for 14 days for the colonies to grow. This step was repeated to obtain colonies free from contamination. These colonies or isolates were used for further studies as described in section 3.5.5.

3.4.3 Isolating fungi from the soil

Soil borne fungi were isolated from disease suppressive soils known to harbour pathogens using the method described by Weller and Thomashow, (1990) and Gilman (1966). The isolation of fungi from all the 48 soil samples was done using Potato Dextrose Agar (PDA) amended with 0.0035g/litre rose bengal and adjusted to simulate their respective pH levels as already described for the starch-casein agar.

Ten g of soil (oven-dry equivalent) from each sample were weighed and transferred into bottles containing 90 mls of sterile water, and shaken at 95 rev/min. for 30 minutes using electrical shaker to detach fungal propagules from the soil particles. This made the dilution of 10^1. Ten-fold dilutions were made to obtain the dilutions required for plating. One ml portions from two dilutions, i.e. 10^3 and 10^4 were plated in duplicate using the pour plate method and incubated at room temperature for one week. After this period fungal colonies were counted from plates showing even distribution of colonies at each dilution level. Individual colonies were further purified and then transferred into fresh PDA petri-dishes to establish pure colonies for microscopic identification.

3.4.4 Identification of soil borne fungal pathogens

Colonies in the PDA petri-dishes were left to sporulate. A small portion of a sporulating fungal mycelium was picked using a sterile needle and placed on a microscope slide. Then a drop of glycerin was added and a cover slip was placed on top. The mounted specimen was observed using a light microscope (Nikon 98455) at the magnification of ×40. Observed fungi were identified using keys from illustrated manuals and textbooks. With this method soil fungal pathogens *Phytopthora sp., Pythium sp., Phoma sp., Asperigillus,* and *Penicellium sp.* were identified. These were used for antibiosis testing (section 3.5.5). Parallel to this, diseased tissues were obtained from the infested plants and incubated in PDA. Tomato plants with damping-off symptoms were up-rooted and roots were washed. Tissues from roots with necrotic symptoms were also incubated in PDA. Colonies from all these tissues were transferred to fresh PDA in order to obtain pure cultures of each fungal pathogen, which were observed under the microscope and then compared with those isolated from the soil. *Phytopthora sp.* and *Pythium sp* were isolated from root tissue of the plants showing damping-off symptoms (Agrios, 1978).

3.4.5 Testing the *Streptomyces* isolates for the ability to inhibit growth of soil borne fungal plant pathogens

Using the isolating loop, the isolates of *Streptomyces* were transferred from oatmeal agar plates to starch casein-agar plates (Johnson and Curl, 1972). A straight line of *Streptomyces* inoculum was streaked across the starch casein agar medium in a petri dish. The inoculated plates were incubated for five days after which the test soil borne plant pathogens isolated from the soil and plant tissues (*Phytopthora sp. Pythium sp. Phoma sp. Penicellium sp Asperigillus sp.*) were streaked at right angles to the *Streptomyces* line as described by Alexander (1983), Brock *et al.* (1994) and Prescott and Dunn (1959). Plates were incubated for a further five days and observed, with final evaluation made on the tenth day. The inhibition of fungal growth was assessed by measuring the length of inhibition zone, in mm using a metre ruler.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Brief description of the properties of the soils

The properties of experimental soils are presented in Table 1. The pH of the soils ranged from 6.75 (neutral) to 7.79 (mildly alkaline) (Baize, 1993; ILACO, 1991; Landon, 1991). Soils at the Horticultural Unit (SUA) had lower pH value while those of Vitonga village (Mlali) had higher pH values. The organic carbon contents of the soils used in this study were 0.88 and 3.19% for Horticultural Unit and Mlali, respectively, which are rated as low and high, respectively. Total N of soils at the horticultural unit was 0.14%, which is considered to be low while that of Mlali was 0.06%, which is very low. Extractable P was 0.31 ppm for soils at Horticultural Unit and 0.263 ppm for soils at Mlali, which is generally low. Clay content was 14 and 43%, making the soils to have textures of silt loam and clay loam for Horticultural Unit and Mlali, respectively. Generally these soils should provide a fairly good environment for the growth of *Streptomyces*.

Table 1. Characteristics of the soil used

Site	pH (1:2.5) H_2O	Organic Carbon (%)	Nitrogen (%)	Extractable Phosphorus (ppm)	%Sand	%Silt	%Clay	Textural class
Horticultural Unit Composite sample (HUC)	6.75	0.88	0.14	0.31	77	8	14	Silt Loam
Mlali Composite sample (VTC)	7.79	3.19	0.06	0.26	37	20	43	Clay Loam

4.2 Populations of *Streptomyces* in soils of the study areas

4.2.1 Effect of the tomato varieties/lines on the populations of *Streptomyces* in soils of the study areas

Table 2 shows the populations of *Streptomyces* obtained from soils sampled in rhizospheres of different tomato varieties/lines. There was a significant (p=0.05) difference in the populations of *Streptomyces* under different tomato lines in soils of the Horticultural Unit but not in those of Mlali (Table 2).

The significant difference in the *Streptomyces* populations under different tomato lines may be an indication of the ability of the different tomato lines to support different populations of *Streptomyces*. From a study carried out by Keswani *et al.* (1977), it was observed that microorganism species in the rhizosphere soil were mainly affected by plant species, and even by varieties of a given crop. The varieties/lines which supported higher populations in the current study may have produced different types or amounts of exudates from their roots that were superior sources of carbon and nitrogen, these are known to be substrates for growth of the *Streptomyces*. Similar observations were made by Crawford (1988), McCarty and Broda (1984), that *Streptomyces* increased in the growth and multiplication, where there was a good source of carbon and nitrogen. This may have contributed to high numbers of *Streptomyces* in the tomato lines, which resulted in higher populations in the present study.

Table 2. Populations of *Streptomyces* in soils under different cultivation of tomato varieties/lines

Location	Tomato varieties/lines	*Streptomyces* population (Log_{10})
Horticultural unit, SUA	CLN 21160 BC1F1-180-10-25-8-12-0 (line 1)	4.89 ± 0.29a
	CLN 21160 BC1F1-180-39-4-8-15-0. (line 2)	4.65 ± 0.18b
Mlali	CAL-VF (variety 1)	4.90 ± 0.32a
	CAL-J (variety 2)	4.88 ± 0.27a

Means within a column followed by the same letter are not significantly different (P=0.05) according to Duncan's New Multiple Range Test.

4.2.2 Effect of *Streptomyces* populations on disease severity in soils of the study areas

When the data was analyzed separately for each site, there was no significant ($p=0.05$) differences between populations of *Streptomyces* obtained from soils sampled under different disease severity levels (Table 3). However, when the data for the two locations was subjected to a combined statistical analysis, the populations of *Streptomyces* under different disease severity levels were significant ($p=0.05$) different (Table 4). The soils associated with intermediate and severely diseased plants haboured significantly lower populations of *Streptomyces* than the soils associated with healthy plants. From diseased plants perhaps the pathogens were more competitive within the rhizosphere, therefore reducing populations of *Streptomyces*. However, such competition did not affect the general fungal population (section 4.4.2).

Table 3. *Streptomyces* populations in soils under plants with varying levels of disease severity in each location

Location	Disease severity levels	*Streptomyces* populations (Log_{10})
Horticultural unit, SUA	Healthy plants	4.90 ± 0.31a
	Intermediate diseased plants	4.70 ± 0.20a
	Severely diseased plants	4.71 ± 0.28a
Mlali	Healthy	5.06 ± 0.29a
	Intermediate diseased plants	4.81 ± 0.31a
	Severely diseased plants	4.80 ± 0.21a

Means within a column for each location followed by the same letter are not significantly different (p=0.05) according to Duncan's New Multiple Range Test.

Table4. *Streptomyces* populations in soils under plants with varying levels of disease severity at both locations combined

Disease severity levels	*Streptomyces* populations (Log_{10})
Healthy	4.98 ± 0.31a
Intermediate diseased	4.76 ± 0.25b
Severely diseased	4.88 ± 0.24b

Means within a column followed by the same letter are not significantly different (P=0.05) according to Duncan's New Multiple Range Test.

The low populations *Streptomyces* associated with severely or intermediately diseased plants might have been caused by the fact that pathogens which attacked the plants were more competitive for the substrates and other necessary physiological factors that are also crucial for the survival of the *Streptomyces*.

4.3 Populations of fungi in soils of the study areas

4.3.1 Effect of varieties/lines on populations of fungi in soils of the study areas

Table 5 shows the fungal populations obtained under different varieties/lines from both study sites. There were no statistical differences observed in fungal populations on tomato varieties /lines grown at Horticultural Unit or Mlali sites.

Table 5. Populations of fungi in soils under different tomato varieties/lines

Location	Tomato varieties/lines	Fungi populations (Log_{10})
Horticultural unit, SUA	1	5.09 ± 0.18a
	2	5.05 ± 0.23a
Mlali	1	4.89 ± 0.20a
	2	4.88 ± 0.31a

Means within a column followed by the same letter are not significantly (p=0.05) different according to Duncan's New Multiple Range Test.

The lack of significant differences in the fungal populations from different tomato lines/varieties imply that the different varieties/lines did not significantly affect the environmental conditions which influenced fungal growth.

4.3.2 Effect of fungi population on disease severity levels

The populations of fungi in soils of the study areas were not significantly (p=0.05) different for the different disease severity levels (Tables 6). The same results were also obtained when the analysis of the two sites was combined.

Table 6. Population of fungi in soils under varying levels of plant disease severity each site

Location	Disease severity levels	Fungi populations (Log_{10})
Horticultural unit, SUA	Healthy	5.08 ± 0.19a
	Intermediate diseased	4.98 ± 0.26a
	Severely diseased	5.14 ± 0.15a
Mlali	Healthy	4.79 ± 0.24a
	Intermediate diseased	4.82 ± 0.31a
	Severely diseased	4.86 ± 0.25a

Means within a column followed by the same letter are not significantly different (P=0.05) according to Duncan's New Multiple Range Test.

The lack of significant difference observed (Table 6) may be caused by the soils used in this study were sampled from the rhizosphere of the tomato plants, that contained similar populations of microorganisms. The rhizospheres of the same crop plant have been reported to have similar influences on populations of micro-organisms including fungi. Alabouvette (1986) and Lazarovits (1997) reported that regardless of the disease level of the crop plant, the populations of certain microorganisms found under the rhizosphere of those crop plants were the same.

4.4 Occurrence of pathogenic fungi in soils of the study areas

4.4.1 General

Table 7 shows the occurrence of pathogenic fungi in the soils of the study areas. Table 8 shows the characteristics of the pathogenic fungi that were isolated and identified.

This study shows generally, that the soils were harbouring at least one or more of the identified plant pathogens. It is clearly shown that the soil samples associated with severely diseased plants contained more plant pathogens than the soil associated with healthy plants. Also the frequencies of pathogen occurrence obtained under different locations or varieties/lines were different.

Factors affecting the frequency of occurrence of the plant pathogens in the soil may be is the location and soil pH differences. Type of organic matter and methods of incorporation in the two areas may also influenced the differences in the frequencies of pathogens occurrence. Type of organic matter and methods of incorporation in a given soil are said to influences the quantity of organic matter in the soil, which on the other hand do influence the populations of micro-organisms found in the soil (Patterson, 1990). At Horticultural Unit compost is always incorporated in the soil while plant remains are used as organic fertilizer at Mlali, leading to higher organic matter in the former location than in the latter.

Varieties/lines can be the other factor which caused differences in the occurrence of these pathogens. Differences in susceptibility of varieties/lines to attack by the plant pathogens can also be a factor that influenced the populations of pathogens around the plants. This may be because the composition and quality of root exudates of the variety/line may influence occurrence and proliferation of the plant pathogens differently. The detailed discussions on all these factors are narrated under the following sections.

Table 7. Summary of the occurrence of plant pathogens in the study areas

Location	*Streptomyces* isolates (disease level)	*Phytopthora*	*Phytopthora*	*Pythium*	*Asperigillus*	*Aspergillus*	*Penicillium*	*Phoma*	*Phoma*	*Botrytis*
		*sp.*1	*sp.*2	*sp.*	*sp.*1	*sp.*2	*sp.*	*sp.*1	*sp.*2	*sp.*
Horticulture	$HV_8(H_1)$	+	-	-	-	-	-	+	+	-
Horticulture	$HV_8(I_1)$	-	-	-	+	-	-	-	-	-
Horticulture	$HV_8(D_1)$	+	-	+	+	-	+	+	+	-
Horticulture	$HV_{10}(H_1)$	+	+	-	-	-	-	-	-	+
Horticulture	$HV_{10}(I_1)$	-	-	-	-	+	-	-	-	+
Horticulture	$HV_{10}(D_1)$	-	-	-	+	-	-	-	-	+
Horticulture	$HV_8(H_2)$	+	+	-	-	-	-	-	-	+
Horticulture	$HV_8(I_2)$	-	-	-	-	+	-	-	-	-
Horticulture	$HV_8(D_2)$	+	+	-	-	-	+	+	+	-
Horticulture	$HV_{10}(I_2)$	-	-	+	-	-	-	-	-	-
Horticulture	$HV_{10}(I_2)$	+	+	-	-	+	-	-	-	+
Horticulture	$HV_{10}(D_2)$	+	+	-	-	+	+	-	-	+
Horticulture	$HV_8(H_3)$	-	-	+	-	-	-	-	-	+
Horticulture	$HV_8(I_3)$	+	+	-	-	+	+	+	-	+
Horticulture	$HV_8(D_3)$	-	-	+	-	+	-	+	-	+
Horticulture	$HV_{10}(H_3)$	+	+	-	-	-	+	-	+	+
Horticulture	$HV_{10}(I_3)$	-	-	-	+	+	-	-	+	-
Horticulture	$HV_{10}(D_3)$	+	-	--	-	+	-	+	+	+
Horticulture	$HV_8(H_4)$	+	-	-	-	-	-	+	-	-
Horticulture	$HV_8(I_4)$	+	-	+	+	-	+	+	-	-
Horticulture	$HV_8(D_4)$	-	+	+	-	-	-	-	-	+
Horticulture	$HV_{10}(H_4)$	+	-	+	-	-	+	-	+	-
Horticulture	$HV_{10}(I_4)$	+	-	-	-	+	+	+	-	-
Horticulture	$HV_{10}(D_4)$	-	+	-	+	-	+	+	+	-
Mlali	$VCVF(H_1)$	-	-	-	-	+	-	-	-	+
Mlali	$VCVF(I_1)$	-	-	+	-	+	-	-	-	-

Table 7 Continued

Location	*Streptomyces* isolates (disease level)	*Phytopthora* *sp.*1	*Phytopthora* *sp.*2	*Pythium* *sp.*	*Asperigillus* *sp.*1	*Asperigillus* *sp.*2	*Penicillium* *sp.*	*Phoma* *sp.*1	*Phoma* *sp.*2	*Botrytis* *sp.*
Mlali	VCVF(D_1)	-	-	-	-	-	-	+	-	-
Mlali	VC(H_1)	+	-	-	-	-	+	+	+	-
Mlali	VC(I_1)	-	+	-	+	-	-	-	+	-
Mlali	VC(D_1)	+		+	-	+	-	-	-	+
Mlali	VCVF(H_2)	-	+	-	-	+	-	+	-	+
Mlali	VCVF(I_2)	-	-	-	-	+	+	+	-	+
Mlali	VCVF(D_2)	+	-	-	+	-	+	-	+	+
Mlali	VC(H_2)	-	+	-	-	+	+	-	-	+
Mlali	VC(I_2)	-	-	+	-	-	+	+	+	-
Mlali	VC(D_2)	+	+	-	-	-	-	+	-	-
Mlali	VCVF(H_3)	-	+	-	+	+	-	+	-	+
Mlali	VCVF(I_3)	-	-	-	-	+	-	-	-	-
Mlali	VCVF(D_3)	+	-	-	-	-	-	-	-	-
Mlali	VC(H_3)	-	-	+	-	+	+	-	+	-
Mlali	VC(I_3)	-	+	-	-	-	-	-	+	+
Mlali	VC(D_3)	+	+	-	-	-	-	+	+	-
Mlali	VCVF(H4)	-	-	+	+	+	-	-	-	-
Mlali	VCVF(I_4)	-	+	-	-	-	-	-	-	+
Mlali	VCVF(D_4)	+	-	-	-	-	+	-	+	+
Mlali	VC(H_4)	+	-	-	-	+	-	-	-	+
Mlali	VC(I_4)	+	-	+	-	-	-	-	-	+
Mlali	VC(D_4)	+	-	-	+	+	-	+	-	+

+ = presence of pathogenic fungi

- = absence of pathogenic fungi

Table 8. Characteristics of the plant pathogens observed

Plant pathogen	Septation	Mycellium	Sporangia shape	Colony colour	Disease caused
*Phytopthora sp.*1	Septate	Branched	Lemon shaped	Dark brown	Late blight
*Phytopthora sp.*2	Septate	Branched	Lemon shaped	White	Late blight
Pythium sp.	Septate	Branched	Spherical	Yellowish green	Damping-off and root/stem rots
Penicellium sp.	Septate	Unbranched	Lemon shaped	Dark green	Blue mold rot
*Phoma sp.*1	Septate	Unbranched	Lens shaped	Dark brown	*Phoma* rot, seedling blight and black leg of crucifers
*Phoma sp.*2	Septate	Branched	Elliptical	Brown	*Phoma* rot, seedling blight and black leg of crucifers
*Asperigillus sp.*1	Septate	Unbranched	Hemispherical	Dark green	Black mold and rots of stored seeds
*Asperigillus sp.*2	Septate	Unbranched	Hemispherical	Yellowish	Black mold and rots of stored seeds
Botrytis sp.	Septate	Branched	Elliptical	Dark green	Grey mold rot, blights and ghost spot

4.4.2 Effect of location on the occurrence of plant pathogenic fungi in the study areas

The occurrence of plant pathogens in the two location is presented in Table 9. The trend shows that Horticultural Unit had higher frequency of occurrence of pathogens (a total of 88 isolates) than Mlali (77). Also in terms of individual pathogens, *Phytopthora sp* 1 was the highest (14) at Horticultural Unit, while the highest at Mlali was *Penicillium sp.* (13) and *Phoma sp* 2 was the lowest at both locations (i.e. 6 and 5 for Horticultural Unit and Mlali, respectively). The differences in the occurrence of pathogens may have been caused by the differences in soil management that exist between the two locations. At the Horticultural Unit irrigation was used to maintain a high moisture status of the soil while at Mlali they did not practise irrigation, which caused the soil to become dry at sometimes. This practice might have contributed to the overall higher populations of pathogens in the Horticultural Unit soils, since moisture has a direct influence in the abundance of pathogens. In the case of fertilizer use, farmers at Mlali plough under crop plant remains while at Horticultural Unit compost is used as organic fertilizer. Compost is easily decomposed and the nutrients so released easily utilized by plants, resulting in fast growth and maturity of the plant which, in turn, may influence multiplication and survival of plant pathogens in the Horticultural Unit soils. The farmers at Mlali practise rainfed agriculture, which is seasonal. At the Horticultural Unit, planting is done at any time as irrigation facilities are available. Continual presence of a crop in the Horticultural Unit soils might have influenced the abundance of pathogens by continued presence of susceptible hosts for pathogen multiplication and survival.

Another factor could be the difference in the soil pH from the two locations. Mangenot and Diem (1979) reported that some disease-causing fungi were favoured by neutral or alkaline environments (e.g. some *Phytopthora spp.*) while others preferred more acid environments (e.g. some *Fusarium spp.*). This can also be observed in this study where *Phytopthora sp.* 1 had the highest frequency in the Horticultural Unit where pH was 6.75 (neutral). However, the frequency of the occurrence of *Phytopthora sp.* 2 in the Horticultural Unit was lower compared to that at Mlali where pH was 7.79 (slightly alkaline).

Tale 9. Summary of occurrence of plant pathogens by locations from which they were obtained

Location	Plant pathogen	Number of frequency of pathogen identified	% out of all pathogen
Horticultural Unit, SUA	*Phytophtora sp.*1	14	16.9
	*Phytophtora sp.*2	9	10.8
	Pythium sp.	8	9.6
	Penicillium sp.	12	14.5
	*Phoma sp.*1	7	8.4
	*Phoma sp.*2	6	7.2
	*Asperigillus sp.*1	9	10.8
	*Asperigillus sp.*2	8	9.6
	Botrytis sp.	10	12.0
	Total	83	99.8
Mlali	*Phytopthora sp.*1	9	11.7
	*Phytopthora sp.*2	8	10.4
	Pythium sp.	8	10.4
	Penicillium sp.	13	16.9
	*Phoma sp.*1	6	7.8
	*Phoma sp.*2	5	6.5
	*Asperigillus sp.*1	11	14.3
	*Asperigillus sp.*2	8	10.4
	Botrytis sp.	9	11.7
	Total	77	100.1

4.4.3 Effect of varieties/lines on the frequency of occurrence of plant pathogenic fungi

Table 10 shows the frequency of occurrence of the plant pathogenic fungi by tomato varieties/lines from which they were isolated. Tomato line1 from Horticultural Unit was associated with more plant pathogens (44 isolates) followed by the variety 2 from Mlali (43) while variety 1 (Mlali) had the least number of pathogens (34). *Phytopthora sp.* 1 was the most dominant pathogen in line 1 from Horticultural Unit (18.2%), while *Asperigillus sp.* 1 and *Penicillium sp.* were the dominant pathogens for Mlali (20.6% each). *Penicillium sp.* was among the dominant pathogens in three out of the four varieties/lines studied, accounting for 18.9%, 20.6% and 14% for the line 2, variety 1 and variety 2, respectively.

The higher frequencies pathogens observed in line 1 (Horticultural Unit) and variety 1 (Mlali) might have been caused by the pathosystem which existed around the rhizosphere of the variety/line. Garret (1975) observed, at least in the case of *Lycopersicon*, that the composition and quality of root exudates, and the host genotype, played a major role in determining the varieties with high populations of plant pathogens. If the C/N ratio, for example, of the host plant debris and exudates were of high quality to a particular pathogen, then this would lead to the multiplication and ultimately increase in numbers of that pathogen. So in this study the plant pathogens associated with variety 1 and line 1 might have been favoured by the exudates and debris from these plants, leading to the high frequencies of pathogens observed. A similar observation was made by Mangenot and Diem (1979).

Table 10. Occurrence of plant pathogens by varieties/lines from which they were obtained

Location	TomatoVariety/lines	Plant pathogen	Frequencies of occurrence of plant pathogen	% of the plant pathogen
Horticultural Unit, SUA	Line 1	*Phytophtora sp.*1	8	18.2
		*Phytophtora sp.*2	5	11.4
		Pythium sp.	4	9.1
		Penicillium sp.	5	11.4
		*Phoma sp.*1	4	9.1
		*Phoma sp.*2	3	6.8
		*Asperigillus sp.*1	4	9.1
		*Asperigillus sp.*2	4	9.1
		Botrytis sp.	7	15.9
		Total	44	100.1
	Line 2	*Phytophtora sp.*1	4	10.8
		*Phytophtora sp.*2	4	10.8
		Pythium sp.	4	10.8
		Penicillium sp.	7	18.9
		*Phoma sp.*1	3	8.1
		*Phoma sp.*2	3	8.1
		*Asperigillus sp.*1	5	13.5
		*Asperigillus sp.*2	4	10.8
		Botrytis sp.	3	8.1
		Total	37	99.9
Mlali	Variety 1	*Phytophtora sp.*1	3	8.8
		*Phytophtora sp.*2	3	8.8
		Pythium sp.	2	5.9
		Penicillium sp.	7	20.6

Table 10 continued

Location	TomatoVariety/lines	Plant pathogen	Frequencies of occurrence of plant pathogen	% of the plant pathogen
		*Phoma sp.*1	2	5.9
		*Phoma sp.*2	3	8.8
		*Asperigillus sp.*1	7	20.6
		*Asperigillus sp.*2	3	8.8
		Botrytis sp.	4	11.8
		Total	34	100
	Variety 2	*Phytophtora sp.*1	6	14.0
		*Phytophtora sp.*2	5	11.6
		Pythium sp.	6	14.0
		Penicillium sp.	6	14.0
		*Phoma sp.*1	4	9.3
		*Phoma sp.*2	2	4.7
		*Asperigillus sp.*1	4	9.3
		*Asperigillus sp.*2	5	11.6
		Botrytis sp.	5	11.6
		Total	43	100.1

4.4.4 Occurrence of plant pathogens in relation to disease severity

Table 11 shows the occurrence of plant pathogens by disease severity level from which they were obtained. Generally, with the exception of ***Phoma sp.*** 1 and ***Asperigillus sp.*** 1, all other pathogens occurred in higher frequencies in the severely diseased plants than in the other (lower) disease levels. This signifies that there were positive correlation between disease severity levels and the populations of the plant pathogens in the study areas, i.e. the more severe the disease, the higher the populations of the pathogens. Similar observation was made by Alabouvette (1991).

Lowe frequencies of occurrence of *Phoma sp.* 1 and ***Asperigillus sp.*** 1, in the severe disease level, may be these pathogens were out-competed by other plant pathogens.

The higher frequencies of certain pathogens observed under severely diseased plants might have also been caused by an inherent susceptibility of those tomato varieties/lines to the plant pathogens. The same effect was also reported by Baker and Cook (1974).

There is a possibility that the points in the field where the severely diseased plants were observed might have been associated also with the same pathogen from previous cropping seasons. Similar observations were reported by Atkinson *et al.* (1975).

Table 11. Summary of occurrence of plant pathogens by disease severity levels

Plant pathogen	Disease level	Number of plant pathogens	% of the pathogens
Phytopthora sp. 1	Healthy	7	30.4
	Intermediate	5	21.7
	Diseased	11	47.8
	Total	23	99.9
Phytopthora sp. 2	Healthy	4	23.5
	Intermediate	6	35.3
	Diseased	7	41.2
	Total	17	100
Pythium sp.	Healthy	5	31.3
	Intermediate	4	25.0
	Diseased	7	43.8
	Total	16	100.1
Penicillium sp.	Healthy	8	32
	Intermediate	7	28
	Diseased	10	40
	Total	25	100
Phoma sp. 1	Healthy	5	38.5
	Intermediate	4	30.8
	Diseased	4	30.8
	Total	13	100.1
Phoma sp. 2	Healthy	3	25.0
	Intermediate	4	33.3
	Diseased	5	41.7
	Total	12	100
Asperigillus sp. 1	Healthy	7	35
	Intermediate	9	45

Table 11 continued

Plant pathogen	Disease level		Number of plant pathogens	% of the pathogens
	Diseased		4	20
		Total	20	100
Asperigillus sp. 2	Healthy		5	31.3
	Intermediate		3	18.8
	Diseased		8	50.0
		Total	16	100.1
Botrytis sp.	Healthy		6	31.6
	Intermediate		4	21.1
	Diseased		9	47.4
		Total	19	100.1

4.5 Ability of *Streptomyces* to produce antibiotics

4.5.1 General results

The extent of antibiosis or inhibition (mm) of plant pathogens by the *Streptomyces* isolates is presented in Table 12. Overall, some *Streptomyces* isolates showed strong antibiosis (>25mm inhibition zone) against plant pathogenic fungi, for example, isolates HV_8I_2, $HV_{10}I_2$, $VCVFI_1$, VCH_4 and VCI_3 showed high antibiosis, while others like (HV_8H_1, HV_8D_1, $HV_{10}H_4$, VCD_2, and VCD_4) showed moderate (11 – 25mm). Some *Streptomyces* e.g. (HV_8H_2, $HV_{10}D_4$, VCH_1, and VCD_3) showed weak antibiosis (1 - 10mm). There are some *Streptomyces* isolates which inhibited all of the test pathogens, for example, isolates HV_8I_3 and HV_8D_1, while isolates like $HV_{10}D_1$ and $VCVFH_3$ inhibited none of the test pathogens. Each test pathogen was inhibited by at least twelve of the *Streptomyces* isolates.

Some isolates from the same disease level, variety/line, or from the same location, inhibited the same test plant pathogens to different extents. For example, isolate VCI_1 showed 5mm inhibition zone against *Phytophtora sp.*1 and *Phytophtora sp.*2, while isolate VCI_3, $VCVFI_1$ and $VCVFI_3$ showed strong inhibition of 29mm or more against the same pathogen species. Isolates from the same location inhibited the same pathogen differently, for example, isolate HV_8I_3 showed 10mm inhibition zone against *Asperigillus sp.*1, while $HV_{10}I_2$ showed 35mm against the same pathogen.

Table 12. Extent of antibiosis indicated by zone of inhibition (in mm) shown by *Streptomyces* isolates

Location	Soil pH	*Streptomyces* isolate	*Phytopthora* *sp.*1	*Phytopthora* *sp.*2	*Pythium* *sp.*	*Asperigillus* *sp.*1	*Asperigillus* *sp.*2	*Penicellium* *sp.*	*Phoma* *sp.*1	*Phoma* *sp.*2	*Botrytis* *sp.*
Horticulture	6.75	HV_8H_1	21	3	5	9	0	0	0	2	0
Horticulture	6.75	HV_8I_1	0	0	0	0	0	3	0	7	0
Horticulture	6.75	HV_8D_1	10	2	4	3	13	5	3	9	0
Horticulture	6.75	$HV_{10}H_1$	0	0	3	5	0	0	0	0	0
Horticulture	6.75	$HV_{10}I_1$	0	0	0	0	3	0	0	0	0
Horticulture	6.75	$HV_{10}D_1$	0	0	0	0	0	0	0	0	0
Horticulture	6.75	HV_8H_2	6	0	0	4	0	0	0	0	0
Horticulture	6.75	HV_8I_2	0	20	0	0	0	6	25	0	0
Horticulture	6.75	HV_8D_2	0	0	0	0	0	5	3	3	0
Horticulture	6.75	$HV_{10}H_2$	0	0	4	0	0	5	0	0	5
Horticulture	6.75	$HV_{10}I_2$	10	0	5	35	3	3	2	15	2
Horticulture	6.75	$HV_{10}I_2$	0	0	0	0	0	0	0	0	0
Horticulture	6.75	$HV_{10}D_2$	0	0	0	4	0	5	7	0	0
Horticulture	6.75	HV_8H_3	0	0	0	0	0	0	0	0	0
Horticulture	6.75	HV_8I_3	15	17	9	10	0	3	3	22	0
Horticulture	6.75	HV_8D_3	0	0	0	0	0	0	0	0	0
Horticulture	6.75	$HV_{10}H_3$	0	0	0	8	5	0	0	15	10
Horticulture	6.75	$HV_{10}I_3$	0	0	0	0	5	0	0	0	0
Horticulture	6.75	$HV_{10}D_3$	5	0	0	3	0	0	0	0	0
Horticulture	6.75	HV_8H_4	0	0	0	0	0	0	0	0	0
Horticulture	6.75	HV_8I_4	0	0	0	0	0	0	0	0	0
Horticulture	6.75	HV_8D_4	0	0	0	0	0	3	4	4	9
Horticulture	6.75	$HV_{10}H_4$	3	0	17	12	0	2	2	0	0
Horticulture	6.75	$HV_{10}I_4$	0	0	2	15	6	0	0	0	0
Horticulture	6.75	$HV_{10}D_4$	0	0	0	0	0	5	0	0	0
Horticulture	6.75	$HV_{10}D_4$	0	0	0	0	0	5	0	0	0
Mlali	7.79	$VCVFH_1$	0	3	9	0	0	5	0	0	3
Mlali	7.79	$VCVFI_1$	30	0	0	7	5	0	6	9	2
Mlali	7.79	$VCVFI_1$	0	25	0	20	0	0	0	0	30
Mlali	7.79	$VCVFD_1$	0	4	0	0	0	0	0	10	7
Mlali	7.79	VCH_1	7	0	0	4	3	0	0	0	0
Mlali	7.79	VCH_1	0	0	0	0	0	0	0	0	0

Table 12 continued

Location	Soil	*Streptomyces*	*Phytopthora*	*Phytopthora*	*Pythium*	*Asperigillus*	*Asperigillus*	*Penicellium*	*Phoma*	*Phoma*	*Botrytis*
	pH	isolate	*sp.*1	*sp.*2	*sp.*	*sp.*1	*sp.*2	*sp.*	*sp.*1	*sp.*2	*sp.*
Mlali	7.79	VCI$_1$	8	2	0	3	0	0	0	6	0
Mlali	7.79	VCD$_1$	0	5	0	0	0	0	0	0	3
Mlali	7.79	VCVFH$_2$	5	0	3	10	0	0	0	5	0
Mlali	7.79	VCVFI$_2$	2	0	0	0	10	0	4	0	0
Mlali	7.79	VCVFI$_2$	0	0	0	0	0	0	0	0	12
Mlali	7.79	VCVFD$_2$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCH$_2$	7	0	2	5	3	3	0	0	6
Mlali	7.79	VCH$_2$	0	0	0	22	0	0	0	0	0
Mlali	7.79	VCI$_2$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCI$_2$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCD$_2$	16	0	0	0	4	0	0	11	7
Mlali	7.79	VCVFH$_3$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCVFH$_3$	10	12	8	32	10	11	8	0	17
Mlali	7.79	VCVFI$_3$	0	16	0	5	24	0	0	15	0
Mlali	7.79	VCVFI$_3$	0	0	0	0	0	8	0	0	30
Mlali	7.79	VCVFD$_3$	0	0	0	4	0	0	0	0	0
Mlali	7.79	VCH$_3$	10	0	7	6	0	6	6	0	0
Mlali	7.79	VCH$_3$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCH$_3$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCI$_3$	0	30	0	0	0	0	0	13	29
Mlali	7.79	VCD$_3$	0	0	0	0	0	0	0	4	0
Mlali	7.79	VCVFH$_4$	0	0	0	0	0	0	0	0	5
Mlali	7.79	VCVFI$_4$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCVFD$_4$	0	0	0	3	0	0	0	0	0
Mlali	7.79	VCH$_4$	14	0	0	0	29	6	0	6	0
Mlali	7.79	VCH$_4$	0	13	0	4	0	0	0	0	0
Mlali	7.79	VCH$_4$	0	0	0	38	0	4	0	0	6
Mlali	7.79	VCI$_4$	0	0	0	0	0	0	0	0	0
Mlali	7.79	VCD$_4$	14	0	0	0	0	0	0	0	0
Mlali	7.79	VCD$_4$	0	0	0	0	0	0	0	0	0

The summary of extent of antibiosis of the *Streptomyces* isolates against plant pathogens is presented in Tables 13 and 14. Almost all of the isolates inhibited one or more of the plant pathogens tested, with only 27% inhibiting none of the test pathogens

Among the tested plant pathogens, *Asperigillus sp.*1 was the most inhibited pathogen, followed by P*hytophtora sp.*1 and 2, *Phoma sp.*1 and 2, *Penicillium sp.*, and lastly *Botrytis sp.* and *Pythium sp.* in that order. Most (73%) of the isolates in present study exhibited antibiosis which agrees with reports by Alexander (1983), Ndonde (1998) and Senkondo (2001) that a majority of *Streptomyces* isolates produced antibiotics. Alexander (1983) reported that 75% of the soil *Streptomyces* produce antibiotics. Ndonde (1998) observed higher percentage (95.78%) while Senkondo (2001) reported lower percentage (69.8%) of isolates that are able to produce antibiotics.

Table 13. Summary of extent of antibiosis by the *Streptomyces* isolates

Extent of pathogen inhibition	No. of *Streptomyces* isolates	% of isolates
Strong (>25mm)	9	14.3
Moderate (11 – 25mm)	12	19.0
Weak (1 - 10mm)	25	39.7
None	17	27.0

Table 14. Categorization of the *Streptomyces* isolates according to extent of antibiosis against each test plant pathogens

Extent of antibiosis	Plant pathogen inhibited	No. of *Streptomyces* isolates showing antibiosis	% of isolates in each antibiosis category
Strong antibiosis	*Phytopthora sp.*1	1	10
	*Phytopthora sp.*2	2	20
	Pythium sp.	0	0
	Penicillium sp.	0	0
	*Phoma sp.*1	0	0
	*Phoma sp.*2	0	0
	*Asperigillus sp.*1	3	30
	*Asperigillus sp.*2	1	10
	Botrytis sp.	3	30
Total		10	100
Moderate antibiosis	*Phytopthora sp.*1	5	17.9
	*Phytopthora sp.*2	6	21.4
	Pythium sp.	1	3.6
	Penicillium sp.	1	3.6
	*Phoma sp.*1	1	3.6
	*Phoma sp.*2	6	21.4
	*Asperigillus sp.*1	4	14.3
	*Asperigillus sp.*2	2	7.1
	Botrytis sp.	2	7.1
Total		28	100
Weak antibiosis	*Phytopthora sp.*1	12	11.1
	*Phytopthora sp.*2	6	5.6
	Pythium sp.	11	10.2
	Penicillium sp.	18	16.7
	*Phoma sp.*1	11	10.2
	*Phoma sp.*2	11	10.2
	*Asperigillus sp.*1	17	15.7
	*Asperigillus sp.*2	11	10.2
	Botrytis sp.	11	10.2
Total		108	100.1

The sensitivity of test plant pathogens to antibiotics produced by the *Streptomyces* isolates may imply a potential of such compounds/antibiotics in the control of those pathogens. The practical question remaining to be answered would be what formulations of these antibiotics to be developed and the stage of plant growth (and disease development) at which that control would be most effective.

On the other hand the fact *Streptomyces* isolates which did not show any antibiosis might imply that they did not produce any antibiotics or that any antibiotic they produced was ineffective against those pathogens. Ndonde (1998) observed that some *Streptomyces* did not inhibit some plant pathogens. Bhuyan (1962) and Hancock (1977) observed that main factors that can cause failure in antibiotics production by *Streptomyces* are type of carbon source, nitrogen, pH and temperature. These need to be optimized first before concluding that a given isolate is unable to produce antibiotics.

Table 15 shows the total numbers of *Streptomyces* isolates inhibiting each pathogen. *Phytopthora sp.*2 and *Pythium sp.*, for example, were inhibited by 13 *Streptomyces* isolates each while *Phytopthora sp.*1, *Phoma sp.*1 and 2, *Penicellium sp. Botrytis sp.* and *Asperigillus sp.*1 and 2, were inhibited by 18, 16, 12, 20, 17, 26, and 14, *Streptomyces* isolates respectively. This may imply that for many pathogens there may exist many *Streptomyces* species capable of inflicting antibiosis to the pathogens.

Table 15. Summary of number of *Streptomyces* isolates inhibiting the plant pathogens growth

Plant pathogen	No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
*Phytopthora sp.*1	18	12.0
*Phytopthora sp.*2	13	8.7
Pythium sp.	13	8.7
Penicillium sp.	20	13.4
*Phoma sp.*1	16	10.7
*Phoma sp.*2	12	8.1
*Asperigillus sp.*1	26	17.4
*Asperigillus sp.*2	14	9.4
Botrytis sp.	17	11.4

4.5.2 Evaluation of *Streptomyces* isolates for antibiotic production against test plant pathogens by locations from which they were obtained

Table 16 shows the antibiosis of the *Streptomyces* isolates from the two locations. In soils sampled from Horticultural unit *Penicillim sp.* 1 and *Asperigillus sp.* 1 were inhibited by morey isolates (16.7% each), while *Phytophtora sp.* 2 and *Botrytis sp.* were the ones inhibited by fewer isolates (6.1% each). On contrary, in soils from Mlali, *Penicillium sp.* and *Phoma sp.* 1 were the least inhibited (5.1% each), while the same *Asperigillus sp.* 1 was the one inhibited by most of the isolates (17.7%). These differences may be a reflection of variations of *Streptomyces* types across locations. The variations may be due to inability of the *Streptomyces* too produce antibiotics, which are important to inhibit plant pathogens.

Table 16. Summary of *Streptomyces* isolates producing antibiotic against test plant pathogens by locations from which they were obtained

Location	Plant pathogen	No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
Horticulture	*Phytophtora sp.*1	7	10.1
	*Phytophtora sp.*2	4	6.1
	Pythium sp.	8	12.1
	Penicillium sp.	11	16.7
	*Phoma sp.*1	8	12.1
	*Phoma sp.*2	8	12.1
	*Asperigillus sp.*1	11	16.7
	*Asperigillus sp.*2	5	7.6
	Botrytis sp.	4	6.1
	Total	66	100.1
Mlali	*Phytophtora sp.*1	11	13.9
	*Phytophtora sp.*2	9	11.4
	Pythium sp.	5	6.3
	Penicillium sp.	7	8.9
	*Phoma sp.*1	4	5.1
	*Phoma sp.*2	8	10.1
	*Asperigillus sp.*1	14	17.7
	*Asperigillus sp.*2	8	10.1
	Botrytis sp.	13	16.5
	Total	79	100

4.5.3 Evaluation of *Streptomyces* isolates' producing antibioticss against test plant pathogens by varieties/lines with which they were associated

For *Streptomyces* isolates from soils sampled around different varieties/lines, are presented in Table 17; there were variations in the numbers of *Streptomyces* isolates, which inhibited the different pathogens. For tomato line 1 grown at the Horticultural Unit, fungal pathogen *Penicillium sp.* and *Phoma sp.* 2 were the most inhibited by many *Streptomyces* isolates (17.6% each) and the rest of the pathogen were inhibited by at least one or more of the isolates. Line 2 from Horticultural Unit, was associated with *Streptomyces* isolates which mostly inhibited *Asperigillus sp.* 1 (21.9%) with none of the isolates inhibiting *Phytopthtora sp.*2. This implies that the rhizosphere of each tomato variety/line was capable of stimulating or harbouring *Streptomyces* types different from those of another variety/line.

Table 17. Summary of *Streptomyces* isolates producing antibiotics against test plant pathogens by varieties/lines from which they were obtained

Location	Tomato variety/line	Plant pathogen	No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
Horticulture	Line 1	*Phytophtora sp.*1	4	11.8
		*Phytophtora sp.*2	4	11.8
		Pythium sp.	3	8.8
		Penicillium sp.	6	17.6
		*Phoma sp.*1	5	14.7
		*Phoma sp.*2	6	17.6
		*Asperigillus sp.*1	4	11.8
		*Asperigillus sp.*2	1	2.9
		Botrytis sp.	1	2.9
		Total	34	99.9
	Line 2	*Phytophtora sp.*1	3	9.4
		*Phytophtora sp.*2	0	0
		Pythium sp.	5	15.6
		Penicillium sp.	5	15.6
		*Phoma sp.*1	3	9.4
		*Phoma sp.*2	2	6.3
		*Asperigillus sp.*1	7	21.9
		*Asperigillus sp.*2	4	12.5
		Botrytis sp.	3	9.4
		Total	32	100.1
	Variety 1	*Phytophtora sp.*1	4	9.1
		*Phytophtora sp.*2	5	11.4
		Pythium sp.	3	6.8

Table 17 continued

Location	Tomato variety/line	Plant pathogen	No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
		Penicillium sp.	4	9.1
		*Phoma sp.*1	3	6.8
		*Phoma sp.*2	5	11.4
		*Asperigillus sp.*1	8	18.2
		*Asperigillus sp.*2	4	9.1
		Botrytis sp.	8	18.2
		Total	44	100.1
	Variety 2	*Phytophtora sp.*1	7	21.9
		*Phytophtora sp.*2	2	6.3
		Pythium sp.	2	6.3
		Penicillium sp.	3	9.4
		*Phoma sp.*1	1	3.1
		*Phoma sp.*2	2	6.3
		*Asperigillus sp.*1	7	21.9
		*Asperigillus sp.*2	3	9.4
		Botrytis sp.	5	15.6
		Total	32	100.2

The tendency shown by some pathogens, for example *Phytophtora sp.* 2, to be inhibited by almost equal numbers of *Streptomyces* isolates from line 1 and variety 1, from Horticultural Unit and Mlali, respectively, may imply that the variety 1/line 1 created equally favorable conditions around the root zone which stimulated *Streptomyces* and that these *Streptomyces* may have produced similar types of antibiotics. Brock *et al.* (1994) reported that different species of *Streptomyces*, even from different parts of the world, were able to produce the same antibiotics.

4.5.4 Evaluation of number of *Streptomyces* isolates showing antibiosis against test plant pathogens as related to disease levels

The inhibition of test plant pathogen as related to disease severity is presented in Table 18. Regardless of the disease severity level, isolates from all levels were able to inhibit the tested plant pathogens (Table 18).

However, in most cases high incidences of antibiosis were associated with the healthy, followed by the intermediately diseased tomato plants. Presence of healthy plants might have been influenced by presence in soil of high levels of pathogens that induced a proliferation of *Streptomyces* strains with higher levels of antibiosis, which lead to the protection of the plants. The opposite might have applied to the intermediate and severely diseased plants, explaining why they had fewer numbers of antibiotic producing *Streptomyces* compared to the healthy ones.

Table 18. Summary of number of *Streptomyces* isolates showing antibiosis against test plant pathogens by disease levels

Plant pathogen inhibited	Disease level	No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
Phytopthora sp. 1	Healthy	10	55.6
	Intermediate	4	22.2
	Diseased	4	22.2
	Total	18	100
Phytopthora sp. 2	Healthy	4	30.8
	Intermediate	6	46.2
	Diseased	3	23.1
	Total	13	100.1
Pythium sp.	Healthy	9	69.2
	Intermediate	3	23.1
	Diseased	1	7.7
	Total	13	100
Penicillium sp.	Healthy	8	42.1
	Intermediate	5	26.3
	Diseased	6	31.6
	Total	19	100
Phoma sp. 1	Healthy	3	25.0
	Intermediate	5	41.7
	Diseased	4	33.3
	Total	12	100
Phoma sp 2	Healthy	4	23.5
	Intermediate	7	41.2
	Diseased	6	35.3
	Total	17	100
Asperigillus sp. 1	Healthy	13	52

Table 18 continued

Plant pathogen inhibited	Disease level		No. of *Streptomyces* isolates inhibiting the pathogen	% of the isolates inhibiting the pathogen
	Intermediate		7	28
	Diseased		5	20
		Total	25	100
Asperigillus sp. 2	Healthy		5	35.7
	Intermediate		7	50.0
	Diseased		2	14.3
		Total	14	100
Botrytis sp.	Healthy		7	41.2
	Intermediate		6	35.3
	Diseased		4	23.5
		Total	17	100

In those cases where many *Streptomyces* isolates from healthy levels inhibited the test plant pathogens, the implication may be that presence of high levels of pathogens somehow induced higher levels of *Streptomyce* antibiosis which then protected the plants against pathogens and made the plants healthy.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary and conclusions

The aim of this study was to explore *Streptomyces* from the infested soils that have ability to inhibit plant pathogens. The results obtained revealed that there were no significant (p=0.05) differences in the *Streptomyces* populations from the two study areas (Horticultural Unit and Mlali). However, there was a significant (p=0.05) difference in the *Streptomyces* populations obtained under different tomato varieties/lines. *Streptomyces* populations obtained under different tomato disease severity levels were not statistically significantly (p=0.05) different. From fungi there were also, no significant (p=0.05) differences between fungi populations under different disease severity levels.

Most of the *Streptomyces* isolates (73%) produced antibiotics against the test plant pathogenic fungi isolated from the same soils, namely ***Phytophtora sp.*1, *Phytophtora sp.*2, *Phoma sp.* 1, *Phoma sp.* 2, *Asperigillus sp.* 1, *Asperigillus sp.* 2, *Pythium sp.*, *Botrytis sp.*** and ***Penicillium sp.***. While some isolates showed strong antibiosis, others showed moderate and others weak. There were some isolates that inhibited none of the test plant pathogens. At least each plant pathogen was inhibited by 12 or more isolates. ***Asperigillus sp.*** 1 was the weakest pathogen, followed by ***Penicillium sp.*** and the least was *Phoma sp.* 2.

In conclusion, *Streptomyces* that were potential antibiotic producers were found in all pathogen infested soils of the study areas. *Streptomyces* isolates from all the disease severity levels and varieties/lines were able to show antagonistic properties to the test plant pathogens.

5.2 Recommendations

The present results lead to the following recommendations.

1. Further research needs to be undertaken so that this antimicrobial potential can be further evaluated, and attempts made to test them under field conditions.
2. Studies should be undertaken to include more varieties/lines that were not included in this study so that the effect of varieties/lines on the *Streptomyces* can be better evaluated.
3. Further studies could include plant pathogenic bacteria, as well as other groups of fungi (*Rhizoctonia spp.* and *Fusarium spp.*) that affect tomato plants.
4. In the long term, studies need to be carried out to extract the antibiotics from these *Streptomyces* and evaluate their potential for disease control in foliar or other applications.
5. Further research is needed to come out with proper formulations of these antibiotics.

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