MOLECULAR CHARACTERIZATION OF *RHIZOCTONIA SOLANI* STRAINS CAUSING TOMATO (*Lycopersicon esculentum* Mill) DAMPING-OFF AND ITS MANAGEMENT

BY

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DECLARATION

This thesis is my original work and has not been submitted for award of degree in any other University before.

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DEDICATION

I dedicate this work to my late wife Felistas Muthoni for her guidance when writing up the proposal and love and kindness during our time together. May the almighty God rest her in peace.

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LIST OF ABBREVIATION

DAP=Days after planting

HCDA= Horticultural Crop Development Authority.

UM1 – LM3 =Agro-ecological zones 1 to 5 respectively.

SAR=Systemic Acquired Resistance

ISR=Induced Systemic Resistance

R1 –R56= Pathogenic *Rhizoctonia solani* isolates 1-56 respectively

Mbr= Methyl bromide

UN=United Nations

EPA = Environmental Protection Agency (of the United nations).

UNDP = United Nations Development Program

G1-GIII= Tomato fruits grade 1 to 3 respectively

ANOVA=analysis of variance

AMOVA=Analysis of molecular variance.

PCR=polymerase chain reaction

FST=F-Statistics

PAGE=Polyacrylamide gel electrophoresis.

SIMQUAL=Similarity for qualitative data.

UPGMA=Unweighted pair group method with arithmetic average.

TBE=Tris borate EDTA

TE=Tris-EDTA.

IPM=Integrated Pest Management

AG=Anastomosis group.

KARI=Kenya Agricultural Research Institute.

AEZ=Agro Ecological Zone.

PDA= Potato dextrose agar

APDA=Acidified potato dextrose agar

NA= Nutrient agar

PSA=Potato sucrose agar

ABSTRACT

The objective of this study was to determine the genetic diversity among the *Rhizoctonia solani* strains causing damping off of tomato in Kenya and assess the efficacy of the disease control by nonchemical methods. Survey was conducted in the year 2002-2003 to determine the current status on the occurrence and distribution of Rhizoctonia damping- off and root rots of tomato in the 4 major tomato growing agro-ecological zones of Kenya. The genetic diversity of the damping off pathogens was evaluated from the 56 pathogenic *Rhizoctonia solani* isolates from infected tomato and rhizosphere soil using microsatellite technique. Efficacy of nonchemical control methods for damping off of tomato was evaluated by screening 7 tomato varieties grown in Kenya for resistance to the disease, assessment of antagonism between pathogenic *R. solani* and other soilborne pathogens on the disease control under greenhouse conditions, and field evaluation of the efficacy of various cultural practices on the disease management. The efficacy of the various cultural practices on the Rhizoctonia damping off of tomato control were compared with the conventional control methods such as chemical fumigation with Basamid and chemical seed treatment.

The results of the survey indicated that the disease prevalence, incidence, severity and the number of pathogenic *R. solani* isolates recovered significantly ($P \leq 0.05$) varied with agro-ecological zones, season of production and the various crop production practices. The disease control was being hampered by lack of adequate information on causal agents, the disease epidemiological factors, and lack of cost-effective and sustainable control strategies. Laboratory isolation and characterization of soilborne fungal pathogens from infected plant and rhizosphere soil indicated that although *R. solani* was the most frequently isolated pathogen, other soilborne fungal pathogens such as *Pythium, Fusarium* and bacterial pathogens like *Pseudomonas* were

also isolated. The various *R. solani* isolates differed on their morphological and cultural characteristics and pathogenicity on common tomato varieties.

Molecular characterization of the 52 pathogenic *Rhizoctonia solani* isolates from the 4 agro-ecological zones using 7 microsatellite markers indicated a high genetic diversity among the *Rhizoctonia solani* causing damping off of tomato as indicated by high polymorphism, large number of alleles typed, high average heterozygosity, large variances of genetic distance and large number of molecular clusters generated. The deficiency of heterozygotes and lack of Hardy-weinberg equilibrium in 5 out of the 7 loci typed indicated a disquilibrium in group of populations from various agro-ecological zones implying low level of intermating within populations. However, the excess of average heterozygotes in the group of populations indicated high degree of gene flow between ecological zones probably due to efficient inoculum dispersal by run-offs and irrigation water.

Evaluation of the efficacy of different management strategies in the control of Rhizoctonia damping off of tomato indicated that a potential exist for the disease control by microbial antagonism and cultural methods but not by use of resistant varieties. The efficacy of the disease control achieved by use of transplanting as opposed to direct seeding and raised beds + watering every 2 days compared favourably to the disease control by conventional strategies such as chemical fumigation with Basamid and chemical seed dressing with Gaucho 390 FS MT. The antagonism in causing Rhizoctonia damping off of tomato observed between pathogenic *R. solani* and nonpathogenic multinucleate *R. solani* and binucleate *R. solani* under greenhouse conditions implied a potential for the biocontrol of the disease by microbial antagonism. On the other hand, the positive synergism in causing Rhizoctonia damping off of tomato observed

between pathogenic *R. solani* and pathogenic *Pythium* and *Fusarium* isolates implied the need to employ control measures that also suppresses *Fusarium* and *Pythiuum* for effective control of Rhizoctonia damping off of tomato.

The high genetic diversity among *R. solani* isolates could have a bearing on their wide host range and virulence levels, all of which influence the efficacy of the disease control methods. Future research should aim at race typing of the different isolates to evaluate their virulence on different host cultivars and evaluate the response of each isolate to various control methods. The effective disease control by microbial antagonism and cultural methods demonstrated in this study will form part of a sustainable cost-effective disease control strategy especially for smallholder resource poor vegetable farmers in Kenya and be part of integrated pest management systems.

CHAPTER ONE

GENERAL INTRODUCTION

1.0: Background information

Before 1950, Vegetable and fruit growers in many parts of the world can best be described as nomadic. One to 4 successive crops were produced on same land after expansive clearing operations had been performed or after long pasture rotations to avoid build up of soilborne pathogens and pest problems (Christie, 1959: Johnson *et al.,* 1962: Overman *et al.,* 1978; Bewick, 1989). As the population increased in late 80s and urban growth increased, suitable land for crop production became more difficult to locate and expensive to acquire and develop (Rodgruez – Kabana, 1992). This led to fast build up of economically important soilborne pathogens such as *Rhizoctonia solani, Fusarium, Pythium,* bacteria wilt pathogens and nematodes beyond economic threshold levels. Because of these constraints, growers increasingly adopted chemical methods of soil pest management. Reverting to such a shift production system is no longer a viable option because of the unavailability of suitable land, as well as other environmental, water and forest reserves management regulatory policies (Noling *et al.,* 1994).

Currently in Kenya, vegetable production is an intensive commercial monoculture practiced in small parcels of land due to encroachments of agricultural land by human settlement and urbanization (HCDA, 2008). Tomato cultivation in Kenya is mainly produced by small scale farmers in the open fields though greenhouse production is rapidly growing (Ministry of Agriculture, Republic of Kenya, 2004).

1.2: Problem statement and justification

Over recent years there has been a great increase in smallholder tomato cultivation in parts of Africa, both as food and as a cash crop; but in some countries after a few good yields this crop is now failing on production drastically. The main reason seems to be a joint disease/nematode complex which has built up partly owing to lack of feasible crop rotation (C.O.P.R, 1983). Tomato cultivation in the tropics is generally more restricted by diseases than by insect pest in most locations (Hill and Waller, 1988; Danesha *et al.,* 1999). The most important soilborne diseases are root-rot caused by *Rhizoctonia solani* and *Fusarium solani,* fusarium wilt, bacterial wilt and root-knot nematodes. The foliar diseases includes early and late blights, leaf mould, mosaic virus, leaf curl virus, bacterial canker, powdery mildew, septoria leaf spot, grey leaf spot and stem canker among others (Hill and Waller, 1988).

In Kenya *Rhizoctonia solani* is a serious problem of tomato and other vegetables such as kales, beans, okra, eggplant and flowers where it causes damping-off and wirestem diseases (Kenya Agricultural Research Institute, Republic of Kenya, 2002; Githinji, 2005). The disease is prevalent in warm tomato production areas of Kenya where it causes major losses both in the nursery as seed decay and damping- off, and in the field as stem and fruit rot (Ministry of Agriculture, Republic of Kenya, 2004). The intensity of production and other crop production practices such as ploughing in of infected plant debris and cow manure application has led to build up of *Rhizoctonia solani* in most production locations beyond economic threshold levels. Some farmers have estimated losses of upto 60% of seedlings in the nursery especially where a lot of cow manure has been applied without chemical fumigation practiced and yield reduction of upto 30% in the field has been reported (Ministry of Agriculture, Republic of Kenya, 2004).

Rhizoctonia damping off of tomato is difficult to control as no adequate information is available on the disease etiology and epidemiology. Most of the popular tomato varieties grown locally are susceptible and economical chemical control is not economically viable. Rotations are often ineffective due to the wide host range and lack of adequate land. This calls for urgent screening of other nonchemical methods for disease control (KARI, 2002).

Rhizoctonia is currently being diagonised by use of plant symptoms, isolation and characterization of isolates on artificial media. It is difficult to quantify the amount and range of inoculum but this could be done using molecular techniques (Kerry, 1993; Herdina and Whisson, 1996). Due to numerous advantages of molecular techniques as opposed to the conventional ones, polymerase chain reaction (PCR) has become one of the most widely used techniques in molecular biology (Kerry, 1993; Herdina and Whisson, 1996; Connie, 2000).

DNA probes are being developed to detect other soilborne fungal pathogens such as damping off fungi, *Pythium* spp. and take-all caused by *Gaeumannomyces graminis* (Herdina *et al.*, 1996). Ultimately, it should be possible to develop a DNA based diagnostic technique for rapid detection of *R . solani* strains in infected tomato plant and infested soil. This could form the basis of a predictive test, and be a sensitive tool to investigate the biology of the disease and evaluate new methods of control.

In view of the economic importance of *Rhizoctonia* stem and root-rot as a constraint to increased yields in tomato and other vegetables in Kenya, this study was designed to assess pathogenic variations of the causal agent, assess yield loss and evaluate the efficacy of different non-chemical methods in the management of the disease.

Therefore the specific objectives of this study were;

(1) To determine the distribution of Rhizoctonia damping off/wirestem disease on tomato in Kenya.

(2) To determine the genetic variability among the population of *Rhizoctonia solani* strains causing tomato damping off using microsatellite technique.

(3) To evaluate the efficacy of host resistance, microbial antagonism and cultural control strategies in the management of *Rhizoctonia* damping off of tomato.

CHAPTER TWO

LITERATURE REVIEW

2.1: Importance of tomato

Tomato (*Lycopersicon esculentum* Mill) originated from South America in the Peru Ecuador region and was taken to the Philippines and Malaya by 1650. It was not cultivated in the tropics until the 20th century but is now cultivated widely throughout the world (Hill and Waller, 1988; Danesha *et al.*, 1999). It is a variable annual herb and grows 0.7 – 2.0m high and the fruit for which it is grown is a fleshy berry, red or yellow when ripe containing vitamins A and C. The fruit is eaten raw or cooked, made into soup, sauce, juice, kentchup, paste, puree or powder, canned and used unripe in chutneys (Hill and Waller, 1988; Danesha *et al.*, 1999). The main production areas are in the USA, Italy and Mexico (Hill and Waller, 1988; Danesha *et al.*, 1999; HCDA, 2008). Included in the tomato varieties grown in Kenya are the early maturing ones such as Monset F1 TM, Beauty, Cal-J VF, Kento (2) (F1 hybrid), Kenform(1) (F1hybrid), Hofit M82; medium maturing ones such as Onyx VF2, Caltana F1 VF hybrid , Roma VF and Money maker and late maturing ones such as Marglobe (KARI, 2002).

Tomatoes are grown in Kenya mainly for local consumption as fresh or processed product and are extremely important as a high value cash crop for small and large scale growers alike (Madumadu, 1979). The recent export outlets for both fresh and processed tomato to the neighbouring countries such as Uganda and Rwanda is becoming important thus supplementing the foreign exchange earnings from such traditional Kenyan horticultural export crops such as beans, eggplant, okra and cut flowers (HCDA, 2008). Horticultural production is extremely important in Kenya as a source of employment, food and foreign exchange earning. In the year

2007/2008 the sub-sector grew by about 20%, employing 1 million people of which 75% were women. It was the country's largest foreign exchange earner, raking in 1 billion USD in foreign exchange. This was far above earnings from tourism and tea which were second and third respectively (HCDA, 2008; Ministry of planning and National development, government of Kenya, 2008). In the year 2008/2009, the sector retained the top most foreign exchange earner bringing in 0.945 billion USD (Ministry of Planning and National Development, Republic of Kenya, 2009).

2.2: Damping off diseases of vegetables

The single term used to describe underground, soil line or crown rots of seedlings due to unknown causes is damping-off. The term actually covers several soil borne diseases of plants and seed borne fungi (Royal Horticultural Society, 2009). Damping off is the term used for a number of different fungus-caused ailments which can kill seeds or seedlings before or after they germinate. The term is used most often in horticulture where seeds or seedlings are specifically planted to be transplanted especially if in warm, wet conditions which speed growth of seedlings but are considered conducive to fungal attacks (Royal Horticultural Society, 2009). The incidence and severity of the damping off diseases and their resultant damage have been observed to vary considerably depending on prevailing environmental and soil conditions, the number and type of damping off pathogens that are present and active in inciting disease under given conditions. Severity and damage of damping off on seedlings are greatest when prevailing environmental or soil conditions during the early part of the growing season are favourable to infection and are followed by stressful conditions during the middle of the growing season such as drought, excess water, poor aeration, insect injury or infection by

other pathogens (Abawi *et al.*, 1990; Damping off wikipedia, 2008; Royal Horticultural Society, 2009).

Seeds may be infected as soon as moisture penetrates the seed coat or a bit later as the radicle begins to extend, all of which rot immediately under the soil surface causing pre-emergence damping-off. This condition results in a poor, uneven stand of seedlings often confused with low seed viability. Cotyledons may break the soil surface only to wither and die or healthy looking seedlings may suddenly fall over causing post-emergence damping-off. Infection results in lesions at or below the soil line. The seedling will discolor or wilt suddenly or simply collapse and die. Weak seedlings are especially susceptible to attack by one or more fungi when growing conditions are only slightly unfavorable. Damping-off is easily confused with plant injury caused by insect feeding, excessive fertilization, high levels of soluble salts, excessive heat or cold, excessive or insufficient soil moisture or chemical toxicity in air or soil (Abawi *et al.*, 1990; .Damping off wikipedia, 2008).

A number of different fungi cause these problems including *Rhizoctonia solani, Botrytis, Macrophomina phaseoli, Phytophthora, Pythium, Sclerotium, Thielaviopsis, Aphanomyces, Fusarium, Cylindrocladium* and others hence the need for the collective term known as damping-off (Royal Horticultural Society, 2009).

Rhizoctonia root rot caused by *Rhizoctonia solani* is a fungal disease which causes damping-off of seedlings and foot rot of cuttings (Danesha *et al.*, 1999; Damping off Wikipedia, 2008). Infection occurs in warm to hot temperatures and moderate moisture levels. The fungi is found in all natural soils and can survive indefinitely. Infected plants often have slightly sunken lesions on the stem or below the soil line. Transfer of the fungi to the germination room or greenhouse is easily accomplished by using outdoor gardening tools inside or vice versa

(Koike, 1996). Pythium Root Rot caused by *Pythium spp.* is similar to Rhizoctonia in that it causes damping-off of seedlings and foot rot of cuttings. However, infection occurs in cool, wet, poorly-drained soils, and by overwatering. Infection results in wet odorless rots. When severe, the lower portion of the stem can become slimy and black. Usually, the soft to slimy rotted outer portion of the root can be easily separated from the inner core. Species of *Pythium* can survive for several years in soil and plant refuse (Plaats-Niterink, 1981; Abawi *et al.*, 1990).

Phytophthora root rot caused by *Phytophthora spp.* are usually associated with root rots of established plants but are also involved in damping-off. These species enter the root tips and cause a water-soaked brown to black rot similar to *Pythium*. These fungi survive indefinitely in soil and plant debris (Damping off Wikipedia, 2008; Royal Horticultural Society, 2009). Black root rot caused by *Thielaviopsis basicola* is a problem of established plants (Abawi *et al.*, 1990). It does not occur in strongly acid soils with a pH of 4.5 to 5.5. It usually infects the lateral roots where they just emerge from the taproot. The diseased area turns dark brown and is quite dry. The fungi survive for 10 years or more in soil. Miscellaneous fungi causing similar symptoms include *Sclerotium rolfsii* (white mold), *Macrophomina phaseoli*, some species of *Botrytis* (gray mold), *Aphanomyces*, *Fusarium*, *Cylindrocladium*, and others (Damping off Wikipedia, 2008).

2.3: Rhizoctonia damping off of tomato

2.3.1. Distribution and host range

Rhizoctonia solani is a basidiomycete that occurs worldwide and causes economically important diseases such as damping –off, root and crown rots of many crops as well as fruit decay to a large variety of vegetable and field crops, turfgrasses, ornamentals and fruit and

forest trees (Flentje, *et al.*, 1970; Anderson, 1982; Carol and Dona, 1986; Adams, 1988; Koike, 1996; Sneh *et al.*, 1996) inflicting yield losses averaging up to 20% yearly in over 200 crops worldwide. In South Australia, *Rhizoctonia solani* is estimated to reduce the yield of Barley where it causes 'bare patch' disease of cereals by a factor of 5 to 10% with individual farmers suffering greater losses (Herdian and Whisson, 1996).

Root or foot rot of tomato caused by *Rhizoctonia solani* is a serious disease in the tropics (Sherf and Macnab, 1986; Hill and Waller, 1988). *Rhizoctonia* is a nonspecialised fungi mostly found associated with decomposed organic matter in all natural soils where it can survive indefinitely (Abawi *et al.,* 1990; Danesha *et al.,* 1999; Damping off Wikipedia, 2008)

2.3.2. Etiology

Rhizoctonia root and stem rot is caused by *Rhizoctonia solani* Kuhn, which is the asexual state of *Thanatephorus cucumeris* (Frank) Donk. The form genus *Rhizoctonia* contains approximately 15 species. Because conidia are not produced, the taxonomy of the genus is very difficult (Parmeter *et al.*, 1969, 1970). According to Parmeter *et al* (1967) and Parmeter (1970), the genus *Rhizoctonia* is a heterogenous assemblage of mycelia of basidiomycetes, ascomycetes and deuteromycetes. These fungi occur in the soil as many strains that differ in appearance in cultures, physiology and pathogenicity. However all the isolates have a characteristic brown mycelium and they show basal constriction of hyphal branches from prominent septa in new branches near the point of origin and exhibit a multinucleate condition of the young hyphal tip cells.

The majority of isolates produce loose sclerotia that along with thick-walled hyphae in host tissues, are the survival structures in soil. Although most of *Rhizoctonia spp* are pathogenic, there exist a non-pathogenic group belonging to the anastomosis group AG-G and is commonly associated with members of the *Rhizoctonia solani* complex and provides an effective protection against root rot caused by *Rhizoctonia solani* AG-4 (Ogoshi, 1987; Sneh, 1996). Both fungi are morphologically similar and it is difficult to differentiate between them without using laborious conventional techniques (Ogoshi, 1987). Fortunately, markers common to all AG-G have been identified by use of RAPD assay (Jabaji *et al.*,1999; Leclerc *et al.*,1999). Examples of pathogenic isolates of *Rhizoctonia* includes *R. solani, R. zeae* and *R. oryzae* (Parmeter, 1970; Yang *et al.,* 1994). Although for a long time the pathogenic strains were believed to belong to the multinucleate group whereas binucleate were generally regarded as nonpathogenic, Fennile *et al* (2005) reported root rot in Yacom caused by binucleate *Rhizoctonia* spp in Brazil.

2.3.3. Symptoms

Damping off and bottom rot are two aspects of infection by *Rhizoctonia solani* in most vegetables (Koike, 1996; Hulbert *et al.*, 2008). Infected plants often have slightly sunken lesions on the stem at or below the soil line (Damping off Wikipedia, 2008).

Damping off or wirestem occurs on newly emerged or very young seedlings when *Rhizoctonia* attacks the hypocotyls or lower stem tissue in contact with the soil. This results in browning and cracking of the epidermis and the formation of lesions. As infection progress, the outer stem decays leaving only the fibrous inner xylem intact hence the name wirestem. Affected plants wilt, turn purple and remain stunted. Seedling may break off at the soil line. The

pathogen can usually be identified by its coarse mycelia that often cause soil particles to adhere to and dangle from diseased stems (Koike, 1996).

Bottom rot is primarily a problem on cabbage and other head-forming crucifers. Once head formation begins, lower leaves in contact with the soil may become infected with *Rhizoctonia.* Dark brown, oval lesions develop where soil touches the leaves. Secondary decay organisms may follow and make these lesions soft and watery. Infected leaves may wilt exposing the head. Occasionally the pathogen may grow up into the inner tissues of the cabbage head (Koike, 1996; Hulbert *et al.,* 2008).

2.3.4. Epidemiology

The inoculum of *Rhizoctonia solani* that causes root rot consists of sclerotia and mycelia. The role of basidiospores as a source of inoculum for root rot is unknown (Anderson, 1982). *Rhizoctonia* is a non specialised pathogen that colonises decomposed organic matter in the soil (Caro *et al.*, 1986; Abawi *et al.,* 1990) hence the natural population of the pathogen is associated with decomposed host plant debris as mycelia or sclerotia in the soil. The pathogen can penetrate intact plant tissue or through natural openings and wounds. Disease severity is favoured by moderate to high soil moisture conditions and moderate temperatures (Caro *et al.*, 1986; Abawi *et al.,* 1990; Damping off Wikipedia, 2008). However, the pathogen grows best at 23 to 28°C on agar media.

The pathogen can be disseminated to new area through the planting of contaminated seeds or the movement of infected host tissues, infested soil or colonized debris by irrigation water, wind and animals (Nik *et al.*, 1979; Abawi *et al.,* 1990). Marshal (1982) reported inoculum

concentration and P^H affect damping off in snap peas. As inoculum of *R. solani* increased from 0.1g/kg soil, there was corresponding increase in disease incidence. Abawi *et al* (1990) reported infection by *Rhizoctonia* is favoured by neutral to slightly alkaline P^H with disease incidence reducing by 32% in soil of P^H 3. 5 compared to that of P^H 5. 6 and 65% in soil of P^H 2. 5.

2.4: Variability in *Rhizoctonia solani*

Rhizoctonia solani is a very complex fungal species which is composed of several strains also referred to as anastomosis groups or AGs that are morphologically similar but which differ genetically and in their ability to cause disease on various plants (Parmeter, 1970). The AG is made up of isolate among which hyphal fusion occurs.

It was initialy divided into 12 anastomosis groups based on hyphal behavior (Ogoshi, 1987) and have been denoted as AG1-11and B1 (Carling *et al.,* 1994). Representative cultures of each AG are used in a complex series of microscopic tests to identify "unknown" Rhizoctonia cultures isolated from plants and soil (Herdina and Whisson, 1996). Currently there are 13 AGs of *R .solani*, AG 1 through AG 13 (Carling *et al*., 2002a, 2002b). Among these AGs seven have been further divided into subgroups according to culture appearance, pathogenicity, thiamin requirements, pectic zymogram patterns and molecular markers (Ogoshi, 1987; MacNash *et al*., 1994; Kuninaga *et al*., 1997; Gonzales *et al*., 2001).

Due to generality of anastomosis group, the concept of intraspecific grouping was introduced as a more specific category of intraspecific variation that recognizes groupings based on combined evidence from anastomosis behavior, pathogenicity and morphology (Ogoshi, 1987). This

grouping is supported by evidence from serological studies (Adams & Lapwood, 1979), fatty acid analysis (John and Jones, 1993), protein electrophoresis (Reynolds *et al.*, 1983) and nucleic acid studies (Jabaji, *et al.,* 1990; Liu and Sinclair, 1992 and 1993; Kanematsu and Naito, 1994; Domier and Sinclair, 1995). Grouping based on DNA hybridization also supports previous grouping based on anastomosis (Kuninaga and Yokosawa , 1985; Viglays, 1988; Carling and Kuninaga, 1990), polymerase chain reaction of the internal transcribed spacer (Liu *et al.*, 1993), sequencing of the ITS region (Boysen *et al.*,1996; Kuninaga *et al.*,1997) and pulsed-field gel eletrophoresis (Keijer *et al.*, 1996).

Studies using restriction fragment length polymorphism analysis of nuclear DNA (Jabaji- Hare *et al.*, 1990) and ribosomal DNA (Kuninaga *et al.,* 1997; Gonzales *et al.*, 2001) have been used to identify genetic differences among the anastomosis groups of *Rhizoctonia solani*. Detection of *R. solani* AG2-2LP isolates causing large -patch disease on Zoysia grass was done using polymerase chain reaction. The banding patterns by RAPD-PCR showed that the three culture types were clearly distinguishable. Lubeck and Pousen (2001) developed universally primed-PCR cross hybridisation assay for rapid identification of isolates of *R. solani*. Weerasena *et al* (2004) developed a DNA probe and a PCR based diagnostic assay for *R. solani* using a repetitive DNA sequence clone. Julian *et al* (1999) used AFLP DNA markers to determine that tuft formation in the phytopathogenic fungi *Thanatephorus cucumeris* is due to heterokaryosis. Fennile *et al.,* (2006) used sequence length and nucleotide similarity to identify isolates of *R. solani* causing foliar blight of beans in Brazil. They reported 95.1-100% and 98.5-100% similarity in the ITS 1 and ITS2 regions among the isolates causing foliar blight in beans. Githinji (2005) used primer RB23, RE14, RE 102, BC5, AF513014 and AY212027 to amply DNA from *R. solani* causing root rot of beans in Kenya by microsatellite technique. Meyer *et al*

(1997) used primers LR and LROR to amplify DNA from 12 AGs causing creater disease in wheat. Nei (1978) used expected heterozygosity as a measure of diversity within a population. The expected herozygosity describes the proportion of expected heterozygotes under random mating (Nei, 1978). It is the probability that at a single locus any two alleles chosen at random from the population are different from each other.

2.5: Management of *Rhizoctonia* damping off

Although many control methods for soilborne diseases have been developed in the last decades, only a small proportion of the success in the laboratory or greenhouse have been translated to practical routine procedures for the field (Katan, 1981). Rhizoctonia root and stem rots of horticultural crops have been managed by various chemical, cultural and biological methods with a varying degree of success (Abawi *et al.*, 1990). Knowledge of the previous history of the particular field such as frequency of root-rot incidence and severity, identity of causal pathogens, cropping sequence and other information is essential for formulating a control programme (Dickson and Boettger, 1977).

2.5.1. Cultural control methods

Almost all crop production activities have direct or indirect impact on root rot incidence and severity. The particular influence are conditions such as level of organic matter, drainage, compaction and structure (Abawi and Pastor, 1990; Burke *et al.*, 1991). In addition, cultural practices that influence plant development and vigor may also affect plant reaction to root rot (Maker *et al.,* 1965; Badry *et al.,* 1979; Rhoades *et al.,* 1986). Soil preparations that reduce compaction, improve tilth, increase drainage and deeply cover infected crop debris will usually reduce damage by *R. solani* (Abawi and Pastor, 1990; Burke *et al.*, 1991). Cultural methods

such as soil amendments and soil solarisation either alone or in combination with other methods are among the most likely substitute to Methyl bromide in the control of soiborne pathogens (Abawi *et al.*, 1990; Albritton *et al.*, 1992; Nolling *et al.*, 1994). Phytosanitary measures including crop rotation and avoiding proximity to other solanaceous crops are important (Carol and Donna, 1998; Royal Horticultural Society, 2009).

Transfer of the fungi to the germination room or greenhouse is easily accomplished by using outdoor gardening tools inside or vice versa. The germination room should not be used for mixing potting soils or transplanting seedlings as a general rule (Damping off Wikipedia, 2008). Removing damaged seedlings controls Rhizoctonia damping off diseases in horticultural crops (Royal Horticultural Society, 2009).

Crop rotation with poor or non-host crops is an effective means of reducing soil population densities of *R. solani* (Rodgruez-kabana, 1992; Kinlock *et al.,* 1993; Todd, 1991; Carol and Donna, 1998). *Rhizoctonia* survives in plant debris particles in the soil and rotating crops for 3 years or more is regarded as one of the best ways to reduce inoculum levels of the fungus. Rotation with nonhost crops such as cereals for 3 to 4 seasons can reduce the pathogen densities and thus lower disease incidence and severity.

Crop rotation should be practiced in relatively clean fields as well as infestesd fields with severe root rot pathogens (Abawi *et al.*, 1990). The length of rotation depends on the severity of the problem. In many cases the major constraints to the use of crop rotation involves the broad host range of most *R. solani* strains, lack of available resistant or tolerance varieties, lack of agronomically adapted cultivars and the long period of rotation required to achieve effective

control (Carol and Donna, 1998). However, a clear understanding of what crops to avoid prior to planting is important. Also it is important to understand the ability of various *Rhizoctonia* strains to survive year after year (Carol and Donna, 1998).

Several scientists have documented the effect of organic matter in the soil on the natural population of *Rhizoctonia* and the damping off disease it causes in various crops. Chitin and inorganic fertilizers that increase ammoniacal nitrogen into the soil suppresses various pathogens due to plasmolyzing effects and to selective proliferation of microbial antagonism (Rodgruez-Kabana *et al.,* 1987). Organic and inorganic amendments have been applied to soil to suppress plant pathogens and to increase yield (Papavizas *et al.*, 1963 and Mannig *et al.,* 1969). Decomposing green plant amendment singly increased the total number of rhizosphere fungi, *Streptomyces* as well as the total number of bacteria and suppressed the Rhizoctonia disease of beans (papavizas *et al.*, 1963; Mannig *et al.,* 1969). Mannig *et al* (1969) reported a decrease in hypocotyls rot of snap beans both in greenhouse and field after amendment with decomposing green maize manure.

Miller *et al* (1966, 1968) and Kirmani *et al* (1975) found that the C: N ratio of organic amendments influences the effect of the various amendments on pathogenesis and disease development with most of the soilbone pathogens including root-knot nematodes being favoured by high nitrogen content relative to carobon. Eno *et al* (1955) examined that different forms of nitrogen such as anhydrous and hydrous forms of ammonia released by organic residues during decomposition determine their relative effectiveness against various soilborne pathogens.

Papavizas *et al* (1963) reported that all the 5 decomposing green plant amendments singly increased the total number of rhizosphere fungi and *streptomyces*, as well as the total number of bacteria and suppressed the Rhizoctonia disease of beans 3-7 weeks after incorporation of the amendments (Papavizas *et al.,* 1963). Decomposing green maize and oats produced the highest number of *Streptomyces* antagonistic to *R. solani* and their effectiveness lasted longer than that of other amendments and gave the susceptible host the highest and longest protection. Sudan grass and no amendment resulted in the least suppression. Green French beans and no amendment produced average results. In the laboratory each amendment retained its relative position in the field (Papavizas *et al.,* 1963). Crop residues of alfalfa, lettuce and cotton gin trash increased root rot severity of Pinto beans caused by *Rhizoctonia solani* and *Fusarium solani phaseoli* whereas residues of barley, sorghum, soybean and Sudan grass reduced it (Maker *et al.*, 1965). Tomato, maize, sesame, cotton and bean plant residues either produce inconsistent effects or did not alter the disease severity. Saadia *et al* (2007) reported a remarkable reduction in the percentage of cotton damping off caused by *R. solani* by addition of fish meal to soil infested with the pathogen.

The ability of fully decomposed animal manure such as cow manure to support microorganisms depends on the quantity of biological energy present in them, mostly in form of cellulosic substances (Hoitink *et al., 1997*).

Soil solarisation, covering moist soil with clear plastic is a nonchemical soil pest management practise that have reduced the population of some soilborne pathogenic fungi, bacteria and nematodes as well as weed seeds (Overman, 1985; Mcsorley *et al.,* 1986; Gaur *et al.*, 1991; Chellemi *et al.*, 1993). Solarisation has been found to be effective against *Rhizoctonia,*

Verticillium, Fusarium and nematodes in tomato, eggplant, potato, onion and weeds (Katan, 1981). High efficacy of solarisation is achieved when 25-30μm thin polythene tarps are used during period of high temperature and on wet soil and if done for at least four weeks (Byrdson, 1970; Katan *et al.*, 1976; Pullman *et al.*, 1976; Elad *et al.,* 1980; Herowitz and Reger 1980). Soil solarisation is most successful in loamy to clay soils within arid and semi-arid regions with intense sunlight and minimal rainfall. In sandy soils with poor water holding capacity and rapid drainage, heat transfer and pest control may be inhibited in deeper soil horizons (Overman, 1985 and Mckenry, 1987).

Apart from thermal inactivation of the pathogen, solarisation induced bio-control may affect the pathogen by raising its vulnerability to soil micro-organisms or increasing the activity of soil micro-organisms towards pathogens or plant which finally leads to decrease in the disease incidence, pathogen survivability or both through mechanisms such as antibiosis, lysis, parasitism or competition (Parpavisas *et al.*, 1980). Organic matter decomposition products such as ammonia, carbon dioxide among others that are toxic accumulate in soils mulched with polythene paper and this may play a role in pathogen decline (Byrdson, 1970). Analysis of solarized soils of various types showed a significant increase in Calcium and Molybdenum ions concentrations as compared to non-treated soils (Chen and Katan, 1980). Since Calcium ions play a role in plant response to various pathogens, it is worthwhile to evaluate the response of plant grown in solarized soils in future research (Chen and Katan, 1980).

Because solarisation alone is unlikely to substitute Methyl bromide, an integrated system with solarisation in combination with other strategies such as seed treatment, solar heated water, soil amendment and biocontrol must be considered (Noling et al., 1994). Alex *et al* (1993) reported

control of Fusarium crown and root rot of tomato with *Trichoderma harzianum* in combination with sublethal dosage of Mbr or solarisation. The high cost of labour and land fill charges to remove and dispose off the plastic mulch also suggests that new recycling technique or spraying biodegradable mulches will be developed and evaluated (Noling *et al.,* 1994).

2.5.2. Chemical control methods

There are many broad-spectrum and highly specific pesticides that effectively control root rot pathogens and their diseases (Munnecke et al., 1979; Sherf and Macnab, 1986). Fumigant soil fungicides such as Methyl bromide, biogas, methyl iodide, toluene and nonfumigants such as metham sodium, vortex, D-D and chloropicrin are highly effective biocides that kill all the pathogens as well as weed seeds (Munnecke *et al.*, 1979; Furgerson *et al* 1994). This approach is highly effective in controlling economically important soilborne diseases such as root rot and wilt but its limited by safety considerations, the need for complicated equipment and highly trained personnel, high cost, pesticide residues, phytotoxicity and re-infestation of the soil resulting from drastic reductions in antagonistic microorganisms (Danson *et al.*, 1965).

However, of particular concern in the chemical control of *Rhizoctonia* and other soilborne pathogens is the recent decision by environmental protection agency of the United Nations to ban the use of fumigant fungicide methyl bromide in agriculture by 2015 due to its hazardous effect on the environment (Watson *et al.*, 1992; Noling and Becker, 1994). Most studies that have been performed to study nonfumigant fungicides and nematicides have not always been consistent either for controlling intended pests or for obtaining consistent returns to the grower particularly when compared with conventional preplant mulched fumigation with Mbr (Noling and Becker, 1994).

Fungicides used as seed dressing or soil treatment should have a broad spectrum of activity to control the wide range of pathogenic strains in a given production location (Sherf and Macnab, 1986). Applying copper fungicide controls Rhizoctonia damping off diseases in horticultural crops (Royal Horticultural Society, 2009). Rhizoctonia root rot have been controlled by applying quintozene, pencycuron, pentachloro-nitrobenzene, terraclor and thiram (Crossman, 1969; Briston *et al.,* 1973; NIK *et al.,* 1979; Maramba, 1982 and Lewis *et al.,* 1983). However not all isolates of *Rhizoctonia* are controlled by a certain active ingredient, raising the need to apply a combination of active ingredients where several isolates exist. Summer (1987) reported differential control of *R. solani* in the greenhouse by pencycuron. From 14 isolates of *R. solani* and binucleate –like fungi, pencycuron at 100, 1.0, and 0.1 micrograms/gram of soil controlled all but one isolate of *R. solani* that was resistant. Pozo (1988) reported the iniability of the fungicide benzyltriazole at different rates, thiabendazol, thiram and carboxin thiram used as seed dressing to control *Rhizoctonia*, *Sclerotium* and *Fusarium* root rot in beans variety caricola. Captafol, trade name Captan controls most pathogens, but not *Rhizoctonia* (Damping off Wikipedia, 2008).

Most studies that have been conducted to evaluate seed dressing have not always been consistent, either for controlling the disease or for obtaining consistenct economic returns to the grower, particularly when compared with conventional preplant mulched fumigation with Mbr. This is due to their biodegradation after continued use and narrow spectrum of activity (Noling and Becker, 1994). Because many are reasonably mobile and readily leached, particularly in sandy low organic soils, additional research will be required to minimize environmental risks (Hornsby, 1991, and Norris *et al.,* 1991) while maximizing root zone retention.

2.5.3. Biological control methods

Considerable information is available on the influence of antagonists, competitors, mycoparasites and predators of soilborne fungal and nematode pathogens (Backman *et al.*, 1975). Several soilborne fungi and bacteria have been reported to be antagonistic to *R. solani* and reduces damping off incidence and severity in many vegetable and field crops (Hadar *et al.*, 1979; Cardoso *et al.,* 1987; Crsistiane, 2008). *Rhizoctonia solani* has been controlled in the laboratory and the damping off disease it causes in several crops under greenhouse and field conditions by *Trichoderma* species, binucleate *Rhizoctonia* like fungi, *Gliocladium* species and *Laetisaria arvali* (TU and Vaartaja, 1982; Hadar *et al.*, 1979; Huber *et al.*, 1966).

Crsistiane (2008) reported biological control of *R. solani* on Soybean with *Bacillus pumilus*. Saadia *et al* (2008) examined biological control of Rhizoctonia damping off on cotton by 4 isolates of Actinomycetes and 8 isolates of bacteria introduced by addition of fish meal to the pathogen infested soil. Cardoso *et al* (1987) reported biocontrol of Rhizoctonia root rot of snap beans by 4 binucleate *Rhizoctonia* isolates as shown by significant ($P \leq 0.05$) reduction in disease incidence and severity under greenhouse and field conditions. Asaka *et al* (1996) repoterted an isolate of *Bacillus substilis* RB14 which produced antibiotic Iturin A and surfurin controlled *Rhizoctonia* under invitro conditions and suppressed damping of tomato under greenhouse conditions. Krishnamurthy *et al* (1996) reported control of *Rhizoctonia solani*, the rice sheath blight pathogen by *T. viride* and *T. harzianum.*

Hennis *et al* (1968) documented that 8 out of 10 strains of *Bacillus subtilis* and 1 out of 8 strains of *B. licheniformis* inhibited growth and reduced sclerotia formation in *Rhizoctonia*. Durman *et al* (1999) reported that 4 out of 5 isolates of *Trichoderma* species controlled

R. solani in the laboratory and reduced growth and survival of sclerotia in the greenhouse condition. Reduction of Rhizoctonia root rot severity in beans has been demonstrated with *Trichoderma* species, binucleate *Rhizoctonia*-like fungi, *Gliocladium* species, *Laetisaria arvalis*, as biocontrol agents (Papavizas, 1985). Duijf *et al* (1988) reported suppression of Fusarium wilt of tomato by *Pseudomonas fluorescens* WCS4117r and by non-pathogenic *Fusarium oxysporum* Fo47.

Mechanisms of biological control are numerous and one or more of them can be involved in any given situation. They include competition for substrates, antibiosis, mycoparasitism, cross protection and induced resistance (Chester, 1933; Kuc *et al.,* 1959; Papavizas *et al*., 1985; Fravel, 1988; Duijf *et al.,* 1988; Baker *et al*., 2001) Furthermore, reduction of bean root rot severity by incorporating organic amendments and the use of crop rotation may well be a result of enhancing beneficial soil-borne micro-organisms (Abawi *et al.,* 1990). Papavizas (1963 and 1985) and Mutitu *et al* (1988) reported a substantial reduction of microbial antagonism in bean rhizosphere as a result of soil amendment with oat straw and supplement nitrogen. This could be a result of competition resulting from increased number and type of microorganisms due to this amendment.

2.5.4. Host resistance

Resistance is the ability of a plant to rermain relatively free from disease because of its inherent structural or functional properties (Onkar *et al.*, 1986). Resistance is an active dynamic response of a plant to a pathogen and it excludes passive phenomena like immunity and disease escape (Onkar *et al.,* 1986). Genetic resistance to various fungal pathogens such as *Verticillium dahliae*, *Fusarium oxysporum f.sp lycopersici* are known for various tomato cultivars and

varieties (Danesha *et al.*, 1999). The highest levels of resistance have been identified for the so called "specialized" soilborne pathogens such as *F. oxysporum fsp solani* and *Sclerotium rolfsii* and low levels of nonspecialised pathogens such as *Rhizoctonia* (Beebet *et al.*, 1981).

Hurbert *et al* (2008) examined resistance to two (AGs) groups of *R. solani* among various Brassicae species and varieties under greenhouse conditions. Danesha *et al* (1999) reported that out of the 10 tomato cultivars that are grown commercially in East Tennessee, only 2 were intermediately resistant to the local strains of *R. solani*. Stockwell *et al* (1987) reported resistance in beans (*Phaselous vulgaris*) variety red kidney to *Rhizoctonia solani* isolate PB three weeks after planting. The changes in bean resistance to *R. solani* with age was associated with elongation and maturation of the hypocotyls and concomitant changes in the pectic substances and calcium content of cell wall (Bateman *et al.*, 1965). Cardoso *et al* (1994) and Haran *et al* (1996) used molecular techniques to show that lignification of host cell wall was the main mechanism of resistance to *Rhizoctonia* invasion in tomato and beans. Panella *et al* (1995) registered two sugar beet germplasms resistant to *Rhizoctonia solani*.

Induced resistant and cross protection may also be involved in biological control of *Rhizoctonia* in various crops (Duijif *et al.*, 1988; Kuc, 2001). Induced resistance theory was suggested in the 19th century and its existence was confirmed by Kuc *et al* (1959) and Chester (1933). Two kinds of induced resistance to disease are recognised in plants depending on the inducing factor. Systemic acquired resistance (SAR) is activated after infection by a necreotising pathogen rendering distant, uninfested plant parts resistant towards a broad spectrum of normally virulent pathogens (Ross, 1961; Kuc, 1982; pieterse *et al.*, 1996). The resistance induced by nonpathogenic microorganisms such as rhizosphere bacteria is called induced

systemic resistance (ISR) (Van loom *et al.,* 1998). SAR is similar to ISR in that the induced resistance is systemically activated, it extends to aboveground plant parts and it is effective against a broad spectrum of plant pathogens (Van loon *et al*., 1998). ISR is most effective against fungi (Kuc, 2001). Mwangi *et al* (2002) reported rhizobacterium induction of systemic resistance in tomato against *Fusarium oxysporum fsp lycopersici.*

CHAPTER THREE

DISTRIBUTION AND OCCURRENCE OF DAMPING OFF AND ROOT ROTS OF TOMATO IN MAJOR PRODUCTION REGIONS OF KENYA

3.1: Abstract

The present survey was conducted in the year 2002-2003 to determine the current status on the prevalence, incidence and severity of Rhizoctonia damping off and root rots of tomato in the major tomato growing agro-ecological regions of Kenya spread in 9 districts and 3 provincies.

The survey results indicated that in the recent past, Rhizoctonia damping off of tomato have increased in importance with up to 30% yield loss reported in most production areas. Damping off of seedlings was the commonest aspect of infection observed on tomato and other vegetables both in the nursery and field conditions. Most commercial tomato varieties were susceptible and no disease control method was being practiced. The disease prevalence, incidence, severity and the number of pathogenic *R. solani* isolates were significantly ($P \leq 0.05$) higher in the low to medium altitude agro-ecoological zones than the high altitude agro-ecological zones; during the wet season than the dry season; in the nurseries than in the field; in irrigated fields than rainfed and where cow manure was applied than in the fields with no manure.

Though *R. solani* was the most frequently isolated pathogen by the general and specific methods of Tsao (1970) and Ko and Hora (1971) respectively, other soilborne pathogens including *Fusarium, Pythium* and *Pseudomonas* bacteria were also isolated from Rhizoctonia infected tomato and rhizosphere soil. This implied the possibility of synergism involving several soilborne pathogens in causing the damping off disease complex on tomato.

The *R. solani* isolates differed on various cultural and morphological characteristics and pathogenicity on the common tomato varieties indicating that they could be genetically different.

3.2: Introduction

Kenya has a surface area of approximately 580,000 Km^2 and the land rises from the sea level through Savannah or Nyika woodlands to cool highlands ascending to the snowy peaks of Mt. Kenya at a height of 6000 meters above the sea level (Jaezolds *et al.*, 1983).

Kenya is hot and humid at the coast, temperate inland and very dry in the north and northeast parts of the country. The average annual temperature for the coastal town of Mombasa at altitude 17m is 30.30^{0}C maximum and 22.40^{0}C minimum; the capital city, Nairobi at altitude 1,661m is 25.20^{0}C maximum and 13.60 ^{0}C minimum; Eldoret at altitude 3,085m is 23.60 ^{0}C maximum and 9.50 ^{0}C minimum; Lodwar at altitude 506m and the drier north plain-lands is 34.80 ^{0}C C maximum and 23.70^{0}C minimum (Jaezolds *et al.*, 1983; Kenya Agricultural Research Institute, 2002).

The rainfall characteristic over most parts of the country is strongly seasonal although its pattern, timing and extent vary greatly from place to place and from year to year. The relatively wet coastal belt along the Indian ocean receives more than 1,000 mm of rainfall per year much of which falls during the long rainy season from March to May as a result of the south-easterly monsoon. The short rains occur from October to December.

Being at the equator, there are no defined seasons as in the temperate areas of the world where the seasons are well defined into summer, spring, autumn and winter. On the contrary the temperature regime is determined mainly by the altitude (Jaezolds *et al.,* 1983). The rainfal mainly influences seasons. Kenya has a bimodal rainfall pattern with long rains from March to May which to an extent carries on into June and reverts to drizzles in July whereas the short rains season start from October to November (Jaezolds *et al.,* 1983; KARI, 2002).

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The incidence and severity of the damping off diseases and their resultant damage have been observed to vary considerably depending on the prevailing environmental and soil conditions and from the number and type of damping off pathogens that are present and active in inciting disease under given conditions (Abawi *et al.*, 1990; Royal Horticultural society, 2009). Several soilborne and foliar diseases attack tomato and other vegetables in Kenya resulting in severe yield reduction (KARI, 2002; Mwangi, 2002); the important soilborne pathogens including *Rhizoctonia solani, Fusarium, Pythium* and plant parasitic nematodes especially of the genera *meliodogyne* and bacteria wilt cause considerable yield losses especially in light textured soils (Samson, 1997; Githinji, 2005; Mwangi, 2002). Species of all these soilborne pathogens can attack singly or in combination resulting to greater damage than when they attack separately resulting in root rot disease complexes in various vegetables (Abawi *et al.,* 1900; Hill, *et al.,* 1988; Githinji, 2005).

Rhizoctonia is a cosmopolitan soilborne pathogen that occurs in all natural soils especially in association with decomposed organic matter (Carol *et al.*, 1986; Abawi *et al.,* 1990). The pathogen has a wide host range in most vegetables and field crops with infection being favoured by warm and moist conditions during the growing seasen (Koike, 1996; Sneh *et al.*,

1996; Royal Horticultural society, 2009). *Rhizoctonia solani* may attack germinating seeds, young seedlings before or after emergence and established plants (Abawi *et al.*,1990; Danesha *et al.*,1999). Accordingly, it is important to determine the incidence and severity of *Rhizoctonia* infection at different times during the growing season (Abawi *et al.*, 1990).

Since aboveground symptoms are not usually diagnostic for most root rot pathogens and are often delayed in appearance, it is necessary to determine the severity and at times incidence by employing destructive sampling technique (Abawi *et al.*, 1990). Such sampling technique involves the digging up of representative plants and the careful washing of their roots.

In addition a large number of plants collected from several locations from field plots or commercial fields are usually needed for accurately assessing Rhizoctonia root rot incidence and severity (Abawi *et al.*, 1990; Danesha *et al.*,1999). Root rot pathogens are unevenly distributed in the field, and thus their disease incidence is variable. Thus the assessment of the disease incidence and even at times disease severity may not reflect the real damage or effect on plants. This possibility dictates the measuring and collecting of data on the effect of of the pathogen on crop growth and yield parameters (Abawi *et al.*, 1990; Alex *et al.*, 1993).

The objective of the present survey was to assess the occurrence and distribution of Rhizoctonia damping off of tomato from the major production agro-ecological regions of Kenya. The information gathered will shed light on the economic importance of the disease on tomato and other vegetables, the effect of various crop production and environmental factors on disease development and the success of disease control methods used.

3.3: Materials and methods

3.3.1. Survey of root rots and damping off

The prevalence, incidence and severity of Rhizoctonia damping off of tomato was assessed from the main tomato producing agro-ecological zones spread in 9 administrative districts and 3 provinces. The districts covered were Nyeri, Kiambu, Maragua and Kirinyaga from central province, Makueni, Embu and Meru Central from Eastern province, and Kajiado and Nakuru from Rift Valley province. The survey was done during the dry season of July-October 2002 and the wet season of March-May 2003. The same fields were surveyed during the two seasons. The targeted area covered 5 agro-ecological zones, upper midland 1 (UM1), upper midland 2 (UM2), upper midland 3 (UM3), upper midland 4 (UM4) and Lower midland 3 (LM3) with altitude ranging from 671 to 2254m above sea level, stretching from E34°56[1] to E38°30[1], and S00°18[1] to S02°45[1](Table 1).

Table 1: Average altitude and climate in the agro-ecological zones covered by the survey

Agro-ecological zone	Altitude (m)	Annual mean Temp (^{0}C)	Annual average rainfall (mm)
UM1: High potential/Cereal/Coffee-Tea	1590-1830	18.9-17.5	1400-1800
UM2: Marginal agriculture/Mainly Coffee	1400-1590	20.1-18.9	1200-1500
UM3: Marginally Coffee	1280-1460	20.7-19.6	1000-1250
UM4: Agropastoralists/Sunflower –Maize	1280-1400	20.7-20	980-1100
LM3: Mainly pastoralists/Sorghum	500-1000	25-31	250-700

Source: Jaezold, R and Schmidt, H., 1983. Farm management Handbook of Kenya, Ministry of Agriculture.

The number of fields surveyed were sub-divided among the administrative divisions of the 9 districts depending on the size and the density of tomato production in each district. Selection of locations in each division was done at random. Field selection within each division was done

at 2-5 km intervals along a road. Sampling was done both in nursery and the field. For each field visited, fifty plants were randomly picked by making two diagonal transects across the field in the form of 'X'. Twenty five plants were picked along each diagonal. Tomato plants with the symptoms typical of *Rhizoctonia* damping off/wirestem were counted and disease incidence expressed as a percentage of the total counted. Intervals between sampled plants depended on the size of the field or nursery but at least 5 points were sampled. The distribution pattern of the Rhizoctonia damping off disease and other diseases and insect pests, their association with low or high areas and the uniformity of infection were determined. At each point three diseased plants of 1-6 weeks were randomly selected for assessment of *Rhizoctonia* infection. This involved carefull digging out of the plants with a shovel and washing the soil to reveal the characteristic root symptoms for recording. The stem and lower stem tissue for each sampled plant was observed with naked eyes and with the aid of a magnifying glass for distinct symptoms and structures of potential pathogens. For any wilted plants, preliminary diagnosis was done by uprooting the plants and either spliting open the stem using a knife to reveal any vascular discolorotion or cutting one end and the cut end immersed in water to check for any milky exudates. This helped in the initial assessment of the cause of wilting.

The disease assessment was based on damping off prevalence, incidence and severity rating of the sampled fields. The disease assessment protocol was adopted from Danesha *et al* (1999) and Abawi *et al* (1990). Damping off prevalence was determined by counting the number of infected fields per site, field, district or agro-ecological zone and finally expressing the value as a percentage of the total fields sampled. Damping off incidence was determined by counting the number of emerged but dead seedlings or those exhibiting the root rot symptoms per site and then total number of all the infected plants per field calculated as a percentage of the total

plants sampled. Finally the mean incidence for all the sampled farms per district or agro-ecological zone were calculated.

Disease severity was based on the disease level of each plant sampled and the mean value for each field calculated. The following scale of 1 to 4 adopted from Abawi *et al* (1990) and Danesh *et al (*1999) was used to categorise the various infection levels, where 1=no disease/healthy seedling, 2=diseased seedling, 3=post-emergence damped-off/dead seedling, and 4=pre-emergence damped off seedling. The number of established plants per field at flowering were counted and finally expressed as a percentage of the total planted to give the stand count.

For each field sampled, the stem and root portions of 1-3 plants showing the characteristic *Rhizoctonia* symptoms together with 100g of rhizosphere soil collected from upto 10cm deep were bulked together into paper bags and taken to the laboratory for isolation, identification and characterization of the pathogen(s) involved. The sampling method was adopted from Johnson and Curl (1972). For each field sampled global positioning system was used to give the exact altitude, longitude and latitude of the sampled site. Sampling favored localities where tomato production is highest while ensuring that wide range of agro-ecologies and socio-economic types of farms were included. Other supplementary information such as age of the crop, host range, variety, planting methods, soil fertilization, cropping patterns, disease control methods and source of seeds were recorded. Appended structured questionnaire was used to get adequate information from the growers (appendix 1.0).

3.3.2. Isolation of root rot and damping off pathogens

This was done from 222 infected tomato plants and 222 soil samples from the rhizosphere. Isolation was done immediately the samples were taken to the laboratory. Both the general and specific methods of Tsao (1970) and that of Ko and Hora (1971), respectively were used. Root and stem sections with early stages of infection were selected, washed under running tap water for 15 to 30 minutes. Then the infected tissue was surface sterilized by dipping in dilute solution of 0.5% sodium hypochlorite for 2 - 5 minutes and then rinsed with distilled water and blot dried between two filter papers. The surface sterilized infected pieces or segments from the margin of the lesions were placed onto APDA (Appendix 9.6) plates containing 1.0ml lactic acid for 1 litre of media and incubated at 22-25 °C in the dark. *Rhizoctonia solani* grew very fast at this temperature and usually the hyphal tip transfer was made within 24 hours after plating.

Isolation of soilborne pathogens from soil was done by placing 10g of soil in an Erlenmeyer flask with 100 – 200ml of water and mixing thoroughly for 2 – 3 minutes. After waiting for a few seconds, the suspension was decanted onto a 50 mm mesh with 300 µm pores screen. Similarly the original soil was repeat washed 2 – 3 times and decanted over the same screen. All the accumulated material was collected on the screen on a paper towel or filter paper, divided into 30 parts and 10 parts placed in each of three petri dishes containing APDA. The setting was incubated at 22-25°C and examination done after 16 to 24 hours for *Rhizoctonia solani* and *Rhizoctonia*-like colonies using the diagnostic features of the mycelium. Where the number of colonies on each plate were too high or too low, the procedure was repeated using 5g or fewer or 20g or more soil per sample.

Pure isolates of *Rhizoctonia* pathogen were obtained by using the specific method of Ko and Hora (1971). The modified Ko and Hora medium was made by adding K_2HPO_4 (1g), $MgSO_4.7H_2O$ (0.5g), KCL (0.5g), $FeSO_4$. $7H_2O$ (0.01g), $NaNO_2$ (0.2g), chloramphenicol (0.05g) and agar (20g) in 1000 ml distilled water. After sterilization in autoclave at 121°C, 15 psi for 20 minute, the medium was cooled to 50°C, after which gallic acid (0.4g), streptomycin (0.05g), metalaxyl (0.2532g of Ridomil 2E: 25%), prochloraz (0.0131g of Prochloraz 38.1%) were added. After 24 -48 hours of incubation at 26 °C, single hyphal tip from the margin of each developing colony was placed on acidified potato dextrose agar medium.

3.3.3. Identification of the root rot and tomato damping off pathogens

This was done based on the cultural and morphological characteristics of pure isolates on artificial media. *Rhizoctonia solani* was identified from cultural and morphological features taken on cultures grown on APDA in the dark over a period of 3 weeks according to the procedure by Ogoshi (1987) and Parmeter (1970). Characters such as color, growth form and rate were determined on cultures by use of naked eyes whereas mycelial type was determined under the light microscope at x100 magnification under oil immersion.

Nuclei count was done for identification of the isolates into multinucleate *Rhizoctonia solani* and binucleate Rhizoctonia-like fungi using the procedure adopted from Carol and Dona (1998). The multinucleate *R. solani* have more than two nuclei per hyphal tip cells whilst binucleate Rhizoctonia-like fungi have two nuclei per hyphal tip cells. The number of nuclei per hyphal tip cell was determined by staining vegetative cells with 1µg/ml 4, 6-diamidino-2-phenyl-indole (Cardoso *et al.*, 1987). Twenty randomly selected cells of each of the 76 isolates

were examined using a flourescent microscope at x300 maginification to count the number of nuclei. The random sampling and counting was replicated 5 times and the mean calculated Radial growth was determined by inoculating APDA plates in triplicates with *Rhizoctonia* isolates. The cultures were maintained at 25°C in the dark, and radial growth was recorded every second day until the colony reached the edge of the plate. Growth rate was expressed as increase in colony diameter per day.

Fusarium identification was done under the microscope from 14 days old cultures on PSA (Appendix 9.8) and was based on morphological and reproductive structures (Booth, 1971). *Pythium* identification was done under a microscope from a 7 days old culture on PDA and was based on morphological, reproductive and vegetative structures (Plaats-Niterink, 1981). Identification of *Pseudomonas* bacteria was frm 36 hours old cultures on NA (Appedix 9.7) and was based on the colony and morphological characteristics such as cell shape, motility and flagellation, gram stain reaction and catalase test on glucose fermentation using Hugh and Leisfson oxidation/fermentation procedure (Krieg, 1984).

3.3.4. Pathogenecity test of damping off pathogens on tomato

Pathogenicity test with the representative cultures of each of the soilborne pathogens obtained from infested rhizosphere soil and infected tomato plants were done on 3 tomato varieties grown in Kenya under greenhouse conditions. The tomato varieties used were Onyx VF2, Cal-J VF and Caltana F1 VF hybrid. These tomato varieties were the most commonly grown by farmers and displayed the *Rhizoctonia* damping off symptoms during survey.

3.3.4.1. Experimental design and layout

The experiment was designed as a 3 by 82 factorial in a completely randomized block design with 5 replicates. The 3 tomato varieties were the factors with the 76 *Rhizoctonia* isolates coded as R1-R76, 2 *Fusarium* isolates coded as F1 and F2, 1 *pythium* isolate coded as P, and 2 *Pseudomonas* isolates coded as Pb1 and Pb 2 as the treatments with the uninfested as the control. Randomisation of the 2nd, 3rd, 4th and 5th replicates was done in the greenhouse during trial set up. Two experiments were concurrently conducted. Ten seeds of each tomato variety were planted per 8-cm diameter polythene pot containing sterilized soil mixture (sand: manure: soil=3:1:1). The pots were watered to about 70% field capacity daily and this translated to about 250-500cm^3 per pot. Infection by blight was controlled by regular foliar spray of propinep, trade name Antracol 70 WP. The experiment was repeated twice. The tomato seeds used in this experiment and in the future studies were purchased from a reputable local dealer; Regina seeds company. The tomato seeds used were not dressed with any fungicide and had a high germination rate. Prior invitro germination test showed each variety had over 98% germination.

3.3.4.2. Prepararation of inoculum and inoculation

Rhizoctonia inoculum was prepared by mixing 50g of finely chopped potato with 500cm^3 of light textured soil and placed in a 1-litre flask. The mixture was autoclaved for 20 minutes at 121^{0}C and 15 psi and cooled. Under aseptic conditions, three small mycelial agar discs obtained from the margin of each of the young colonies of Rhizoctonia isolates growing on APDA plates were transferred to the mixture. Each culture type was produced in triplicate. The containers were incubated at 22-25°C for 12 days at the end of which all the containers were emptied and the infested soil mixed thoroughly to make soil-potato inoculum. Concentration of

R. solani inoculum was done by the soil dilution plate method as following: Ten grams of Rhizoctonia colonised soil-potato mixture was suspended in 90ml sterile distlled water in an Erlenmeyer flask and shaken for 30 minutes on a shaker at 300-500 rpm. While the suspension was in motion, 10ml was withdrawn and added to 90ml of sterile water blank in a conical flask. The procedure was repeated upto 10^{-6} dilution. For each of the last three dilutions 0.1ml of the suspension were mounted under a microscope and mycelia fragments determined by use of a haemocytometer. Three samples were taken per isolate and the mean number of mycelial fragments per 0.01ml concentration calculated at each dilution. This was multiplied by 10 to give the number per ml. To get the number of mycelial fragments per ml of stock suspension, each of the three averages were multiplied by the reciprocal of the dilution of 10^{-5} to 10^{-7} respectively. Where the the fragments were too few or too many the procedure was repeated with few (2- 8g) or more (15-20g) of the potato-soil inoculum. The concentration was adjusted to 10^{6} mycelial fragments per gram of the soil-potato inoculum and 2- 4cm of the soil-potato inoculum added to cover tomato seeds planted on steam sterilized potting soil mixture in the greenhouse.

Inoculum of *Fusarium* pathogen was readily produced on APDA plates after 2 weeks of incubation at 25°C. Spore suspensions were then prepared by adding about 5 ml of distilled water to each APDA plate and scraping the surface of the pathogen culture with a sterile glass slide. The suspension was then passed through four layers of cheesecloth to remove mycelial fragments. The spore suspension was then cleaned by centrifuge at 3000-5000 rpm for 30 seconds, the supernanant discarded, and the spore pellet suspended in distilled water and centrifuged again. The spore pellet was re-suspended in water. Spore suspension prepared in

this manner consisted mostly of macroconidia, microconidia and a few clamydospores. Spoe concentration was determined by the viable count method as follows: Serial dilution of upto 10^{-7} was prepared by taking 1 ml of suspension into 9ml distilled water repeatedly for 7 times. From the dilutions of 10^{-5} to 10^{-7}, 1 ml of the well agitated suspension was transferred to a sterile petri dish using a one mililitre pipette. This was done in triplicate. Into each seeded petri dish, about 12ml of cooled PDA at 42^{0}C was poured and swirled in both anti-clockwise and clockwise direction by hand to ensure through mixing. The plates were incubated at room temperature for one week and *Fusarium* colonies that developed were counted. Three samples were taken per isolate and the mean number of spores per 0.0ml concentration calculated at each dilution. This was multiplied by 10 to give the number per ml. To get the number of fungal propagules per ml of stock suspension, each of the three averages were multiplied by the reciprocal of the dilution of 10^{-5} to 10^{-7} respectively. The concentration was adjusted to 10^{6} spores per ml. Spore suspension containing 10^{6}/ml of each isolate was used to inoculate tomato seeds planted in steam sterilized soil mixture in the greenhouse by placing 5ml of the inoclum suspension into a 3cm deep hole made at about 2cm from each seed.

Pythium was cultured on cornmeal agar containing cornmeal l40g, agar 15g and distilled water 1000ml. The *Pythium* inoculum for inoculation was obtained by flooding 6 days old pure cultures with 5ml distilled water per plate and then scrapping the surface with a sterile microscopic slide. The resultant mycelial suspension was transferred into a sterile test tube and sonicated for 30 seconds. This suspension was filtered through double layer cheesecloth and the hyphal fragments adjusted with sterile distilled water to 10^{5} propagules per ml using a haemocytometer (Dickson and Abawi *et al.,* 1974). Inoculation was done as with *Fusarium.*

The *Pseudomonas* bacteria was cultured on Nutrient agar (Appendix 9.7) at 23 $\pm 2^0$ C for 36 hours while continuosly shaking at 100 rpm. To obtain cells the cultures were centrifuged at 5000x g for 20 minutes at 6^0C and the pellet resuspended in 0.1M $MgSO_4$. For each isolate, the cell suspension in the solution was adjusted to OD_{560}=2, that represent a concentration of about 10^8 cfu per ml and 15 ml of this concentration used per each pot with sterile soil mixture. Soil inoculation procedure used was as described for *Fusarium.*

3.3.4.3. Disease assessment

This was based on incidence and severity of infection by each of the *Rhizoctonia, Fusarium, Pythium* and *Pseudomonas* isolates on tomato variety. Each variety was assessed individually and the mean for the 3 varieties per treatment for the 2 experiments calculated. The ambient temperature inside the greenhouse during the trial period was 18-28°C.

Rhizoctonia damping off incidence and severity was assessed using the procedure adopted from Danesha *et al* (1999) and Abawi *et al (*1990). Damping off incidence was based on the number of emerged but dead seedlings or those exhibiting the root rot/wirestem symptoms per replicate. The total number of the replicates were tallied and a cumulative percentage survival of the total planted calculated at the end of 1st, 2nd, 3rd, 4th and 5th weeks after planting. Two assessments per week at 3 days interval were done and the mean for the two consecutive assessments done to get the weekly tally. Rhizoctonia damping off severity was estimated at the end of the 5th week after planting by destructive sampling of all the plants. The scale of 1 to 4 adopted from Danesha *et al (*1990) was used to categorise the various infection levels where 1=no disease/healthy seedling, 2=diseased seedling, 3=post emerged damped-off/dead seedling and 4=pre-emergence damped-off seedling.

The incidence and severity of infection by *Fusarium* pathogen was assessed the same way as for the *R. solani.* The number of seedlings showing the characteristic Fusarium root rot symptoms were counted weekly. At flowering, the number of plants showing the characteristic yellowing of leaves caused by *Fusarium* were counted and the percentage of the total plants calculated. Severity of *Fusarium* infection was estimated at the end of 5th week after planting by the destructive method. The characteristic brownish vascular discoloration was checked by split opening stems of plants showing chlorosis or wilting symptoms.

Infection assessment protocol by *Pythium* isolate was as for the *Rhizoctonia* isolates. Infection assessment by *Pseudomonas* isolates was as for the *Fusarium*. Presence of milky bacterial exudates was checked by cutting one end of wilted plants and dipping the cut end in water.

3.3.5. Preservation of damping off pathogens

Pure strains of *Rhizoctonia solani* were grown on sterilized barley grains in test-tubes and stored in a refrigerator where they could be retrieved for further studies (Carol and Donna, 1998). Ten grams of sieved barley grains was placed in universal bottles (about 2/3 full). The bottle and seeds were then autoclaved twice at 121^{0}C and 15 psi for 15 minutes at two days interval. A mycelial suspension was prepared by flooding each plate with 5ml of sterile distilled water and gently scrapping the surface of the colonies with an edge of a glass slide to dislodge the mycelium. Two mililitre of this suspension was poured over the sterilized barley grains in the universal bottles aseptically. The tops with their rubber liners were left loosely screwed for two weeks at room temperature to allow the fungus to grow. The caps were then tighted and the cultures stored in the refregirator at 4^{0}C. Each isolate was preserved in triplicate. All *Rhizoctonia* cultures stored on barley grains were maintained by re- inoculating

onto new tubes of sterilized barley grains each year. Retrieval was by placing one gram (about 5 particles) of barley grains from the bottle and sprinkling them into APDA and specific medium of Ko and Hora plates. Sub-culturing was done as soon as *Rhizoctonia* grew out from the barley grains. Identification was done on pure cultures as described in section 3.3.3 to ascertain they were the same isolates preserved.

Fusarium was stored in finely sieved sterile sandy soil (Booth, 1971). Ten grams of sieved loam soil was placed in universal bottles (about 2/3 full). The bottle and soil were then autoclaved twice at 121^0C and 15 psi for 15 minutes at two days interval. A spore suspension was prepared was prepared by flooding the plate with 5ml of sterile distilled water and gently scrapping the surface of the colonies with an edge of a glass slide to dislodge the spores. Two mililitre of this suspension was poured over the sterilized soil in the universal bottles aseptically. The tops with their rubber liners were left loosely screwed for two weeks at room temperature to allow the fungus to grow. The caps were then tighted and the culture stored in the refregirator at 4^0C. Each isolate was preserved in triplicate and maintained by inoculating onto new tubes of sterilized soil each year. Retrieval was by placing one gram (about 5 particles) of soil from the bottle and sprinkling them onto PDA plates. Sub-culturing was done as soon as *Fusarium* grew out from the soil particles. Identification was done on pure cultures as described in section 3.3.3 to ascertain they were the same isolates preserved.

Pythium isolate wase preserved in PDA slants in test tubes at 4^0C and in a deep freezer at -70^0C plates as per the procedures by Lester *et al* (199). The PDA medium was prepared by mixing 500ml of potato infusion (100g of potatoes boiled in 500ml of distilled water and then filtered through cheesecloth), 5g of glucose, and 7.5g of agar. The medium was sterilized and then

dispensed in 5ml aliquots into screw-cap glass test tubes (16 by 125mm). Small mycelial discs measuring about $2cm^3$ of pure *Pythium* cultures growing on PDA at 25^0C was transferred onto the surface of the medium in each tube. This was done in triplicate. After 10 days of incubation at 25^0C, the test tubes were placed directly into a 4^0C temperature freezer. The preserved cultures were maintained by inoculating into new tubes each year. This was by choping a small portion of the colony measuring about $2cm^3$ from the PDA slant and then placing onto new PDA in the sterile test tubes. Retrieval was by inoculating the chopped off portion of the colony onto the surface of PDA at 25^0C for at least 2 weeks. Sub-culturing was done as soon as *Pythium* grew out from the mycelia discs. Identification was done on pure cultures as described in section 3.3.3 to ascertain they were the same cultures preserved.

Pseudomonas bacteria isolates were preserved in nutrient broth as per the procedures by Lester *et al* (1992 and Krieg *et al.*(1984). For each isolate, 10^3 cfu per ml prepared as described in section 3.3.4 were dispersed into universal bottles aseptically. The inoculated tubes were incubated at 35 ± 2^0C for 18-24 hours aerobically and then preserved at 4^0C. A set of each isolate was also stored in the deep freezer. Each of ther isolates was preserved in triplicates and maintained by periodic inoculation into new sterile tubes. Retrieval was by inoculating bacteria colonies into new NA plates. Sub-culturing was done as soon as bacteria colonies grew out in the media. Identification was done on pure cultures as described in section 3.3.3 to ascertain they were the same isolates preserved.

3.3.6. Statistical data analysis

The data collected was analysed by One Way Anova using Genstat 6th edition software and means separated using fisher's LSD procedure at a P≤0.05. Alternatively means were compared graphically using standard deviations calculated using Microsoft Excel soft ware (Microsoft corp). Incase of zero values on incidence, the data was converted to their respective arch sine values before analysis was done (Ahmad and Baker, 1987).

3.4: Results

3.4.1. Distribution of damping off and root rot disease

Two aspects of infection by Rhizoctonia solani in vegetables were observed and these were damping off or wirestem and root rot. Damping off or wirestem occured on newly emerged or very young seedlings of tomato, eggplant, kale or okra when Rhizoctonia attacks the hypocotyls or lower stem tissue in contact with the soil. This resulted in browning and cracking of the epidermis and the formation of lesions. As infection progresses, the outer stem decays leaving only the fibrous inner xylem intact. Affected plants wilted, turned purple and remained stunted. Seedling may break off at the soil line. Coarse white mycelia that often caused soil particles to adhere to and dangle from diseased stems were observed during the wet season. Infected older plants exhibited dark dry canker covering 30-70% of the stem and most of them developed adventitious roots from the lower end of the canker.

The disease was more during wet season than dry season, in irrigated fields than in rainfed, nurseries than in the fields, where cow manure was applied than fields without, under monoculture than mixed cropping and medium to lower altitude agro-ecological zones UM3, UM4 and LM3 than in the higher altitude agro-ecological zone UM2 (Table 2 and 3).

Table 2: Mean prevalence, incidence and severity of *Rhizoctonia* damping off of tomato in various production conditions

Production Disease system	Disease prevalence (%)	Disease incidence (%)	Disease severity
Cow manure	68.4	19.0	1. 3
No manure	31.6	13.0	1.1
Irrigated	65.0	17.6	1.3
Rainfed	35.0	14.4	1.1
Nurseries	75.5	20.0	1.3
Field	24.5	12.0	1.1
Direct planted	59.3	17.0	1.3
Transplanted	39.7	15.0	1.1
Monoculture	52.4	16.3	1.2
Mixed crop	47.8	15.7	1.2
Grand mean	50.0	16.0	1.2
LSD ($P \leq 0.05$)	19.0	2.5	0.1
CV ((%)	77.3	15.8	7.9

The disease symptoms were clearly and consistently associated with infected plants and poor growth in the nursery and field. Other symptoms of soilborne pathogens observed included soft rot of young seedlings especially in high altitude areas and during the wet season, and wilting mainly on older tomato plants at flowering and fruiting stages.

None of the farmers or local extension personnel interviewed was aware of the causal agent of the disease and no control measure was being applied. Most of the farmers interviewed concurred that the disease was a major production constraint for the popular tomato varieties grown such as Cal-J VF, Monset F1 TM, Caltana F1 VF, Money maker, Onyx VF2, Grifatton and Marglobe improved, and other vegetables such as okra, beans, kales and eggplant. Some wilted tomato plants which were sampled had a brownish vascular discoloration characteristic of *Fusarium oxysporum fsp lycopersici.*

In all agro-ecological zones, tomato planted in *Rhizoctonia* infested fields during wet season consistently produced significantly ($P \leq 0.05$) higher prevalence, incidence and severity of damping off disease than those planted in the same fields during the dry season (Table 3). During the wet season tomato planted in agro-ecological zones UM4 and LM3 had high disease prevalence of 42.5% and 36.1% respectively, incidence of 22.8% and 22.1% respectively, and severity of 1.7 and 1.9 respectively as compared to 23.3% and 27.9%, 8.5% and 7.0%, and 1.3 for prevalence, incidence and severity respectively for crop planted in the same agro-ecological zones during the dry season (Table 3).

During the wet season agro-ecological zones UM3 and UM2 had low mean disease prevalence, incidence and severity of 30.3% and 22.7% respectively, 9.3% and 1.9% respectively, and 1.4 and 1.1 respectively and these were not significantly ($P \leq 0.05$) different from each other but were significantly lower than UM4 and LM3 prevalence, incidence and severity indicated above (Table 3). For both seasons tomanto planted in *Rhizoctonia* infested fields in the lower altitude agro-ecological zones UM4 and LM3 had higher mean damping off prevalence, incidence and severity of 32.9% and 32.0% respectively, 15.7% and 14.6% respectively and 1.5 and 1.6 respectively than those planted in the higher altitude agro-ecological zones UM2 and UM3 with prevalence, incidence and disease severity of 25.0% and 22.7%, 5.8% and 1.9%, and 1.3 and 1.1 respectively (Table 3). Due to high prevalence of the disease during the wet season than in dry season most of the farmers referred to it as "cold" or being caused by cold weather.

Table 3: Prevalence, incidence and severity of *Rhizoctonia* damping off of tomato in the various agro-ecological zones and seasons

Agro-ecological zone	Disease prevalence (%)			Disease incidence (%)			Disease severity		
	wet	dry	mean	wet	dry	mean	wet	dry	mean
UM2	22.7	0.0	22.7	1.9	0.0	1.9	1.1	1.0	1.1
UM3	30.3	19.6	25.0	9.3	2.4	5.8	1.4	1.1	1.3
UM4	42.5	23.3	32.9	22.8	8.5	15.7	1.7	1.3	1.5
LM3	36.1	27.9	32.0	22.1	7.0	14.6	1.9	1.3	1.6
Mean	32.9	17.7	28.2	14.0	4.5	9.5	1.5	1.2	1.4
LSD ($P \leq 0.05$)			5.1	10.2	4.0	6.7	0.4	0.1	0.2
CV (%)			18.0	72.7	88.4	70.8	23.0	28.4	46.1

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

There were some differences though not significant at $P \leq 0.05$ in damping off prevalence, incidence and severity with the administrative boundaries of the district (Table 4) but not provinces or the soil type hence not shown here. Maragua, Makueni, Kirinyaga and Embu districts had high damping off prevalence of over 60.0%. Meru central and Nakuru had moderate disease prevalence of over 40.0% whilst Kiambu and Kajiando had low disease prevalence of less than 40.0% (Table 4). Makueni, Nyeri and Maragua districts had high disease incidence and severity of more than 15.0% and 1.7 respectively. Kirinyaga, Embu, Nakuru and Kajiando districts had moderate damping off incidence and severity of more than 8.0% and 1.3 respectively. Meru central district had low damping off incidence of 6.4%. Kiambu district recorded the lowest mean damping off incidence and severity of 1.6% and 1.1 respectively (Table 4).

Table 4: Mean prevalence, incidence and severity of *Rhizoctonia* damping off of tomato in various districts

District	Disease Prevalence(%)	Mean disease incidence (%)	Disease severity
Kirinyaga	68.1	13.6	1.5
Kiambu	37.0	1.6	1.1
Maragua	85.9	22.3	2.0
Nyeri	50.0	23.3	2.0
Makueni	68.5	17.4	1.7
Embu	60.0	6.4	1.3
Meru Central	42.9	11.2	1.4
Nakuru	42.9	9.1	1.2
Kajiando	23.5	8.8	1.3
Mean	53.2	12.6	1.5
LSD (≤0.05)	20.0	7.3	0.3
CV%	34.2	57.4	22.1

3.4.2. Isolation and identification of the tomato damping off pathogens

Both the multinucleate *R. solani* and the binucleate Rhizoctonia-like fungi were isolated by the general and specific methods of Tsao (1970) and Ko and Hora (1971) respectively. *Fusarium, Pythium* and *Pseudomonas* bacteria were isolated by the general method.

Rhizoctonia solani colonies on PDA plates exhibited a light to dark brown color, course mycelial growth and at times, zonation and sclerotial formation by some isolates. Under the light microscope, all the isolates displayed the characteristic brown coarse myclelium of *R. solani;* they also showed basal constriction of hyphal branches from the prominent septa in new branches near the point of origin and exhibited a multinucleate condition of the young hyphal tip cells (Table 5). Nuclei staining indicated that 66 isolates were multinucleate with an average of 3-4.9 nuclei per cell and 10 were binucleate with an average of 1.7-2.4 nuclei per hyphal tip cells. Fifty eight of the isolates produced loose sclerotia. Growth rate was 13.8-31 mm per day

for all the isolates. Based on physical, cultural and nuclei count, the 76 isolates were placed into 9 discernible culture types (Table 5): 10 binucleate *Rhizoctonia* with average number of 1.7-2.4 nuclei per hyphal tip cell and 66 mutininucleate *Rhizoctonia* with average number of 3-4.9 nuclei per hyphal tip cell. The 10 binucleate *Rhizoctonia* could be further divided into 6 slow growing isolates with average radial growth of less than 15.0 mm/day and 4 fast growing isolates with average radial growth of more than 15.0mm/day. No sclerotia were produced by binucleate *Rhizoctonia*.

The 66 multinucleate isolates could be put into 2 culture types: 25 slow growing isolates with average radial growth of less than 15mm/day and 41 fast growing isolates with average radial growth of more than 15mm/day. Eight isolates could not produce sclerotia whilst 58 produced sclerotia. The 58 that produced sclerotia could further be categorised into 4 culture types: 18 isolate cultures were initially white to cream, turning yellowish light brown after 10 days. Concentric rings of dark and light mycelial growth were evident. Mycelium was appressed to the agar surface, with sparse aerial growth. Cultures produced a limited number of light-coloured, irregularly shaped sclerotia after 2 weeks. These turned dark brown with age, producing a brownish exudate. Ten cultures had aerial raised mycelium that appeared dark brown and cottony, no concentric rings were evident, and sclerotia were small and few in number. Sixteen isolates were dark brown and had mycelium appressed to the agar surface, and did not form concentric rings. Sclerotia were mainly found at the centre of colonies and occasionally at the margin. Fourteen isolates produced sclerotia that often aggregated into large compound clusters that were darker brown than mycelium, aerial mycelium was sparse (Table 5).

Table 5: Preliminary characterisation of the Rhizoctonia solani isolates

Isolates	Average Nuclei count	Average growth rate (mm/day)	Sclerotia	Aerial mycelium	Texture of mycelium	Color of mycelium	Substrate color
6	2.2	≤15.0	-	Sparse	Appressed	Dark-brown	Clear
4	2.0	≥15.0	-	Cottony	Raised	Dark-brown	Clear
8	3.1	≤15.0	-	Cottony	Raised	White to cream	Clear
7	2.8	≤15.0	+	Sparse	Appressed, light dark concentric rings	White to cream initially Yellowish-brown after 10 days	Brownish
11	4.2	≥15.0	+	Sparse	Appressed, light dark concentric rings	White to cream initially Yellowish-brown after 10 days	Brownish
10	3.5	13.3	+	Cottony	Raised	Dark-brown	Clear
16	3.6	3.8	+	Sparse	Appressed	Dark-brown	Clear
14	4.3	4.9	+	Sparse	Appressed	Dark-brown	Clear

The two isolates of *Fusarium* were identified by their characteristic off-white to cream buff color on 7 days old culture on APDA and mass production of pinkish orange macroconidia with a foot cell bearing some kind of a heel. *Pythium* isolate was identified by its characteristic production of coenocytic hyphae, oogonia containing a single spore/oospore and antheridia in an elongated club-shaped antheridium. *Pseudomonas* bacteria was identified by its characteristic rod- shaped, aerobic, gram-negative, non-spore forming, singly polar flagellated motile cells that gave positive catalase test with absence of gas formation from glucose fermentation.

3.4.3. Pathogenicity of damping off pathogens on tomato

Fifty six out of the 76 *R. solani* isolates were pathogenic to the common tomato varieties grown in Kenya under greenhouse conditions with mean percent seedling survival of less than 80% and damping off severity of more than 1.7 whilst 26 isolates were nonpathogenic with mean percent survival of more than 80.0% and disease severity of less than 1.2 (Table 6). The pathogenic *Rhizoctonia* isolates when applied at the rate of 10^6 mycial fragments/gram of soil caused damping off and root rot symptoms on tomato under greenhouse conditions similar to the ones observed in the field during the survey.

The effect of pathogenic *Rhizoctonia* isolates on the percent survival of tomato seedlings was significant at 1st, 2nd, 3rd, 4th and 5th weeks, and on damping off severity at the 5th week after planting (Table 6). The uninfested control had significantly higher percent survival of seedlings and lower disease rating value than the plants exposed to pathogenic *Rhizoctonia* isolates.

The characteristic Rhizoctonia damping off and necrotic lesions on the lower stem and roots from which Rhizoctonia was readily reisolated was observed on the susceptible tomato

varieties. This indicated that the 56 *Rhizoctonia solani* isolates were pathogenic to the common tomato varieties grown in Kenya with damping off incidence and severity significantly ($P \leq 0.05$) higher than for the uninfested control (Table 6).

The 2 *Fusarium* amd 1 *Pyhtium* isolates were pathogenic to the Rhizoctonia susceptible tomato varieties under the greenhouse condition with mean percent survival of less than 80.0% and disease severity of more than 1.7 and this were significantly different from the uninfested control at $P \leq 0.05$. The pathogenic *Fusarium* and *Pythium* isolates produced significant reduction in tomato seedlings survival at 1st, 2nd, 3rd, 4th and 5th week after planting and higher disease severity at the 5th week as compared to the uninfested control. *Fusarium* isolates induced wilting and brownish vascular discoloration on the 5 week old plants. The *Pythium* isolate caused soft rot on 2 weeks old seedlings. *Pythium* isolate was more virulence on tomato varieties than the 2 *Fusarium* isolates and some *Rhizoctonia* isolates as shown by the lower percent survival at the end of 5th week after planting and higher damping off severity (Table 6).

By the 5th week, the 2 *Pseudomonas* bacteria isolates had mean percent tomato seedling survival of more than 85.0% and disease severity of less than 1.1 and they were not significantly different from the uninfested control at $P \leq 0.05$ (Table 6) indicating they were nonpathogenic to the tomato varieties under the greenhouse conditions. All the 26 nonpathogenic *R. solani* and 2 *Pseudomonas* bacteria isolates had no significant ($P \leq 0.05$) effect on percent tomato seedling survival at 1st, 2nd, 3rd, 4th and 5th week after planting and disease severity at the 5th week and were not different from uninfested control. All the pathogenic isolates had significantly higher mean percent seedling survival by the 5th week than the nonpathogenic isolates and the uninfested control at a $P \leq 0.05$.

Table 6: Effect of various damping off pathogens on percent tomato seedling survival and diseaase severity

Pathogen isolate	Mean weekly survival (%)					Disease Severity
	1st week	2nd week	3rd week	4th week	5th week	
R1	47.5	85.2	81.0	75.3	70.0	2.6
R2	50.0	81.3	72.3	70.4	69.0	2.5
R3	60.0	88.0	75.0	73.0	73.0	3.1
R4	45.4	81.2	72.0	70.0	69.0	2.8
R5	48.0	77.0	68.0	66.6	65.0	2.9
R6	43.0	78.0	67.0	66.0	64.0	2.6
R7	58.0	82.0	79.0	76.0	75.0	2.5
R8	45.0	89.0	75.0	70.0	69.0	3.2
R9	45.0	75.0	72.0	69.0	67.0	3.4
R10	55.0	81.0	73.0	70.0	70.0	3.3
R11	53.0	87.0	80.0	78.0	77.0	2.8
R12	42.0	74.0	71.0	70.0	70.0	2.2
R13	43.0	82.0	69.0	67.0	65.0	2.0
R14	70.0	89.0	76.0	74.0	73.0	2.9
R15	66.0	80.0	72.0	70.0	68.0	1.8
R16	55.0	87.0	80.0	78.0	76.0	1.9
R17	50.0	85.0	76.0	74.0	74.0	1.7
R18	60.0	83.0	79.0	77.0	77.0	2.7
R19	48.0	71.0	65.0	60.0	58.0	2.6
R20	54.0	80.0	66.0	66.0	64.0	2.7
R21	55.0	88.0	74.0	66.0	66.0	2.5
R22	44.0	83.0	84.0	77.0	74.0	2.6
R23	56.0	78.0	80.0	75.0	74.0	2.8
R24	51.0	82.0	83.0	77.0	76.0	2.4
R25	61.0	87.0	76.0	72.0	71.0	2.3
R26	45.0	56.0	68.0	67.0	61.0	3.0
R27	53.0	88.0	75.0	71.0	69.0	2.9
R28	40.0	74.0	66.0	63.0	60.0	3.2
R29	39.0	74.0	68.0	67.0	67.0	3.0
R30	43.0	81.0	74.0	71.0	70.0	3.1
R31	55.0	79.0	77.0	72.0	72.0	2.1
R32	44.0	81.0	72.0	68.0	67.0	3.0
R33	49.0	85.0	73.0	72.0	72.0	2.0
R34	56.0	86.0	79.0	77.0	74.0	1.8
R35	47.0	78.0	78.0	69.0	67.0	1.7
R36	45.0	83.0	74.0	72.0	71.0	1.7
R37	41.0	75.0	70.0	68.0	68.0	1.8
R38	53.0	80.0	72.0	66.0	65.0	2.7
R39	44.0	86.0	73.0	71.0	71.0	2.5
R40	48.0	83.0	74.0	69.0	68.0	2.6
R41	55.0	84.0	72.0	70.0	70.0	2.9
R42	54.0	76.0	70.0	65.0	64.0	3.0

Table 6. Continued

Pathogen isolate	Mean weekly survival (%)					Disease Severity
	1st week	2nd week	3rd week	4th week	5th week	
R43	66.0	85.0	73.0	70.0	69.0	2.7
R44	48.0	66.0	64.0	62.0	62.0	2.6
R45	31.0	70.0	67.0	65.0	65.0	3.5
R46	33.0	68.0	65.0	63.0	62.0	3.0
R47	40.0	76.0	68.0	66.0	64.0	2.2
R48	44.0	79.0	70.0	66.0	65.0	2.0
R49	44.0	85.0	76.0	75.0	75.0	1.8
R50	55.0	85.0	78.0	77.0	74.0	1.6
R51	69.0	90.0	81.0	77.0	77.0	2.9
R52	50.0	66.0	67.0	62.0	62.0	3.0
R53	57.0	88.0	79.0	76.0	73.0	3.4
R54	45.0	78.0	60.0	59.0	56.0	2.7
R55	48.0	89.0	75.0	73.0	72.0	2.6
R56	44.0	88.0	69.0	74.0	71.0	2.8
R57	60.0	95.0	95.0	94.0	94.0	1.0
R58	70.0	96.0	97.0	97.0	96.0	1.0
R59	68.0	98.0	96.0	96.0	96.0	1.0
R60	69.0	99.0	97.0	97.0	97.0	1.0
R61	68.0	93.0	94.0	94.0	94.0	1.0
R62	75.0	99.0	99.0	99.0	99.0	1.0
R63	60.0	96.0	95.0	95.0	95.0	1.0
R64	67.0	97.0	97.0	97.0	97.0	1.0
R65	70.0	96.0	96.0	95.0	95.0	1.0
R66	69.0	98.0	98.0	98.0	97.0	1.0
R67	75.0	96.0	98.0	98.0	98.0	1.0
R68	71.0	95.0	96.0	96.0	96.0	1.0
R69	67.0	99.0	99.0	98.0	97.0	1.1
R70	67.0	96.0	96.0	96.0	96.0	1.0
R71	66.0	94.0	95.0	95.0	95.0	1.0
R72	68.0	97.0	97.0	97.0	97.0	1.0
R73	71.0	93.0	95.0	95.0	95.0	1.0
R74	65.0	96.0	97.0	97.0	97.0	1.0
R75	73.0	98.0	98.0	97.0	97.0	1.0
R76	66.0	97.0	97.0	97.0	97.0	1.0
F1	44.0	86.0	78.0	75.0	71.0	2.4
F2	53.0	89.0	76.0	70.0	68.0	2.6
P	42.0	77.0	68.0	67.0	67.0	3.1
Pb1	66.0	98.0	97.0	97.0	97.0	1.0
Pb2	68.0	99.0	98.0	98.0	98.0	1.0
Control	67.0	98.0	98.0	98.0	98.0	1.0
Mean	54.8	85.3	79.6	77.6	76.6	2.1
LSD (≤0.05)	6.3	11.9	15.7	14.8	1 9.1	0.8
Cv (%)	5.2	7.3	3.5	3.6	14.6	36.3

R1-76=*R. solani*, F1-2=*Fusarium* isolates, P=*Pythium* isolate, Pb1-2=*Pseudomonas* isolates

There were significant (P≤0.05) differences on the mean number of pathogenic *Rhizoctonia solani* isolates with agro-eological zones, season and administrative boundaries of districts but not provinces or soil type. Wet season consistently produced higher number of isolates in all agro-ecological zones and districts than the same fields during the dry season (Table 7 and 8). During the wet season samples from agro- ecological zone UM2, UM3, UM4 and LM3 had high number of 2, 10, 17 and 9 isolates respectively as compared to 0, 4, 4, 7 and 7 isolates respectively for the same agro-ecological zones during the dry season (Table 7). Agro-ecological zones UM4 and LM3 had high number of total isolates of 24 and 16 respectively and not significantly (P≤0.05) different from each other but significantly different from the 14 and 2 isolates in UM3 and UM2 respectively (Table 7).

Table 7: Number of pathogenic *Rhizoctonia solani* isolates in various agro-ecological zones and seasons

Agro-ecological zone	Wet season	Dry season	Total isolates
UM2	2	0	2
UM3	10	4	14
UM4	17	7	24
LM3	9	7	16
Total	38	16	56
LSD(P≤0.05)	7. 9	5.2	9.1
CV (%)	37.0	53.4	66.5

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3

Makueni and Kirinyaga districts had high number of pathogenic *R. solani* of 15 and 14 respectively and not significantly (P≤0.05) different from each but different from the rest of the districts. Maragua and Embu had 7 each, Kiambu and Meru central had 4 each, Kajiando 3, Nakuru 2 and Nyeri 1(Table 8). *Rhizoctonia solani* isolates were recovered from all agro-

ecological zones during the wet and dry seasons. *Pythium* was isolated from agro-ecological zone UM2 during the wet season. *Fusarium* and *Psedudomonas* bacteria were isolated from agro-ecological zones UM3 and UM4 during the dry and wet season respectively.

Table 8: Mean number of pathogenic *Rhizoctonia solani* isolates in various districts and seasons

District	Wet season	Dry season	Total isolates
Kirinyaga	9	6	14
Kiambu	2	2	4
Maragua	7	0	7
Nyeri	1	0	1
Makueni	9	6	15
Embu	4	3	7
Meru central	4	0	4
Nakuru	1	1	1
Kajiando	1	2	3
Total	38	16	56
LSD	5.0	2.8	5.3
CV(%)	27.0	65.0	88.4

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3

3.5. Discussion

Variation in agro-ecological zones and season of production had the highest effect on Rhizoctonia damping off incidence, severity and number of isolates recovered. This is perhaps because agro-ecological zones in Kenya are based on altitude and climatic conditions of which directly influence Rhizoctonia distribution and infection, crop production methods and intensity. Abawi *et al* (1990) and Damping off Wikipendia (2008) reported that Rhizoctonia infection is favoured by moderate to high soil moisture conditions and moderate temperature. Like most fungi, Rhizoctonia requires soil moisture for growth and dissemination. Similarly, in all agro-ecological zones sampled, Rhizoctonia damping off incidence, severity and number of

pathogenic isolates recovered were significantly higher during the wet season than the dry season. Likewise, warmer medium to low altitude agro-ecological zone UM3, UM4 and LM3 had higher Rhizoctonia damping off prevalence than higher and cooler agro-ecological zone UM2. No Rhizoctonia damping off was observed in ecological zone UM1 probably because it was too cold for the pathogen survival hence disease development. Caro *et al* (1986), Abawi *et al* (1990) and Damping off Wikipedia (2008) reported that Rhizoctonia damping of severity in tomato is favoured by moderate to high soil moisture conditions and moderate temperatures. There was also was low tomato production observed in high altitude agro-ecological zones UM1 and UM2 because leaf blight is a serious production constraint for the crop in the higher cool and wet altitude. This is corroborated by Hill and Waller (1988) who reported infection by blight as the major limitation to tomato production in higher cooler altitude areas. Even though agro-cological zone LM3 was too dry for the disease development under normal conditions, the disease was prevalent during the wet season and where irrigation was practiced.

The disease was observed in 80.0% of the agro-ecological zones surveyed and attacked all the popular tomato varieties and other vegetables grown such as okra, beans, eggplant and kales causing damping off and bottom rot diseases. The wide distribution and host range of *Rhizoctonia solani* has been reported by several other researchers. Anderson (1982), Flentje *et al* (1970), Adams (1988), Sneh *et al* (1996) and Koike (1996) reported *Rhizoctonia solani* occurs worldwide and causes economically important diseases such as damping –off, root and crown rots of many crops as well as fruit decay to a large variety of vegetable and field crops, turfgrasses, ornamentals and fruit and forest trees. Similar findings was reported by Danesha *et al* (1999) who documented lack of resistance to Rhizoctonia damping off of tomato in all the 9 cultivars grown in Tennessee region of united states of America. This implied that currently the

disease cannot be managed by use of host resistance or crop rotation. This is despite the fact that use of resistance varieties/cultivars could be the most economical disease control strategy in Kenya especially by the resource poor small scale tomato farmers.

The disease was more prevalence in irrigated fields than rainfed fields is probably because under irrigation the intensity of tomato production is higher than under rainfed fields resulting to higher inoculum build up. This observation on relationship between inoculum build up and Rhizoctonia damping off disease is corroborated by Henis *et al* (1970) description of a linear relationship between *Rhizoctonia* inoculum concentration and rate of infection of beans and damping off in artificially infested soil media upto 0.25g/kg soil. Their report also showed that saprophytic growth was correlated with propagule size and volume. The direct relationship between inoculum density and the damping off incidence and severity established in this study means that by determining the *R. solani* inoculum level in the field before planting can assist in predicting the expected damage by the pathogen later in the season. This can form a sensitive predictive technique in decision making before planting as well as evaluating the success of new control methods. However, such accurate enumeration should employ use of rapid and reliable disease control assessment techniques such as molecular procedures.

The disease was more prevalent where cow manure was frequently applied as an alternative to expensive commercial fertilizers. The association of the pathogen with soil organic matter is further corroborated by findings of Danesha *et al* (1999), Abawi *et al* (1990) and Damping off Wikipedia (2008) who reported Rhizoctonia is a nonspecialised fungi found associated with decomposed organic matter in all natural soils and where it can survive indefinitely either in form of mycelia or sclerotia. This further explains why more isolates were recovered from

rhizosphere soil than from the infected plants. The disease incidence was more on lower areas of the field than in higher areas, suggesting run-offs as a major source of inoculum dissemination. Also Githinji (2005) reported run off is a major dispersal agent for *R. solani* causing damping off of beans in Embu district of Kenya. Nik *et al* (1979) reported seed infection and run-off as the major agent of disperal for the *R. solani* causing damping off in French beans.

That the Rhizoctonia damping off prevalence and the number of pathogenic *R. solani* isolates recovered was higher on young seedlings than from the adult plants may have a bearing in future research on the disease management as currently most of the researchers are devoting more effort to Rhizoctonia diseases in older plants than seedlings. This finding is corroborated by reports from several researchers. Carol and Dona (1998) reported similar findings when they isolated more *Rhizoctonia* strains from sugarbeet seedlings than from adult plants. Koike *et al* (1996) reported seedling damping off and bottom rot as the two major aspects of infection by *Rhizoctonia* in most vegetables. Danesha *et al* (1999) reported seedling damping off as the most severe stage of infection by *Rhizoctonia solani* on tomato and occurs mostly between emergence and 30 days after planting.

The various *R. solani* isolates differed on morphological and cultural aspects such as colony color, sclerotial formation, growth rate, number of nuclei per hyphal tip cells and pathogenicity on tomato plant indicating the possibility of high genetic diversity among them. However, multinucleate and binucleate *R. solani* isolates looked alike in culture but differed only in pathogenicity to tomato and the number of nuclei per hyphal tip cells. Carol and Dona (1996) reported similar findings when they found all strains of multinucleate *Rhizoctonia* caused

damping off in sugarbeet seedlings whereas generally all the binucleate *Rhizoctonia*-like fungi were nonpathogenic to the seedlings. However findings by Fennile *et al* (2005), deviate from this generalization but effectively agreed with the findings of this study when they reported root rot in Yacom caused by binucleate Rhizoctonia spp AG-G in Brazil.

During the pathogenicity test, the number of emerged tomato seedlings were significantly higher from the untreated pots as compared to the treated ones by end of first week indicating that *Rhizoctonia*, *Pythium* and *Fusarium* also causes pre-emergence damping off in tomato. Similar observations were made by Abawi *et al* (1990) who reported seed decay and pre-emergence damping off phases of the *Rhizoctonia*, *Pythium* and *Fusarium* pathogens are component of the total disease complexes they incites in tomato, and therefore need to be quantified. The 2 *Pseudomonas* isolates were nonpathogenic to the common tomato varieties under the greenhouse conditions. This findings deviate from the report by Meyer *et al* (2002) and Brodey *et al* (1991) who reported numerous *Pseudomonas* species that can act as plant pathogens.

Pythium isolate was more virulent on tomato varieties than the 2 *Fusarium* isolates and some *Rhizoctonia* isolates probably because *Pythium* is a major cause of damping off diseases in several crops (Owen, 2002). The different isolates of *R. solani* produced slight variations on disease levels during pathogenicity test indicating differences in virulence among them. It also indicated that they could be genetically different. Such differences could be revealed by genotyping of each isolate (Bruns *et al.,* 1991). Similar findings have been reported by Caro and Dona (1986) who found AG-2 cultures were more virulence than were AG-4 cultures on 6-8 weeks old sugarbeet plants in Ohio.

Other soilborne fungi and bacteria were isolated alongside *Rhizoctonia solani* from Rhizoctonia infected tomato and rhizosphere soil indicating the possibility of synergistic interaction with Rhizoctonia in causing damping off disease complex on tomato. Involvement of other fungal and bacterial pathogens in most production areas was further supported by the presence of some wilted plants that had brownish vascular discoloration of the xylem element and others with soft rot on the lower stem of young seedlings. Hach (1975) and Huber *et al.,* 1966 reported similar findings when they found *R. solani, Fusarium* and *Pythium* were the pathogens frequently isolated from diseased bean plants and rhizosphere soil in Wisconsin' central sand of USA.

CHAPTER FOUR

MOLECULAR CHARACTERIZATION OF *RHIZOCTONIA SOLANI* CAUSING DAMPING OFF OF TOMATO BY MICROSATELLITE ANALYSIS.

4.1: Abstract

The objective of this experiment was to study the genetic diversity among the *R. solani* isolates causing damping off of tomato in Kenya by microsatellite technique. In the study, 8 microsatellite loci were screened for amplification of DNA from 56 *Rhizoctonia solani* isolates. The product of amplification was resolved through high resolution Polyacrylamide gel electrophoresis, the data generated analysed using appropriate softwares and used to estimate the genetic diversity and phylogenesis among the *R. solani* population.

The study indicated high genetic diversity among *R solani* isolates as evidenced from the large number of alleles typed, high average heterozygosity, 100% polymorphism, large variances of genetic distance and large number of genetic clusters generated. Seven distinct molecular clusters with several subclusters were produced from 7 loci typed in 52 isolates based on genetic distance coefficient. The moderate F-statistic and co-phenetic values indicated good precision for the *R. solani* genetic distance estimates and validity for clustering obtained respectively. The variations in isolates clusters with agro-ecological zones was significant but minimal with districts and provinces. The high genetic diversity among the *R. solani* could have a bearing on their wide host range and virulence, all of which influence the efficacy of disease control methods.

Lack of Hardy-Weinberg equilibrium for most of the loci typed and deficiency heterozygotes in group of populations indicated a disquilibrium between random mating and genetic drift among

the *R. solani* within agro-ecological zones. However the excess of average heterozygotes in the group of populations indicated high degree of intermating between various agro-ecological zones probably due to efficient inoculum dispersal by run-offs and irrigation water.

4.2: Introduction

Despite previous progress made to document the various aspects of infection by *R. solani* on tomato and other vegetables in Kenya, there is little information on the genetic diversity of the fungus under farmland conditions (KARI, 2002; Githinji, 2005). Efforts to breed disease resistance to Rhizoctonia root rot must take into account the genetic diversity among the pathogen for it is a prerequisite to understanding the interaction between the pathogen and the host plant (Herdina *et al.*, 1996). Although fungicides are a key component in the integrated management of many plant diseases, the appearance of resistance has become an important factor in limiting the efficacy and useful lifetime of fungicides developed at increasingly higher costs (Zhonghua *et al.*, 2005. Extensive molecular studies have led to advances in understanding of mechanism of fungicide resistance and in developing effective, rapid methods for detection of resistant genotypes of various pathogens (Herdina *et al.*, 1996: Zhonghua *et al.*, 2005).

Conventionally, species level diversity based on morphology, pathology and hyphal anastomosis behaviour attempt to describe genetic variations that exist within *Rhizoctonia* species (Ogoshi, 1987; Sneh, 1996). Since conidia are not produced, the taxonomy of the genus *Rhizoctonia* is very difficult (Parmeter *et al.*, 1969, 1970). According to Parmeter *et al* (1967) and Parmeter (1970), the genus *Rhizoctonia* is a heterogenous ssemblage of mycelia of basidiomycetes, ascomycetes and deuteromycetes. These fungi occur in the soil as many strains

that differ in appearance in cultures, physiology and pathogenicity (Ogoshi, 1987; Sneh, 1996). Use of host differentials to carry out race identification on a new isolate is time consuming, scoring of symptoms is at times subjective and the infection process which is a functional of environmental conditions such as temperature, light and humidity can be quite variable even with the same race and cultivar combinations (Onkar *et al.,* 1986).

Molecular techniques offer a better alternative especially where morphological characteristics are too few to distinguish between isolates of a pathogen (Robinson and Harris, 1999). Molecular techniques make use of natural variation in the DNA of all species as a means for classifying the races of fungal pathogens (Doohan *et al.,* 1998). Molecular markers are used due to their naturally occurring polymorphism (Doohan *et al.,* 1998; Robinson and Harris, 1999). An ideal marker would have sufficient variation for the problem under study: be reliable, and simple to generate and interpret. Unfortunately, an ideal marker does not exist for use in all studies; rather a technique(s) will be suited to a range of investigations (Robinson and Harris, 1999). Different molecular techniques to determine genetic diversity are available and these includes Random Amplified Polymorphic DNA, Amplification Fragment Length Polymorphism, Microsatellite and jeffries probes (Herdina *et al.*, 1995; Robinson and Harris, 1999). Although all the techniques have pro and cons, the choice of the procedure used depends on several factors including the problem under study and costs (Robinson and Harris, 1999).

Microsatellite markers, also known as simple sequence repeats (SSRs), short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs) are tandem repeats of sequence units generally less than 5 bp in lengths eg (TG)n or (AAT)n. They are thought to be produced by errors in DNA replication, when the DNA polymerase"slips" when copying in the repeats

region, changing the number of repeats in the region (Robinson and Harris, 1999). Microsatellites are hypervariable, in addition to which their co-dominace and reproducibility makes them ideal for genome mapping, as well as for population genetics and diversity studies (Robinson and Harris, 1999). The PCR products are separated on a high resolution polyacrylamide gel, and the products detected with a fluorescent detector such as an automated sequencer or an-X-ray film (Robinson and Harris, 1999). Like with most other molecular techniques, the disadvantages are that, it is expensive and also inacurate detection can occur due to slippage, homology, mutations and inaccurate scoring of the so "called" product fragment sizes (Robinson and Harris, 1999). Microsatellite techniques have been used in the biodiversity study of *R. solani* and other soilborne fungal pathogens such as damping off fungi, *Pythium* spp, root rot and wilt, *Fusarium* spp. (Githinji, 2005; Herdina *et al.*, 1995).

The objective of the study was to use microsatellite technique to determine biodiversity of *R. solani* isolated from infected tomato and rhizosphere soil in Kenya representing the different biotypes in the different production regions of the country. This could form the basis of a predictive test, and be a sensitive tool to investigate the biology of the disease and evaluate new methods of control.

4.3: Materials and methods

The fungal isolates stored in sterile barley seeds under refrigeration were reactivated on APDA, and by the Ko and Hora (1971) methods as described in section 3.3.3. One gram of *R. solani* infested barley seeds were plated on APDA and incubated in the dark for 24-48 hours. Hyphal tip transfer to a new APDA plates was done to obtain clean isolates. Preliminary identification

of the isolates were done on 7 day old cultures on APDA to ascertain that they were the initial *R. solani* isolates and was based on the cultural and morphological features.

4.3.1. DNA extraction and quantification

The modification of the methods by Herdina *et al.,* (1996), Raeder *et al* (1985) and Julian, (1997) were used. Plugs of PDA measuring approximately 2 cm^2 containing hyphal tips of *Rhizoctonia solani* were placed in petri dishes containing 20ml of 2.4% Potato dextrose broth and allowed to grow at room temperature in the dark without shaking.

After approximately 5 days and fungal growth had reached near the edge of the dish, the visible outer ring of young hyphae (50g) containing *R. solani* was scrapped off using a glass slide and rinsed with sterile double distilled water. The mycelia was filtered through 2 layers of cheese cloth and blot dried between two filter papers. Total DNA was extracted from the mycelia essentially as described by Raeder and Broda (1985) except that 0.1-1.0g of fresh material was ground in liquid nitrogen using a mortar and pestle and transferred to 5 volumes of the extraction buffer (TE). The suspension was extracted with an equal volume of phenol/chloroform (1:1, v/v) and centrifuged at 1200X-1500X for 5 minutes. The resultant aqueous phase was incubated with 0.1mg ml^{-1} RNase A (Appendix 9.1) at 37^0C for 30 minutes and extracted with an equal volume of chloroform/isoamylalcohol (24:1, v/v).

The DNA was visibly precipitated from the aqueous phase by the addition of 0.54 volumes cold isopropanol, centrifuged briefly, washed with 70% ethanol, 10mM MgAC, dried in *vacuo,* and resuspended in 0.1 to 1 ml of TE buffer (10mM Tris, 1 mM EDTA, PH 8.0). After the last wash with alcohol the cell pellet were resuspended in 20µl of TE buffer in 1.5ml

microcentrifuge tube. The DNA was further purified by CsCl density gradient centrifugation. After purification, the samples were placed on a 95°C heating block for 5 minutes to deactivate the DNAase molecule.

A dilution factor of 50 was used in the preparation of DNA sample for quantification. Subsequently, 15µl of each of the sample was added to 735µl of TE amounting to 750µl per cuvet and mixed well. For better results, the cuvet was filled upto at least 1/3 of its capacity. Purity was determined by reading the optical density at 260nm (260nm =50µg/ml) and 280nm wavelengths. Concentration of DNA was determined by programming the spectrophotometer to automatically give the results.

Five microlitre of DNA extract was loaded into 1.5% agarose gel along with 500ng lambda DNA as a standard, ran under electric current at 20mA for 45-60 minutes after when it was stained by dipping in 5% ethidium bromide and put on shaker for 30 seconds. After destainining with excess running water for 10-15 minutes the gel was photographed under ultraviolet light (Appendix 9.2). The DNA concentration was obtained by comparing the relative band intensity between samples and the marker. Where the sample was too dilute then the DNA was concentrated by ethanol precipitation in which 0.6 volumes isopropanol was added, washed with 70% ethanol, dried and resuspended in 1000µl TE buffer (Julian, 1997). Each sample was diluted to a working concentration of about 20ng of DNA per µl and verified spectrometerically and by relative band intensity between the sample and the marker.

4.3.2. Determination of the microsatellite loci

A set of 8 polymorphic primers were assembled and tested to acertain wether they would amplify in a repeatable manner. The sequence of the primers combination were RB23; cag ccg tct ttc tct ctc c/ gcc ttg aat cac-tac-ctc-ca, RB14; tac-cca-ttg- cct-tgt-ttc-c/act-ccg-cgt-tct-gct-aga-g', RE102; gga-ctt-gtc-agc-gtc-aag/tca-acc-atc-tca-agg-tat-gtc, BC5; cgt-ttt-cca-gca-ttt-caa-gt/cat-ctc-ata-ttc-gtt-cct-ca, AF513014; tcg tgc aag gac ttg gta gtc/ acg agt gct tct acc ata ctc, AY212027; gct tct acc ata gtg ac acc/acg agt gct tct acc ata ctc, LR7; tac tac cac can gat ct/ctg ctg gtg gtt cta ga and LROR; acc gct gaa ctt aag c/tgg cga ctt gaa ttc gb. DNA molecular weight marker GENERULER ^{TM} 100 bp ladder plus was used during the analysis as the standard. The primer screening protocol outlined in appendix 8.0 was used.

The primers were synthesized using the Applied Biosystems 394 DNA/RNA synthesizer and supplied by Carramore International limited of UK. Each of the 8 flourescently labelled oligonucleotide primer sets with 3 orange, 3 green and 2 yellow were reconstituted by adding a 50ul of triple distilled water to each, then held on a bench centrifuge at 1300-1500 rpm for 30 seconds before adding 10µl each to each tube. Quantification of the primer concentrations was done according to the procedure by Sambrook *et al* (1989). This involved reading the optical density of each primer at 260nm and then changing the optical desnsity reading to molarity (µ M units) using the following equation: OD_{260}/SIGMA 260 X 10^6 =Concentration (µM) where SIGMA260=10,000 X Number of primer bases (N)/molecular weight of each base (m) X cm. Where the primers were too concentrated they were further diluted by adding 200µl or 350µl of ultra distilled water.

4.3.3. DNA amplification and fragment detection

Tweny nanograms of template DNA samples were mixed with fluorescently labelled oligonucleotide primers, deoxyribonucleotide triphosphate (dNTPs) and thermal stable Taq polymerase in X10 buffer in 100µl total reaction volume. The master mix for the reaction was made by adding 1.5µl of 10 X PCR buffer (100ml Tris, 500 mMKCL, 1.5mM $MgCl_2$, P^H 8.3, 3.2µl nucleotides (1.25mM each of dATP, dCTP, dGTP, dTTP), 0.5µl each primer pair (5pMo/µl), 0.16µl Taq DNA polymerase (8 units/µl, 0.14µl of distilled water added to make 9µl total volume, then 1.0µl of template DNA (20ng/µl) to make 10µl total volume in a 1.5 ml microcentrifuge tube. The samples were ran in duplicate with a control in which the template DNA was replaced by 1.0 µl triple distilled water. All the PCR ingeredients were from the same manufacture, Carramore International limited hence optimized.

The 57 samples, 56 isolates and one control were amplified at one go in a 96-well plate using each of the primer set. The master mix for the 57 samples was premade before adding template DNA to each. The tubes were held on a bench centrifuge for 30 seconds and loaded onto the DNA thermal-cycler, model Gene AMP 9700-Applied biosystems incl. for the PCR to proceed. The DNA was heated to 95°C for 1 minute, 50-58°C (depending on primer set) for 1 minute followed by 72°C for 10 minutes. After 35 cycles, the annealed product was stored at 4°C. Procedure adopted from Meyer *et al* (1997), Fenile *et al* (2005) and Githinji *et al* (2005).

The amplified fragment(s) were detected by taking a sample (5µl) from the reaction product and loading onto 1.5% agarose gel alongside 5µl Gene ruler 100bp plus strategically placed on the gel, ran at 100V until the bromophenol blue migrated to just above the set of the wells. The gel was stained by dipping in 5% ethidium bromide and put on a shaker for 30 seconds. After

destaining the gel by running under tap water for 5 minutes, the DNA bands were visualized under ultraviolent transillumination. The preparation of the horizontal 1.5% agarose gel and 5% etidium bromide was as explained in appendix 9.2. The size of products was verified by comparison with the molecular weight marker.

After the initial amplification of template DNA using the 8 primer sets and electrophoresis through 1.5% agarose gel, 52 isolates were sequenced using a double-stranded DNA template of each PCR product (75ng) and 1μM each of the 7 primers RB23, RE14, RE102, BC5, AF513014, AY212027 or LROR following the protocol supplied by the manufacturer, Amarsham Pharmacia. Each PCR product was purified using microspin S-400 HR columns model Amersham Pharmacia according to the manufacturer's instructions. The PCR product was separated through 5% polyacrylamide gel electrophoresis (Appendix 8.3 and 10.0) for 2 hours at 50V. The DNA samples were prepared by mixing with formamide, loading buffer, dye solution and a denatured internal sized standard DNA. Polyacryalmide gel, loading solution and electrophoresis buffer was prepared and samples elctrophoresed according to the description in the ABI Prism™ User's manual (Appendix 9.3, 9. 4, 9.5 and 10.0 respectively) Applied biosystem (1995).

Gene Ruler ™ 100 bp ladder that has size fragments between 100-3000 base pairs and its denatured molecular lengths in base pairs long were 100, 200, 300, 400,500, 600, 800, 900, 1031, 1200, 1500, 2000 and 3000 bp was used as the internal sized standard. The DNA fragments were sized using the Genescan™ 672 analysis software version 1.2.2 which operates with the Parkin Elmer ABI prism™ 377 DNA sequencer to automatically size and quantify DNA fragments using automated fluorescent detection. The 3rd order least squares size calling

method was used to determine the molecular length of the unknown fragment. Only strongly and reproducible bands were scored. Co-migrating bands were assumed to be identical in base pairs.

4.3.4. DNA fragment analysis

The products of electrophoresis and Genescan analysis were imported into the Genotyper ^{Tm} software for further analysis as described in the genotyper User's manual set, Applied Biosystems (1995). The internal size standard DNA fragments were checked to ascertain whether they were accurately assigned sizes. Lanes that showed anomalies were retracted and reanalyzed using Genescan software, then reimported into the Genotyper. The orange, green and yellow labelled DNA electrograms/fragments representing alleles at microsatellite loci studied were assigned sizes in base pair. The data was exported into Microsoft Excel software application.

The sequence fragments comprising of size and quantity generated by the pairs of each primer were assembled (Appendix 11.0). For each sample, the presence or absence of each band was determined and designated 1 if present or 0 if absent inorder to obtain binary banding data from which the total alleles were typed, and their homology determined according to Hartl and Clark, (1989). Average number of alleles per loci and the whole population was determined by counting the number of alleles with non-zero frequencies (Appendix 12.0). Polymorphism was determined as percentage of all loci typed regardless of allele frequencies.

Heterozygosity per locus and the whole population was estimated from the proportion of observed heterozygosity and expected heterozygosity respectively (Appemdix 13.0). Nei's

(1978) genetic diversity was estimated from the expected heterozygosity using the algorithm of Levene (1949). Hardy- Weinberg equilibrium test was performed from genotypic frequencies observed at each locus using algorithm by levene (1949) and Chi- square (X^2) computation at 0.05. If X^2cal>Htab, then Ho was rejected and vice versa; X^2cal:-chi-square calculatedc, Htab-chi-square calculated, Ho-the hardy-Weinberg principle that the population was in a random mating resulting in equilibrium distribution of genotypes after only one generation so that the genetic variation is maintained.

Similarity matrices from binary banding data of each of the 7 primer combinations were derived with the similarity for qualitative data programme (SIMQUAL) in the Numerical Taxonomy and Multivariante analysis for personal computer (NTSYS-pc) version 2.0 (Rolf, 1993). Estimates for similarity were based on Jaccard's (1908) coefficient, Nei and Li's similarity index and simple matching coefficients (Francis *et al.*, 1999; Nei., 1972, 1978). DISPAN computer progamme was used to calculate Nei's (1978) standard genetic distance (DS) and Nei *et al* (1983) genetic distance (DA) with their standard error for the pair-wise comparisons using 7 loci. The genetic distance calculation was based on allele frequencies.

F-statistic was used for analysis of the molecular structure of *R .solani* as desribed by Wright, (1978). The statistical indexes involved was used to measure variances of genetic distance, the deficiency or excess of average heterozygotes in each population, the degree of gene differentiation among populations in terms of allele frequencies and the deficiency or excess of average heterozygotes in a group of populations (Appendix 6.0). Matrices of similarity were analysed using unweighted pair group method with arithmetic average clustering method.

Dendrograms were generated with the treev option of NTSYS-pc and goodness of fit calculated using COPH and MXCOPH (Appendix 7.0) programs (Rolf, *et al.*, 1981).

4.4: Results

4.4.1. Primers typed in microsatellite screening

DNA samples of optical density 0.053 to 1.343 and 0.024 to 0.774 at wavelengths 260 and 280 nm respectively were obtained. The purity ratio and DNA concentration in µg/ml ranged from 1.4 to 2.00 and 0.09 to 2.70 respectively (Table 9).

Table 9: Concentration and purity ratio of DNA from *Rhizoctonia solani* isolates

Isolate	OD (260nm)	OD (280nm)	Purity ratio	Concn. µg/ml
R1	0.075	0.043	1.74	0.19
R2	1.080	0.585	1.85	2.70
R3	0.254	0.148	1.72	0.64
R4	0.399	0.230	1.73	1.00
R5	0.655	0.397	1.65	1.64
R6	0.660	0.372	1.66	1.65
R7	0.378	0.200	1.89	0.95
R8	0.053	0.029	1.83	0.13
R9	0.515	0.270	1.91	1.29
R10	0.619	0.378	1.70	1.55
R11	0.930	0.603	1.54	2.33
R12	0.600	0.401	1.50	1.50
R13	0.865	0.530	1.62	2.16
R14	1.302	0.670	1.94	3.25
R15	0.795	0.480	2.10	1.99
R16	0.436	0.266	2.00	1.09
R17	0.400	0.295	1.40	1.00
R18	0.046	0.024	1.92	1.15
R19	0.329	0.168	1.96	0.82
R20	0.600	0.330	1.82	1.50
R21	0.554	0.310	1.81	1.39
R22	0.498	0.314	1.60	1.25
R23	1.020	0.591	1.73	2.55
R24	0.288	0.158	1.82	0.72
R25	0.350	0.180	1.94	0.86

Table 9 continued

Isolate	OD (260nm)	OD (280nm)	Purity ratio	Concn. µg/ml
R26	1.450	0.732	1.98	3.63
R27	0.185	0.096	1.93	0.46
R28	0.060	0.040	1.50	0.15
R29	0.160	0.094	1.70	0.40
R30	0.440	0.285	1.50	1.10
R31	0.268	0.143	1.87	0.67
R32	0.290	0.160	1.81	0.73
R33	0.142	0.083	1.71	0.36
R34	0.034	0.018	1.89	0.09
R35	0.498	0.278	1.79	1.25
R36	1.012	0.545	1.86	2.53
R37	0.896	0.472	1.90	0.42
R38	0.736	0.398	1.85	1.84
R39	0.660	0.428	1.54	1.65
R40	0.438	0.305	1.44	1.10
R41	0.098	0.064	1.53	0.25
R42	1.343	0.774	1.74	3.36
R43	0.786	0.423	1.86	1.97
R44	0.875	0.431	2.00	2.19
R45	0.550	0.330	1.70	1.40
R46	0.096	0.066	1.45	0.24
R47	0.778	0.434	1.79	1.95
R48	1.000	0.636	1.58	2.50
R49	0.076	0.040	1.90	0.19
R50	0.938	0.485	1.93	2.35
R51	0.034	0.018	1.89	0.09
R52	0.216	0.130	1.66	0.54
R53	0.060	0.035	1.71	0.15
R54	0.444	0.245	1.81	1.11
R55	0.078	0.046	1.70	0.20
R56	0.088	0.056	1.57	0.22

R1…56- code for *Rhizoctonia solani* isolates numbered 1 to 56

OD=Optical density.

Concn.=Concentration

The Seven primers RB23, RE14, RE102, BC5, AF513014, AY212027 and LROR could successfully amplify DNA from some or all of the 52 out of the 56 *R. solani* isolates producing fragments of sizes 130-1800bp, with average annealing temperature of 50-58 °C. RB23

produced fragments of 183-194bp at 55 °C, RE14 had 160-195bp at 55 °C, RE102 had 130-145bp at 58 °C, BC5 had 140-190bp at 58 °C, AF513014 had 188-238bp at 55 °C, AY212027 had 285-297bp at 55 °C and LROR had 1400-1800bp at 50 °C. The primer LHOR7 could not amplify DNA from any of the 56 isolates. DNA samples from 4 *R. solani* isolates could not be amplied by any of the 8 primer sets.

4.4.2. Alleles typed in the *R. solani* DNA during microsatellite screening

The following 66 alleles were typed in 52 isolates using the 7 microsatellite loci. There was high DNA sequence homology on the lower scale of 130-297 bp representing 84.8% of the alleles. The primer LHOR typed the largest number of null alleles (Table 10).

Table 10: Allele by locus from *Rhizoctonia solani* population.

Isolate	Locus RB 23	Locus RE 14	Locus RE 102	Locus BC 5	Locus AF513014	Locus AY 212027	Locus LHOR
R1	0,0	175,177	143,145	162,168	191,193	285,288	1520,1523
R2	186,192	175,190	137,145	0,0	229,230	0,0	0,0
R3	186,186	189,190	135,137	158,161	191,193	0,0	0,0
R4	183,186	175,177	135,137	156,158	225,229	290,293	1800,1800
R5	183,186	0,0	130,133	140,140	188,188	290,291	1520,1800
R6	186,186	175,180	133,145	162,164	216,219	288,295	1520,1523
R7	183,186	181,181	130,145	178,178	236,238	288,293	0,0
R8	183,186	180,181	130,133	140,140	188,188	290,291	1520,1520
R9	192,192	160,160	135,137	158,158	191,193	291,293	1523,1523
R10	183,186	180,180	0,0	140,140	188,188	290,291	0,0
R11	186,194	189,190	143,145	168,169	222,225	291,293	1520,1795
R12	186,194	180,181	133,137	168,169	213,214	291,293	1410,1415
R13	183,186	180,181	130,133	169,172	219,222	293,295	1415,1480
R14	186,194	180,181	133,137	168,169	213,214	295,297	0,0
R15	186,192	160,160	143,145	174,178	193,199	295,297	1523,1800
R16	183,186	175,181	133,137	168,169	222,225	290,293	1523,1800
R17	183,186	177,180	143,145	140,142	188,191	285,288	1795,1800
R18	183,186	160,181	135,137	140,142	205,208	285,290	0,0
R19	192,194	190,195	0,0	155,156	236,238	0,0	0,0
R20	183,186	0,0	133,135	158,162	229,230	295,297	0,0
R21	186,194	175,177	133,135	162,164	211,213	288,291	0,0
R22	183,186	181,181	130,130	174,178	234,238	293,297	0,0

Table 10. Continued

Isolate	Locus RB 23	Locus RE 14	Locus RE 102	Locus BC 5	Locus AF513014	Locus AY 212027	Locus LHOR
R23	183,186	160,195	130,137	156,158	213,214	285,297	1415,1415
R24	186,186	160,175	137,143	161,162	193,193	293,295	1523,1523
R25	183,186	189,190	130,133	169,172	236,238	291,293	1480,1795
R26	183,186	0,0	130,143	174,178	234,236	293,297	1410,1410
R27	192,194	180,181	130,133	161,162	234,236	290,291	1523,1800
R28	183,186	160,189	135,143	142,155	208,211	285,291	0,0
R29	183,183	181,181	133,135	140,142	188,188	295,297	1410,1415
R30	0,0	175,175	130,143	158,161	214,215	288,291	1415,1523
R31	183,186	160,180	0,0	0,0	199,204	285,288	0,0
R32	183,186	0,0	133,135	174,178	193,199	0,0	1520,1800
R33	183,186	175,195	137,143	172,174	230,234	290,297	1520,1523
R34	186,192	0,0	137,143	169,172	191,193	293,295	1410,1415
R35	183,186	160,177	143,145	164,164	193,199	295,297	1795,1800
R36	0,0	189,190	0,0	156,161	222,225	0,0	0,0
R37	186,186	177,180	143,145	140,142	188,191	285,288	1795,1800
R38	183,192	180,181	130,133	140,140	188,188	290,291	1415,1520
R39	183,192	177,180	130,135	155,156	188,188	290,291	0,0
R40	186,192	175,177	143,143	0,0	188,188	290,291	1415,1795
R41	186,192	175,177	130,145	161,162	215,216	288,293	0,0
R42	183,192	0,0	135,137	140,140	202,204	293,295	0,0
R43	183,192	0,0	135,137	140,140	202,204	293,295	1480,1520
R44	183,186	190,195	130,135	172,174	188,191	290,291	0,0
R45	183,183	0,0	135,135	140,140	188,188	290,291	1520,1795
R46	186,192	189,190	143,145	0,0	202,204	293,295	0,0
R47	183,186	175,189	133,135	169,172	225,229	290,295	0,0
R48	183,186	160,181	133,135	168,169	204,205	288,290	1480,1520
R49	0,0	190,195	143,145	140,140	188,191	290,291	1480,1520
R50	183,186	160,190	135,145	155,156	211,213	285,295	0,0
R51	194,194	177,180	135,137	164,168	213,214	291,293	1410,1800
R52	186,192	160,160	143,143	174,178	193,199	295,297	0,0

Key: R1.....R52- code for *Rhizoctonia solani* isolates numbered 1 to 52.

All loci were found to be polymorphic in the population across all the microsatellite loci. The smallest allele size detected was 130 bp at RE102 and the largest 1800 bp at LHOR. The observable number of alleles at each locus ranged from 4 at locus RB23 to 21 at AF 51301. The mean number of alleles detected per loci was 9.42 (Table 11).

The gene diversity was computed for all the 7 microsatellite loci studied and the values were as follows 0.605, 0.630, 0.766, 0.774, 0.828, 0.813 and 0.770 for RB23, RE14, RE102, AY2120, AF5130 and LROR, respectively. The expected average hetrozygosity (He) which is the same as Nei's (1987) genetic diversity was 0.85 whilst observed heterozygosity (Ho) was 0.84 (Table 11).

Table 11: Heterozygosity within *Rhizoctonia solani* population.

Loci typed	Unbiased HZ	Unbiased HZ SD	Obs.HZ	Obs.HZ SD	No.alleles	No.of Alleles SD
7	0.85	0.09	0.84	0.09	9.42.	0.08

Key: Loci typed=Number of loci typed in the sample; Unbiased HZ=Nei's unbiased gene diversity (Nei, M., 1987); Unbiased SD=unbiased gene diversity inter-locus standard deviation; Obs. HZ=observed heterozygosity; Obs.HZ SD=observed heterozygosity standard deviation; No.Alleles=Mean number of alleles/locus (MNA); No.Alleles SD=MNA standard deviation.

Chi-square (X^2) at $P \leq 0.05$ computation for HW test indicated that 2 out of the 7 loci were in equilibrium (Table 12). The loci LROR and RB 23 were in HW equilibrium whereas AY212027, AF 513014, BC5, RE 102 and RE 14 were not (Table 12).

Table 12: Chi-square for Hardy-Weinberg equilibrium test ($P \leq 0.05$) at various loci

Locus	Chi-square	Degree of freedom	Probability	X^2tab	Ho
RB23	9.09	6	0.17	12.59	Accepted
RE 14	72.65	28	0.00	41.34	Rejected
RE 102	37.21	15	0.00	25.00	Rejected
BC 5	227.73	78	0.00	43.77	Rejected
AF513014	478.44	210	0.00	43.77	Rejected
AY212027	70.64	21	0.00	32.67	Rejected
LROR	26.61	21	0.18	32.67	Accepted

Htab=Chi-square calculated, Ho=the hardy-Weinberg principle

4.4.3. Genetic relationship among the *Rhizoctonia solani* isolates

Jacard's coefficient of genetic distance varied from 0.9000 to 0.000 for the 52 *R. solani* isolates typed (Table 13).

Table 13: Similarity among the *R. solani* isolates based on the coefficient of genetic distance

R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30
R31	R32	R33	R34	R35	R36	R37	R38	R39	R40	R41	R42	R43	R44	R45
R46	R47	R48	R49	R50	R51	R52								

R1	1.000														
R2	3.3333	1.0000													
R3	2.8571	2.3077	1.0000												
R4	2.0000	1.4286	1.2500	1.0000											
R5	2.6667	2.1429	1.8750	2.6667	1.0000										
R6	2.0000	2.3077	1.2500	2.0000	3.5714	1.0000									
R7	3.3333	7.5000	3.3333	1.4286	2.1429	2.3077	1.0000								
R8	2.6667	1.3333	1.8750	1.8750	5.3846	4.6154	1.3333	1.0000							
R9	3.3333	1.6667	2.3077	1.4286	3.0769	2.3077	1.6667	4.1667	1.0000						
R10	1.7647	3.8462	1.7647	1.1111	5.0000	2.5000	2.8571	3.1250	2.0000	1.0000					
R11	7.2727	3.0769	2.6667	2.6667	2.5000	1.8750	3.0769	2.5000	3.0769	1.6667	1.0000				
R12	2.8571	2.3077	1.2500	2.8571	9.0000	3.8462	2.3077	5.8333	3.3333	4.2857	2.6667	1.0000			
R13	1.3333	2.5000	2.1429	6.2500	2.0000	3.0769	2.5000	2.0000	1.5385	1.8750	1.2500	1.3333	1.0000		
R14	1.4286	1.6667	2.3077	1.4286	1.3333	2.3077	1.6667	2.1429	7.6923	5.8824	1.3333	1.4286	3.6364	1.0000	
R15	6.3636	2.3077	2.8571	2.0000	2.6667	2.8571	2.3077	3.5714	3.3333	1.1111	5.8333	2.8571	2.1429	2.3077	1.0000
R16	3.5714	5.4545	1.8750	2.6667	3.3333	4.6154	4.1667	3.3333	2.1429	3.1250	3.3330	3.5714	3.8462	3.0769	4.2857
	1..0000														
R17	8.8889	3.6364	3.0769	2.1429	2.8571	2.1429	3.6364	2.8571	3.6364	1.8750	8.0000	3.0769	1.4285	1.5385	7.0000
	3.8462	1.0000													
R18	2.1429	1.5385	2.1429	6.2500	2.8571	2.1429	1.5385	2.8571	5.0000	2.6667	2.0000	2.1429	3.3333	7.1429	2.1429
	2.0000	2.3077	1.0000												
R19	3.5714	3.0769	3.5714	1.8750	4.2857	1.8750	3.0769	2.5000	3.0769	2.3529	3.3333	3.5714	5.0000	2.1429	3.5714
	3.3333	3.8462	2.8571												
R20	2.5000	2.0000	2.5000	4.2857	3.1250	1.7647	2.0000	1.6667	2.0000	2.2222	2.3529	2.5000	1.1765	5.8824	1.7647
	2.3529	2.6667	1.8750	2.3529	1.0000										
R21	3.5714	3.0769	3.5714	1.8750	4.2857	1.8750	3.0769	2.5000	2.1429	2.3529	3.3333	3.5714	3.8462	2.1429	3.5714
	3.3333	3.8462	2.0000	8.1818	2.3529	1.0000									
R22	2.8571	7.7778	2.0000	2.8571	1.8750	2.0000	6.0000	1.1765	1.4286	3.3333	3.5714	2.0000	2.1429	1.4286	2.0000
	4.6154	3.0769	1.3333	2.6667	2.5000	2.6667	1.0000								
R23	3.1250	2.6667	2.3529	2.3529	4.6667	3.1250	2.6667	3.7500	4.6154	3.5294	2.9412	4.0000	3.3333	1.1765	3.1250
	3.7500	3.3333	5.3846	4.6667	2.7778	3.7500	2.3529	1.0000							
R24	2.5000	2.0000	2.5000	2.5000	2.3529	1.7647	2.0000	2.3529	2.0000	1.5789	2.3529	2.5000	1.1765	1.2500	2.5000
	2.3529	2.6667	1.1765	3.1250	2.9412	3.1250	1.7647	2.7778	1.0000						
R25	3.1250	2.6667	3.1250	1.6667	3.7500	1.6667	2.6667	2.2222	1.8750	2.1053	2.9412	3.1250	3.3333	1.8750	3.1250
	2.9412	3.3333	1.7647	6.9231	2.7778	8.3333	2.3529	3.3333	2.7778	1.0000					
R26	2.8571	6.0000	2.0000	2.8571	1.8750	2.0000	4.5455	1.1765	1.4286	3.3333	3.5714	2.0000			
	2.1429	2.3077	2.0000	4.6154	3.0769	1.3333	2.6667	2.5000	2.667	8.0000	2.3529	1.7647	1.3529	1.0000	
R27	3.0769	3.6364	2.1429	2.1429	2.851	3.0769	3.6364	2.8571	1.5385	1.8750	2.8571	3.0769	3.3333	3.6364	3.0769
	5.0000	3.3333	6.6667	3.8462	1.8750	3.8462	3.0769	2.5000	2.6667	4.2857	3.0769	1.0000			
R28	1.4286	7.6923	1.4286	6.6667	4.1667	2.3077	7.6923	3.0769	2.7273	2.8571	1.3333	3.3333	1.5385	7.6923	1.4286
	1.3333	1.5385	3.6363	1.3333	2.0000	1.3333	6.6667	2.6667	1.2500	1.1765	6.6667	7.1429	1.0000		
R29	1.8750	3.0769	1.8750	1.8750	5.3846	3.5714	3.0769	3.3333	2.1429	5.0000	1.7647	4.6154	2.8571	1.3333	1.8750
	3.3333	2.0000	2.8571	3.3333	2.3529	3.3333	2.6667	4.6667	3.1250	2.9412	2.6667	2.8571	3.0769	1.0000	
R30	1.5384	3.0000	1.5385	1.5385	3.3333	1.5385	3.0000	1.4286	3.0000	4.1667	1.4286	2.5000	2.7273	0.0000	7.1429
	2.3077	1.6667	4.0000	3.3333	2.1429	2.3077	2.5000	5.0000	1.3333	2.0000	2.5000	1.6667	1.8181	4.5455	1.0000
R31	2.2222	1.7647	2.2222	2.2222	2.1053	1.5789	1.7647	2.1053	1.7647	1.4286	2.1053	2.2222	1.0526	1.1111	2.2222
	2.1053	2.3529	1.0526	2.7778	2.6316	2.7778	1.5789	2.5000	8.4615	2.5000	1.5789	2.3529	1.1111	2.7778	1.1765
	1.0000														
R32	1.1111	2.0000	1.7647	1.1111	5.0000	3.3333	2.0000	4.0000	2.0000	4.6667	1.0526	4.2857	2.6667	1.2500	1.7647
	2.3529	1.1765	2.6667	2.3529	1.5789	2.3529	1.7647	3.5294	2.2222	2.1053	1.7647	1.8750	2.8571	6.1538	3.0769
	2.6316	1.0000													

Table 13. Continued

R33	2.1429	3.6364	1.3333	2.1429	2.8571	3.0769	3.6364	2.0000	1.5385	2.6667	2.0000	3.0769	3.3333	1.5385	1.3333
	3.8462	2.3077	1.4286	3.8462	2.6667	3.8462	3.0769	2.5000	1.8750	3.3333	3.0769	3.3333	7.1429	2.8571	2.7273
	1.6667	1.8750	1.0000												
R34	3.5714	2.1429	7.2727	1.8750	2.5000	1.8750	2.1429	2.5000	3.0769	1.6667	3.3333	1.8750	2.0000	2.1429	3.5714
	2.5000	3.8462	2.8571	3.3333	3.1250	3.3333	1.8750	2.9412	2.3529	2.9412	1.8750	2.0000	2.1429	1.7647	1.4286
	2.1053	1.6667	1.2500	1.0000											
R35	3.3333	2.0000	1.7647	2.5000	4.0000	4.2857	2.0000	4.0000	3.8462	2.2222	3.1250	4.2857	1.8750	1.2500	3.3333
	3.1250	3.5714	2.6667	4.0000	2.9412	3.1250	1.7647	4.3750	3.7500	2.7778	1.7647	2.6667	2.0000	3.1250	2.1429
	3.3333	2.2222	1.8750	2.3529	1.0000										
R36	2.8571	3.3333	2.8571	3.8462	1.8750	1.2500	3.3333	1.1765	1.4286	2.5000	2.6667	2.0000	1.3333	2.3077	2.0000
	2.6667	3.0769	6.2500	2.6667	4.2857	2.6667	3.8462	1.6667	2.5000	3.1250	3.8462	3.0769	6.6667	1.8750	1.5385 \
	2.2222	1.1111	2.1429	2.6667	1.7647	1.0000									
R37	5.0000	3.3333	6.3636	2.8571	3.5714	2.0000	3.3333	2.6667	3.3333	2.5000	4.6154	2.8571	2.1429	2.3077	3.8462
	3.5714	5.4545	3.0769	4.6154	4.2857	4.6154	2.8571	4.0000	3.3333	4.0000	2.8571	3.0769	2.3077	2.6667	2.5000
	2.9412	1.7647	2.1429	7.2727	3.3333	3.8462	1.0000								
R38	2.6667	3.0769	1.1765	1.8750	4.2857	3.5714	3.0769	3.3333	4.1667	4.0000	2.5000	4.6154	2.0000	6.2500	1.8750
	3.3333	2.8571	3.8462	2.5000	2.3529	1.7647	2.6667	4.6667	2.3529	1.5789	2.6667	2.0000	4.1667	5.3846	4.5454
	2.1053	4.0000	2.8571	1.7647	4.0000	1.8750	2.6667	1.0000							
R39	2.0000	1.4286	3.8462	1.2500	2.6667	2.0000	1.4286	3.5714	4.5454	1.7647	1.8750	2.8571	2.1429	2.3077	2.8571
	1.8750	2.1429	3.0769	3.5714	1.1111	2.6667	1.2500	4.0000	3.3333	2.3529	1.2500	2.1429	2.3077	2.6667	2.5000
	2.9412	2.5000	1.3333	3.5714	3.3333	1.2500	2.8571	2.6667	1.0000						
R40	2.6667	3.0769	1.1765	2.6667	3.3333	4.6154	3.0769	3.3333	2.1429	2.3529	2.5000	3.5714	5.0000	4.1667	2.6667
	5.3846	2.8571	2.8571	3.3333	1..6667	2.5000	2.6667	3.7500	1.6667	2.2222	2.6667	3.8462	2.1429	3.3333	2.3077
	1.5000	2.3529	2.8571	1.7647	4.0000	2.6667	2.6667	4.2857	1.8750	1.0000					
R41	2.0000	2.3077	1.2500	2.0000	3.5714	8.0000	2.3077	4.6154	2.3077	2.5000	1.8750	3.8462	3.0769	2.3077	2.8571
	5.8333	2.1429	2.1429	1.8750	1.7647	1.8750	2.0000	3.1250	1.7647	1.6667	2.0000	3.0769	2.3077	3.5714	1.5385
	1.5789	3.3333	3.0769	1.8750	4.2857	1.2500	2.0000	3.5714	2.0000	4.6154	1.0000				
R42	2.8571	2.3077	2.0000	2.0000	5.8333	2.8571	2.3077	3.5714	2.3077	4.2857	2.6667	5.0000	1.3333	6.6667	2.0000
	2.6667	3.077	3.0769	2.6667	3.3333	2.6667	2.0000	3.1250	1.7647	2.3529	2.0000	2.1429	3.3333	4.6154	2.5000
	1.5789	3.3333	2.1429	2.6667	3.3333	2.0000	3.8462	3.5714	1.2500	2.6667	2.8571	1.0000			
R43	2.6667	4.1667	1.8750	2.6667	4.2857	4.6154	3.0769	3.3333	2.1429	4.0000	2.5000	3.5714	3.8462	2.1429	2.6667
	6.6667	2.8571	2.8571	3.3333	4.0000	3.3333	3.5714	4.6667	3.1250	2.9412	3.5714	3.8462	2.1429	4.2857	3.3333
	2.7778	3.1250	3.8462	2.5000	4.0000	1.8750	3.5714	3.3333	1.8750	4.2857	4.6154	3.5714	1.0000		
R44	1.7647	2.8571	1.7647	1.7647	6.1538	3.3333	2.8571	4.0000	2.0000	5.7143	1.6667	5.3846	2.6667	1.2500	1.7647
	3.1250	1.8750	2.6667	3.1250	2.2222	3.1250	2.5000	4.3750	2.9412	2.7778	2.5000	2.6667	2.8571	7.5000	4.1667
	3.3333	8.3333	2.6667	1.6667	2.9412	1.7647	2.5000	5.0000	2.5000	3.1250	3.3333	4.2857	4.0000	1.0000	
R45	1.6667	2.6667	1.6667	1.6667	4.6667	4.0000	2.6667	3.7500	1.8750	4.3750	1.5789	4.0000	3.3333	1.8750	1.6667
	3.7500	1.7647	2.5000	2.9412	2.1053	2.9412	2.3529	5.0000	2.7778	2.6316	2.3529	3.3333	2.6667	6.9230	3.8462
	2.5000	5.3333	3.3333	1.5789	2.7778	1.6667	2.3529	4.6667	2.3529	3.7500	4.0000	3.1250	4.6667	6.4286	1.0000
R46	1.7647	3.8462	1.7647	1.1111	5.0000	2.5000	2.8571	3.1250	2.0000	8.3333	1.6667	4.2857	1.8750	5.8823	1.1111
	3.1250	1.8750	2.6667	2.3529	2.2222	2.3529	3.3333	3.5294	1.5789	2.1053	3.3333	1.8750	2.8571	5.0000	4.1667
	1.4286	4.6667	2.6667	1.6667	2.9412	1.7647	2.5000	4.0000	1.7647	2.3529	2.5000	4.2857	4.0000	5.7143	4.3750
	1.0000														
R47	2.3077	4.0000	1.4286	1.4286	3.0769	3.3333	4.0000	2.1428	1.6667	2.8571	2.1429	3.3333	3.6363	1.6667	1.4286
	4.1667	2.5000	1.5385	4.1667	2.0000	4.1667	3.3333	2.6667	2.0000	3.5714	3.3333	3.6364	7.6923	3.0769	3.0000
	1.7647	2.0000	8.7500	1.3333	2.0000	2.3077	2.3077	3.0769	1.4286	3.0769	3.3333	2.3077	4.1667	2.8571	3.5714
	2.8571	1.0000													
R48	2.3529	2.6667	1.6667	1.6667	3.7500	3.1250	2.6667	2.9412	3.5714	3.5294	2.2222	4.0000	1.7647	1.1765	1.6667
	2.9412	2.5000	3.3333	2.2222	2.1053	1.5789	2.3529	4.1176	2.1053	1.4286	2.3529	1.7647	2.6667	4.6667	3.8462
	3.1579	4.3750	2.5000	2.2222	3.5294	1.6667	3.1250	6.9231	3.1250	3.7500	3.1250	3.1250	2.9412	5.3333	4.1176
	3.5294	2.6667	1.0000												
R49	1.7647	3.8462	1.7647	1.1111	5.0000	2.5000	2.8571	3.1250	2.0000	8.3333	1.6667	4.2857	1.8750	5.8824	1.1111
	3.1250	1.8750	2.6667	2.3529	2.2222	3.1250	3.3333	3.5294	1.5789	2.7778	3.3333	1.8750	2.8571	5.0000	4.1667
	1.4286	4.6667	2.6667	1.6667	2.2222	2.5000	2.5000	4.0000	1.7647	2.3529	2.5000	4.2857	4.0000	5.7143	4.3750
	6.9231	2.8571	3.5294	1.0000											
R50	1.4286	7.6923	1.4286	1.4286	4.1667	2.3077	7.6923	3.0769	2.7273	2.0000	1.3333	3.3333	2.5000	1.6667	2.3077
	2.1429	1.5385	5.0000	2.1429	2.0000	2.1429	6.6667	3.5714	1.2500	1.8750	6.6667	1.5385	5.5556	3.0769	1.8182
	1.1111	2.8571	7.1429	2.1429	2.0000	6.6667	2.3077	3.0769	2.3077	2.1429	2.3077	3.3333	3.0769	2.8571	2.6667
	2.0000	7.6923	1.8750	2.0000	1.0000										
R51	3.0769	3.6364	2.1425	2.1429	2.8571	3.0769	3.6364	2.8571	1.5385	1.8750	3.8462	3.0769	3.3333	3.6364	3.0769
	5.0000	3.3333	6.6667	3.8462	1.8750	3.8462	3.0769	2.5000	2.6667	3.3333	3.0769	7.7778	7.1429	2.8571	1.6667
	2.3529	1.8750	3.3333	2.0000	2.6667	3.0769	3.0769	2.0000	2.1429	3.8462	3.0769	2.1429	3.8462	2.6667	3.3333
	1.8750	3.6364	1.7647	1.8750	1.5385	1.0000									
R52	3.5714	2.1429	7.2727	1.8750	2.5000	1.8750	2.1429	2.5000	3.0769	1.6667	4.2857	1.8750	2.0000	2.1429	3.5714
	2.5000	3.8462	2.8571	3.3333	3.1250	3.3333	1.8750	2.9412	2.3529	2.9412	1.8750	2.0000	2.1429	1.7647	1.4286
	2.1053	1.6667	1.2500	8.1818	2.3529	2.6667	7.2727	1.7647	3.5714	1.7647	1.8750	2.6667	2.5000	1.6667	1.5789
	1.6667	1.3333	2.2222	1.6667	2.1429	2.8571	1.0000								

Key: R1…..R52- code for *Rhizoctonia solani* isolates numbered 1 to 52.

There were moderate differences of 0.5517 to 0.9272 between the Nei's original genetic identity and Nei's unbiased genetic identity but high differences of 0.0756 to 0.7162 between the Nei's original genetic distance and Nei's unbiased genetic distance for populations of *Rhizoctonia solani* from the different agro-ecological zones (Table 14 and 15 respectively).

Table 14: Nei's Original Measures of Genetic Identity and Genetic distance for *Rhizoctonia solani* isolates from different agro-ecological zones

Population identity	UM2	UM3	UM4	LM3
UM2	****	0.7147	0.5517	0.4886
UM3	0.3359	****	0.8161	0.8008
UM4	0.5947	0.2032	****	0.8510
LM3	0.7162	0.2222	0.1614	****

**** =Nei's genetic identity are bove diagonal and genetic distance are below the diagonal.

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

Table 15: Nei's Unbiased Measures of Genetic Identity and Genetic distance for *Rhizoctonia solani* isolates from various ecological zones

Population identity	Um2	UM3	UM4	LM3
UM2	****	0.8164	0.6183	0.5612
UM3	0.2029	****	0.8843	0.8894
UM4	0.4809	0.1230	****	0.9272
LM3	0.5777	0.1172	0.0756	****

**** =Nei's genetic identity are above diagonal and genetic distance are below the diagonal.

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

The variances of Nei's genetic identity and standard genetic distance calculated from the Nei's (1972) original genetic identity and genetic distance 'DS,' and Nei's (1978) unbiased genetic identity and genetic distance 'DA' for populations of *Rhizoctonia solani* indicated good genetic differentiation for isolates populations within ecological zones. The values ranged from 0.0666 to 0.1017 and 0.1385 to 0.0808 respectively (Table 16).

Table 16: Variances of genetic identity and genetic distance for *Rhizoctonia solani* isolates from various agro-ecological zones

population identity	UM2	UM3	UM4	LM3
UM2	****	0.1017	0.0666	0.0731
UM3	-0.1330	****	0.0682	0.0886
UM4	-0.1138	0.0802	****	0.0762
LM3	- 0.1382	10.1050	-0.0876	****

**** = Nei's genetic identity are above diagonal and genetic distance are below the diagonal.
The negative sign indicate deficiency of heterozygotes.
UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

There was overall moderate degree of gene differentiation among populations in terms of allele frequencies as indicated by FST value of 0.13, deficiency of heterozygotes in each population as indicated by FIS value of -0.135 and excess of average heterozygotes in the group of 4 populations indicated by FIT value of 0.01.

The 52 *Rhizoctonia* isolates from all the ecological zones produced 7 distinct clusters which further produced several sub-clusters (Appendix 5.0; Figure 1). In terms of percentages, clusters 1, 2, 3, 4, 5, 6 and 7 had 15.4%, 9.6%, 13.5%, 28.9%, 21.2%, 5.8% and 5.8% of the

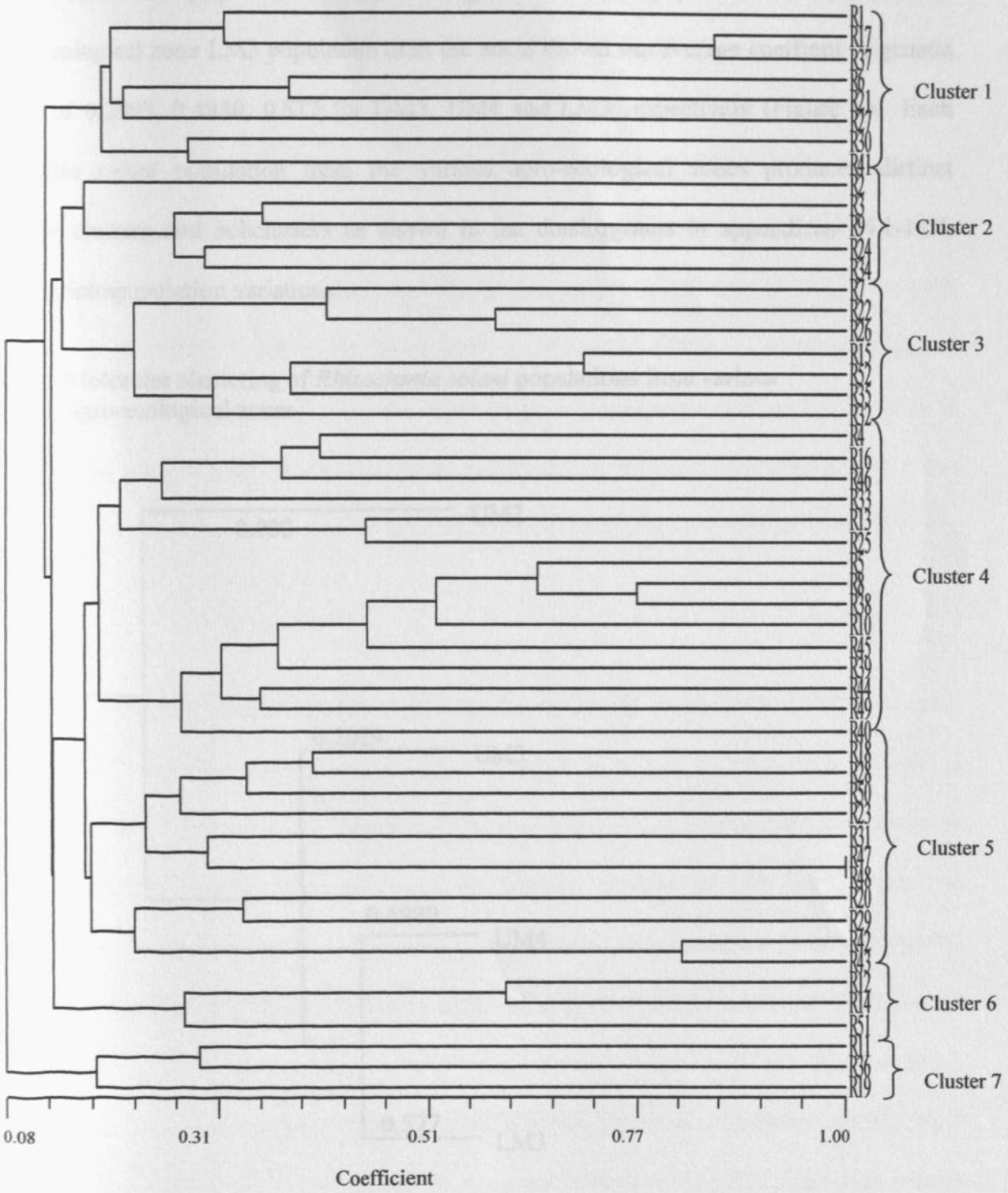
isolates respectively. In terms of ecological zones, cluster 5 and 6 carried 50% each of all the isolates in UM2. The 13 isolates from UM3 were found in 6 clusters 1, 3, 4, 5, 6 and 7 with each of them carrying 7.7%, 15.4%, 30.7%, 30.7%, 7.7% and 7.7% respectively. The 23 isolates from UM4 were found in 6 clusters 1, 2, 3, 4, 5 and 7 with each of them carrying 21.7%, 17.4%, 4.3%, 26.1%, 26.1% and 4.3% of the isolates respectively. The 14 isolates from LM3 were found in all the 7 clusters with clusters 1, 2 ,3, 4, 5, 6 and 7 having 71%, 7,1%, 21.4%, 28.6%, 14.3%%, 14.3% and 7.1% of the isolates respectively (Table 17). During the validation of the clustering by the cophenetic procedure, an average cophenetic value of 0. 4832 was obtained.

Table 17: Molecular clustering of *R. solani* isolates from various agro-ecological zones

Agro-ecological zone	Number of isolates	Molecular cluster						
		1	2	3	4	5	6	7
UM2	2	0.0	0.0	50.0	50.0	0.0	0.0	0.0
UM3	13	7.7	0.0	15.4	30.7	30.7	7.7	7.7
UM4	23	21.7	17.4	4.3	26.1	26.1	0.0	4.3
LM3	14	7.1	7.1	21.4	28.6	14.3	14.3	7.1
Percent of total		15.4	9.6	13.5	28.9	21.2	5.8	5.8

UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

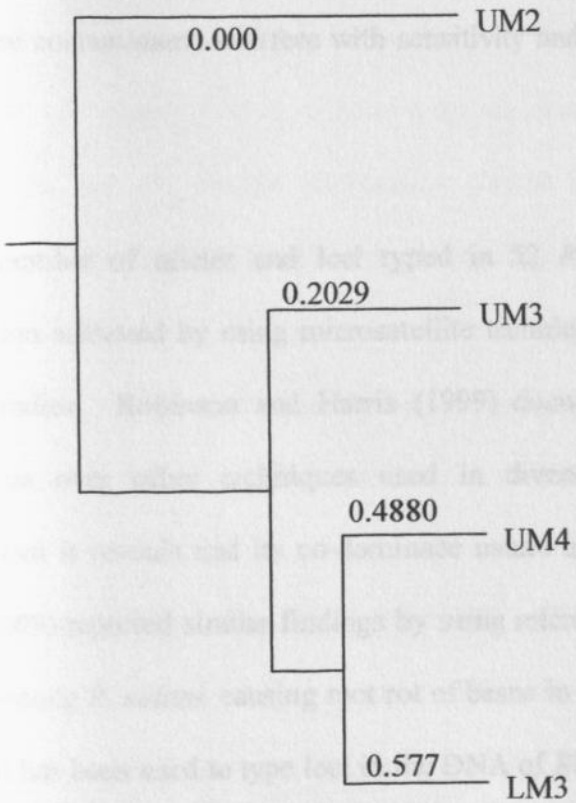
Figure 1: Molecular clustering of *Rhizoctonia solani* isolates causing damping off of tomato



R1-52=code for R. solani isolates numbered 1 to 52.

Phylogenetic analysis of isolate groups from the 4 agro-ecological zones produced distinct genetic groups. Isolate population from agro-ecological zone UM2 was more related to agro-ecological zone UM3 population and agro-ecological zone UM4 population was more related to agro-ecological zone LM3 population than the rest a shown the average coeffient of genetic distance of 0.2009, 0.4880, 0.577 for UM3, UM4 and LM3 respectively (Figure 2). Each *Rhizoctonia solani* population from the various agro-ecological zones produced distinct molecular clusters and subclusters as shown in the dendrograms in appendices 14.1-14.4. indicating intrapopulation variations.

Figure 2: Molecular clustering of *Rhizoctonia solani* populations from various agro-ecological zones



UM2, UM3, UM4 and LM3= Upper midland 2, 3 and 4 respectively; LM3=Lower midland 3.

4.5. Discussion

The specific medium of Ko and Hora (1971), was found to be ideal for culturing *R. solani* for DNA extraction for it prevented contamination by other mycoparasites. Culturing pure *R. solani* isolates on potato dextrose broth and DNA extraction by the procedure of Raeda and Broda (1985), yielded high amount of quality DNA for PCR reaction. Fennile *et al* (2006) and Herdina *et al* (1995) used similar procedure to obtain DNA from *R. solani* for PCR. Addition of RNase to the preparation sample and further purification with CsCl density centrifugation removed RNA and other metabolites respectively that would interfere with polymerization process (Connie, 2000). For *R. solani* clean DNA is difficult to obtain due to the presence of polysaccharides and unwanted DNA from other fungi and bacteria which coprecipitate with target DNA on ethanol and isopropanol, and co-sperate on ion exchange columns (Keijer, *et al.*, 1996). These contaminants interfere with sensitivity and reliability of the assay (Keijer, *et al.*, 1996).

The high number of alleles and loci typed in 52 *R. solani* isolates indicated high gene differentiation achieved by using microsatellite technique implying the procedure is ideal for diversity studies. Robinson and Harris (1999) documented that microsatellite markers is advantageous over other techniques used in diversity studies for the high degree of polymorphism it reveals and its co-dominace nature allows differentiation of heterozygotes. Githinji (2005) reported similar findings by using microsatellite markers to show high genetic diversity among *R. solani* causing root rot of beans in Embu district of Kenya. The fact that a marker that has been used to type loci in the DNA of *Rhizoctonia solani* isolates causing crater disease of wheat could also type some loci in *R. solani* isolates causing damping off of tomato indicates the great variability and unspecialized nature of *Rhizoctonia solani* pathogen.

Flentje *et al* (1970), Anderson (1982), Carol and Dona (1986), Adams (1988), Koike (1996) and Sneh *et al* (1996) reported that *Rhizoctonia solani* occurs worldwide and causes economically important diseases such as damping –off, root and crown rots of many crops as well as fruit decay to a large variety of vegetable and field crops, turfgrasses, ornamentals and fruit and forest trees.

The high genetic diversity as indicated by high number of alleles, high polymorphism of 100% and high expected average heterozygosity of 0.85 implied high genetic variability of the *R. solani* isolates causing damping off of tomato in Kenya. Bjornstad and Roed (2001) described 0.6-0.8 heterozygosity as moderate. These parameters could have a bearing on various characters such as the virulence since the genetic material codes for various proteins that determines such characters as pathogenicity. Barathan and Tavantzis (1991) reported that the high degree of genetic diversity among genomic elements found in natural populations of *R. solani* suggests that the genetic information carried by the genome is more important in phenotype determination than the mere presence of any dsRNA in *R. solani* isolate. Ethan *et al* (2000) reported presence of two segments of dsRNA ranging in size from 86 to 2275 bp and 79 to 2130 bp in *R. solani* isolates that could code for polypeptides of 730 aa that is also found in capsids of other pathogens such as *Fusarium.*

Allele frequencies in 2 out of the 7 loci typed were in Hardy-Weinberg equilibrium. This means that genes controlled by the alleles in those two loci are likely at 95% confidence level to be unchanged in the next generation, but in the absence of forces that shape genetic diversity such as mutation, recombination, selection and genetic drift. It also indicates low levels of random mating in the populations. Although hardy-weinberg equilibrium describes the expectations of

alleles in an idealised situation, its documentation can have a bearing in the management of phenotypes controlled by those alleles-at least in the short run and in the absence of forces that shape the genetic diversity.

The disquilibrium for populations within ecological zones was further demonstrated by the deficiency of heterozygotes in individual populations probably due to large number of null alleles typed. This perhaps could be due to mutations in the binding regions of the microsatellite primers that may inhibit annealing resulting in reduction or loss of the PCR product resulting in null alleles. The primer LROR produced the highest number of null alleles. However, the excess of average heterozygotes and moderate to high variances of genetic distance (Wrights, 1978) in the group of populations indicates substantial rate of gene flow between various ecological zones probably due to inoculum dispersal by run-offs and irrigation water.

The high genetic differentiation among the *Rhizoctonia* isolates causing damping off disease on tomato was further displayed in the large number of genetic clusters found from the overall population of the 4 agro-ecological zones. Similar findings were reported by Githinji (2005) who reported high number of genetic clusters among the *R. solani* isolates causing root rot of beans in Embu district of Kenya using microsatellite technique and UPGMA as a clustering procedure. However, Fennile *et al* (2005) reported somewhat different findings when they found 94-100% genetic similarities among isolates of the binucleate *Rhizoctonia solani* causing root rot of Yacom according to UPGMA analysis using RAPD markers. During the validation of the clustering by the cophenetic procedure an average cophenetic value was obtained indicating that the dendrogram distances reflected the distance data in the original matrix.

The moderate allele frequency differentiation indicated by variance of genetic distance among the 52 isolates using 7 loci indicated good precision in the estimation of population diversity parameters (Wright, 1978). The variance of genetic distance estimate has inverse relationship with the number of loci and isolates sampled per population (Bjornstad and Roed, 2001). The precision could be increased by increasing sample size and or analyzing larger number of loci.

Run-off as a major dispersal agent for *R. solani* between agro-ecological zones was supported by the fact that most of the isolates in clusters found on higher agro-ecological zones UM2 and UM3 were also found on lower ecological zones UM4 and LM3 but not vice versa. Similar studies by Githinji *et al* (2005) indicated that ecological zones had an effect on the distribution of *R. solani* isolates clusters causing root rot of beans in the Embu district of Kenya. Abawi *et al(*1990) and Nik *et al* (1979) reported that *Rhizoctonia* pathogen can disseminate to new area through the planting of contaminated seeds or the movement of infected host tissues, infested soil or colonized debris by irrigation water, wind and animals. The effect of geographical boundaries delimited by districts had moderate effect on isolate cluster distribution whilst the provinces had little effect. This is probably because in Kenya a province usually cut across several regions differing in climate and altitude whereas districts generally occupy a certain region. Meyer *et al* (1997) made similar observations when they reported that various crater disease causing isolates of *R. solani* are similar regardless of geographical origin.

CHAPTER FIVE

EFFICACY OF HOST RESISTANCE, MICROBIAL ANTAGONISM AND CULTURAL PRACTICES IN THE MANAGEMENT OF RHIZOCTONIA DAMPING OFF OF TOMATO

5.1: Abstract

The objective of this chapter was to evaluate the efficacy of nonchemical methods based on host resistance, microbial antagonism and cultural strategies in the control of Rhizoctonia damping off of tomato. Result of a greenhouse experiment indicated that varieties Onyx VF2, Caltana F1 VF and Cal-J VF were moderately susceptible; Monset F1 TM, Roma VF and Marglobe improved were susceptible whilst Money- maker was highly susceptible. The first three weeks after planting were found to be the most susceptible stage of tomato seedlings to Rhizoctonia damping off with the infection reducing by the fourth week. There was biocontrol of Rhizoctonia damping off of tomato indicated by reduced incidence and severity when pathogenic multinucleate *R. solani* and nonpathogenic multinucleate and binucleate *R. solani* but increased damping off of tomato incidence and severity when *R. solani* and pathogenic isolates of *Fusarium* and *Pythium* under the greenhouse conditions.

Results of a field experiment indicated that by reducing coew manure application, use of transplanting, planting on raised beds and increasing irrigation interval significantly ($P \leq 0.05$) reduced *Rhizoctonia* damping off of tomato and resulted in increase of mean percent crop stand and yield. In some cases, the efficacy of these cultural practices either singly or in combination compared favourably to the disease control achieved by using conventional control methods such as chemical soil fumigation with Metham sodium and seed dressing with fungicide. The efficacy of the disease control by microbial antagonism and cultural methods can further be

enhanced by combining with other compatible disease control strategies such as chemical and host resistance , and be part of a sustainable cost-effective disease management program.

5.2: Introduction

In Kenya Rhizoctonia damping off is a serious disease of tomato and other vegetables such as kales, beans, okra, eggplant and flowers (Kenya Agricultural Research Institute, 2002; Githinji, 2005). Lack of sustainable control strategies is a major drawback in the management of the disease in tomato and other vegetables. Planting disease resistant or less susceptible cultivars or varieties is a cost –effective method of lessening the impact of *R. solani* (Abawi *et al.*, 1990; Danesha *et al*, 1999). Although genetic resistance to various fungal pathogens such as *Verticillium dahliae*, *Fusarium oxysporum fsp lycopersi* are known for most popular tomato varieties grown in Kenya, there is no information is available on their susceptibility to *Rhizoctonia* damping off (Regina seeds company, 2004).

Currently, soil disinfestations is mainly accomplished through drastic means such as chemical fumigation with fumigant and nonfumigant fungicides or steaming (Baker 1970; Munnecke *et al.*, 1979; Katan 1980). The recent decision by environmental protection agency of the United Nations to ban the use of fumigant fungicide methyl bromide in agriculture by 2015 due to its hazardous effect on the environment is a major drawback in the control of *Rhizoctonia* and other soilborne pathogens (Noling and Becker, 1994; Watson *et al.*, 1992). Although several nonfumigant and seed dressing chemicals are available for the control of *Rhizoctonia solani*, none is as effective as methyl bromide. Some of these performance inconsistencies after repeated use may be due to enhanced biodegradation and their specificity (Mojtahed *et al.*, 1991; Stirling *et al.*, 1992; Hornsby *et al.*, 1991; Norris *et al.*, 1991). Several soilborne fungi and bacteria have been implicated to be antagonistic to *R. solani* and reduces damping off

incidence and severity in many vegetable and field crops (Hadar *et al.*, 1979; Damping off wikipedia, 2008). *Rhizoctonia solani* has been controlled in the laboratory and the damping off disease it causes in several crops under greenhouse and field conditions by *Trichoderma* species, binucleate *Rhizoctonia* like fungi, *Gliocladium* species and *Laetisaria arvali* (Huber *et al.*, 1966; Hadar *et al.*, 1979; TU and Vaartaja, 1982; Crsistiane Domingoes, 2008). Biocontrol with microbial fungicides is being investigated in several academic laboratories. Typical targets are those plants being mass-produced in nearby commercial greenhouses (Damping off wikipedia, 2008). Early results indicate damping-off prevention comparable to that achieved with the use of standard fungicidal drenches. However, there remains some notable drawbacks in biocontrol of damping-off which includes their narrow host range, some formulations produce phytotoxic substances and changes in environmental conditions have been shown to affect their efficacy in some cases (Damping off wikipedia, 2008).

Laboratory isolation and characterization of Rhizoctonia damping off pathogens from infected plants and rhizosphere soil collected during the survey indicated that although *R. solani* was the most frequently isolated pathogens, other soilborne pathogens such as *Fusarium, Pythium* and *Pseudomonas* bacteria were also present. This implied the involvement of several soilborne pathogens in causing Rhizoctonia damping off disease complex. Effect of such interactions and host resistance on disease levels can only be established through a comprehensive evaluation under controlled greenhouse conditions. Greenhouse screening procedures have the advantage of providing constant favourable conditions for disease development, uniform and high levels of inoculum, and uniform high incidence of infection as opposed to field screening conditions (Dickson *et al.*, 1977). However, promising candidates from the greenhouse evaluation must

eventually be tried in the field in the presence of interacting biological and physical factors (Onkar *et al.*, 1986).

Almost all crop production activities have direct or indirect impact on root rot incidence and severity. Particular influence are by conditions such as level of organic matter, drainage, compaction and structure. In addition, cultural practices that influence plant development and vigor may also affect plant reaction to root rot (Badry *et al.*, 1979; Maker *et al.*1982 and Rodes *et al.*, 1975). However there is no information available data on the the effect of the common cultural practices employed by most tomato growers in Kenya on Rhizoctonia damping off disease (KARI, 2002).

Therefore this Chapter sought to evaluate the 7 popular commercial tomato varieties grown in Kenya for susceptibility to local strains of *R. solani* isolates and assess the effect of interaction between pathogenic *R. solani* isolates and other soilborne pathogens on the disease control under greenhouse conditions, and carry field evaluation of the efficacy of common cultural practices on the diseas control. The efficacy of the various cultural practices on Rhizoctonia damping off of tomato control were compared with the disease control achieved by using conventional control methods such as chemical fumigation with Basamid and chemical seed treatment with Gaucho 390 FS MT and Captan 1200EC.

5.3: Materials and methods

5.3.1. Culture of *R. solani* and inoculum preparation

Preparation of *Rhizoctonia* inoculum and inoculation was as outlined in the pathogenicity test in section 3.3.4.1 except that a mixed inoculum of all the isolates was used. Stored isolates were retrieved as per the procedure in section 3.3.5. Where inoculum was used, the order of

inoculation was as follows: *R. solani* inoculum was added into steam sterilized soil mixture, followed by *Pythium, Fusarium* and finally *Pseudomonas.* For field inoculation, 200cm^3 of 10^6 mycelial fragments of *R. solani* per gram of soil-potato inoculum per hill was used and mixed with the soil gently before tomato seeds were planted.

5.3.2. Evaluation of tomato varieties for susceptibility to *Rhizoctonia solani* damping off

The greenhouse experiment was carried out at the University of Nairobi Kabete Campus.

A mixture of the 56 pathogenic isolates of *Rhizoctonia solani* identified during the pathogenicity test was evaluated against each of the 7 tomato varieties. The 7 tomato varieties used were Cal-J VF, Onyx VF2, Caltana F1 VF hybrid, Monset F1 TM, Marglobe improved, Roma VF and Money maker. The experiment was designed as a 7 by 2 factorial with tomato varieties as treatment factors, the infested and the uninfested pots as the treatment levels in a completely randomized block design with 5 replicates. The ambient temperature inside the greenhouse during the trial period was 18-27°C. The reaction of the different tomato varieties to Rhizoctonia inoculum was categorized as 1.0=immune, 1.1-1.4=resistant, 1.5-1.9=intermediate, 2.0-2.9=susceptible and 3.0-3.9=highly susceptible (Scale adopted from Abawi *et al.*, 1990). The experiment was repeated twice.

5.3.3: Determination of synergism between *Rhizoctonia solani* and other soilborne pathogens on causing damping off of tomato

Laboratory isolation and characterization of *Rhizoctonia* and other soilborne pathogens involved in root rot disease complex from all the tomato growing areas of Kenya had yielded 56 pathogenic isolates of multinucleate *Rhizoctonia solani*, 10 nonpathogenic isolates of multinucleate *Rhizoctonia solani*, 10 nonpathogenic isolates of binucleate *Rhizoctonia*, 2

pathogenic *Fusarium* isolates, 1 pathogenic *Pythium* isolate and 2 nonpathogenic *Pseudomonas* bacteria isolates. This greenhouse experiment was carried out to assess any interaction between these soilborne pathogens and pathogenic *R. solani* in causing Rhizoctonia damping off disease complex on tomato. *Rhizoctonia solani* susceptible tomato variety Roma VF was used.

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The specific treatments were PRS= mixed inoculum of the 56 pathogenic *R.solani* isolates, PF=mixed inoculum of the 2 pathogenic *Fusarium* isolates, P=Pathogenic *Pythium* isolate, NRS= mixed inoculum of the 10 nonpathogenic multinucleate *Rhizoctonia solani* isolates, BR= mixed inoculum of the 10 binucleate Rhizoctonia-like fungi isolates, Pba= mixed inoculum of the 2 *Pseudomonas* bacteria strains, MRS=mixed inoculum from all the different pathogens and untreated=uninfested control. The experiment was designed as a completely randomized design with 5 replicates. The ambient temperature inside the greenhouse during the trial period was 18-27°C. The experiment was repeated twice.

5.3.4. Evaluation of the efficacy of cultural methods in management of Rhizoctonia damping off of tomato

This open field experiment was conducted to evaluate the effect of 4 cultural practices commonly practiced by tomato farmers in Kenya in the management of Rhizoctonia damping off of tomato. The trials were conducted for two seasons at Kabete Campus of the Univeristy of Nairobi farm. Since the trials were conducted in the open, timing was synchronized to coincide with the period of no rains so that the effect of watering regime could be evaluated. The first and second season experiments were conducted during the dry months of July–October 2004 with average temperature of 22-25^{0}C and January– March 2005 with average temperature of 22-26^{0}C respectively. The soils at the experimental site were deep loamy to clay and slightly

alkaline with P^H 7.5-8.5. The screening involved 3 common commercial tomato varieties grown in Kenya against a mixed inoculum of 56 pathogenic strains of *R. solani* extracted from infected tomato seedlings and rhizosphere soil sampled from the major production regions of the country. Prior greenhouse experiment indicated that the 3 tomato varieties Caltana F1 VF, Roma VF and Marglobe improved were susceptible to the *R. solani* strains.

The experiment was a split plot in a completely randomized block design with 4 replicates. Randomization of 2nd, 3rd and 4th replicates was done in the field during the trial set up. Randomisation was done within the treatments in a block and the blocks themselves. Microplots measuring 2.7m long and 2.1m wide were used as experimental unit and replicated 4 times to make an experimental block.

The specific treatments were effect of planting on raised beds, effect of cow manure application, effect of varying watering interval, effect of transplanting,, effect of planting on raised beds + cow manure application, effect of cow manure application + varying watering interval, effect of planting on raised beds + varying watering interval and effect of planting on raised beds + varying watering interval + cow manure application. Chemical fumigation with Basamid 800Gr (80% MIT) and chemical seed dressing with Gaucho 390 FS MT which contain pencycuron 50g/l, thiram 107g/L and imidacloprid 233g/L) and Captan 1200EC which contain captafol-carbendazim were used as standard checks and the uninfested as the control.

For direct seeding, each experimental unit was made of 2 rows per Variety, one for the infested and the other uninfested. Each row had 5 planting hills each planted with 5 seeds/hill making a total of 150 seeds per experimental unit. This was replicated 4 times to make 600 seeds per

treatment block. The seeds were covered with 2-4cm of soil after planting. For transplanted, one seedling per hill and the uninfested as the control made a total of 30 seedlings per experimental unit which was replicated 4 times to make 120 seedlings per treatment block. The spacing of tomato plants was 45cm within the rows and 60 cm between the rows as recommended by the dealer (Regina seed company, 2004). For transplanted, tomato seedlings were raised on Rhizoctonia free nursery till 21 days old when they were taken to *Rhizoctonia* inoculated field. The nursery was finely prepared then fertilized with 1kg diamonium phosphate + 1kg calcium nitrate per m^2 before seeding. Nursery was watered in the morning and evening at 2000 cm^3/m^2 upto 7 days after planting, 4000 cm^3/m^2 twice daily upto 15 DAP and then 4.0L alternate days upto 21 DAP when transplanting was done. This was compared with direct planting used on all the other treatments. Transplanted crop was watered normally till harvesting.

Ten centimeter high bed was raised using a fork Jembe. A flat bed was the normal planting field. Steam sterilized cow manure of C: N ratio 40:1 from a cattle shed was applied by hands. The three watering intervals evaluated were 0.0L/hill, 0.15L/hill and 0.3L/hill. Water volume was measured by use of a graduated measuring glass cylinder. The 4 levels of watering regime evaluated were normal watering, watering daily, watering alternate days and watering after 2 days. Watering was done manually by use of a watering can. Water was applied at the rate of 1.0L/hill during germination and seedling stage, 1.5L/hill during vegetative to flowering phase and 2.0L/hill at the fruiting stage. A particular watering interval was maintained throughout the seaason. Normal watering meant watering the field to about 70% capacity. This watering translated to about watering twice daily during the first week at 1.0 L/hill, once daily at

1.5L/hill from establishment to fruiting and 2.0L/hill till harvesting at ambient temperatures of 22-26^{0}C. Field capacity was monitored daily by use of a tensiometer.

For fumigation with Basamid a flat bed was prepared using a fork jembe and rake and inoculated with *R. solani* containg 10^6 mycelia fragments per gram at 1000cm^3 of soil potato inoculum/m^2 dispersed by hand on the surface. Basamid granular was dispersed on top of inoculated bed at a rate of 5g/m^2 then mixed with the soil by use of a fork Jembe. A soil thermometer was strategically inserted. The bed was then sprinkler irrigated upto 70% field capacity or 30cm deep and covered with a transparent plastic polythene paper of thickness 30µm. At the end of every week the plastic cover was removed, the soil mixed by a Jembe and the cover replaced. This continued for 4 weeks after which the plastic mulch was removed. At the end of fumigation germination test was done using cabbage seeds to test for chemical residues. With no chemical residues tomato seeds were planted. Soil solarisation procedure was adopted from Katan *et al* (1976).

Tomato seeds were dressed with Gaucho MT FS 390 and Captan at the rate of 8ml/1kg and 1.2ml/kg of seeds respectively. Seed dressing for the trials was done manually using a plastic bucket as the mixing chamber for the chemical and seeds. Delinted seeds were weighed into desirable quantities and transferred into 1 Litre buckets and using a syringe, liquid Gaucho 390 FS MT was accurately measured and dispensed into the plastic bucket containing the delinted seeds at the rate of 6 ml/kg of seed. Captan, a wettable powder was measured using a precision metre PM 40 weighing balance with an accuracy of 0.00. Then a slurry was prepared and dispersed into the seeds in a 1 litre plastic bucket at the rate of 1.2ml/kg of seed. Mixing was

done by shaking the plastic bucket through a 360^0 rotation for at least 1 minute. Dressed seeds were transferred into plastic bags for packing and planting.

5.3.5. Disease and yield assessments

Infection assessment was as in the pathogenicity test in section 3.3.4.2 except that in the field experiment, damping off incidence was assessed after every 5 days and severity was based on 12 randomly sampled plants per replicate at 30 DAP. Stand count was determined by counting the number of established plants per treatment at 60 DAP and the mean number of established plants per experimental block and treatment block finally expressed as a percent of the total planted. Yield assessment was done at maturity of the plants by harvesting, grading and weighing all the marketable fruits per replicate and then calculating the quality and quantity of yield per experimental block and finally per treatment block which was then converted to yield in tons per hectare. The fruit quality was assessed on a grading system based on size and weight of fruit as, GI= ≥ 3 - 4cm diameter and ≥80 g/fruit, GII= 2 – 2.9cm diameter and 59 - 79 g/fruit, GIII=≤ 2cm diameter and ≤60g/fruit. The grading procedure was adopted from Kenya Agricultural Research Institute (2002). For each treatment, each fruit grade was expressed as percent of the total. Each tomato variety was evaluated independently and the mean yield calculated.

During planting, diamonium phosphate (18.46.0) and urea (46% N) were applied at the rate of 2.5 and 5 g /hill respectively. Urea was repeat applied at 10g/hill at 30 and 50 DAP. Foliar diseases such as blight were controlled by a regular spray with 70% propineb, trade name Antracol 70WP, a contact fungicide. Insect pests such as aphids and thrips were controlled by use of imidacloprid, trade name Confidor 200 SL which is a systemic insecticide and fruit

borers by regular spray with methomyl, trade name Methomex 90 SP which is a systemic insecticide.

5.3. 6. Statistical data analysis

At the end of the two experiments all the data collected was analysed by One Way Anova using Genstat 6th edition software and means compared using fisher's LSD procedure at P≤0.05. Alternatively means were compared graphically using Microsoft Excel software (Microsoft corp). In case of zero values on the data of percent seedling survival, the data was transformed (Ahmad and Baker, 1987) to respective arc sine values before analysis was done.

5.4: Results

5.4.1. Susceptibility of tomato varieties to Rhizoctonia damping off

There were significant differences between varieties in mean percent survival of seedlings at the end of the 1st, 2nd, 3rd, 4th, and 5th weeks after planting (Table 18). At the end of 1st week, Onyx VF2 and Cal-J VF registered high percent seedling survival whereas Marglobe, Money maker and Monset registered the lowest survival rate and were not significantly different from each other. Caltana F1 VF, Onyx VF2 and Cal-J VF consistently had significantly (P≤ 0.05) higher percent seedling survival at 2nd-5th week than other varieties and were not different from each other. Monset F1 TM and marglobe improved had the intermediate percent survival at all the assessment dates. Varieties Money Maker and Roma VF had the lowest percent survival at 1st- 3rd week. At the 4th week, Caltana F1 had the highest survival rate whilst Money-Maker had the lowest rate (Table 18).

At the end of 5th week varieties Onyx VF2, Caltana F1 VF RN and Cal-J VF had high cumulative survival of above 60.0% and not significantly (P≤ 0.05) different from each other, Monset F1 TM and Marglobe improved had moderate survival of above 40.0% whilst Rom VF and Money Maker had low survival of less than 40.0% (Table 18).

Table 18: Percent seedling survival of *R. solani* infected tomato from various varieties

Variety	Weeks after planting				
	1	2	3	4	5
Onyx VF2	47.3	85.4	76.9	74.6	72.3
Caltana F1 VF RN	45.6	87.9	75.6	72.1	70.1
Cal-J VF	47.3	84.0	72.1	71.7	69.4
Money maker	22.5	54.2	53.0	40.0	32.7
Marglobe improved	30.5	66.8	58.1	50.8	42.5
Roma VF	26.4	64.3	56.5	47.4	38.0
Monset F1 TM	37.8	66.1	65.7	52.2	45.0
Mean	36.8	72.7	65.4	58.4	52.9
LSD (0.05)	10.4	13.0	9.7	14.0	17.1
CV (%)	8.3	17.9	14.9	24.0	32.3

At the end of 5th week, the varieties Onyx VF2, Caltana F1 VF, Cal-J VF, Monset F1 TM and Roma VF had low damping off severity values of 2.2, 2.3, 2.4, 2.6 and 2.8 respectively and were not significantly (P≤0.05) different from each other. Varieties Marglobe improved and Money marker had high damping off severity value of 3.3 and 3.4 and they were not significantly (P≤0.05) different from each other (Figure 3).

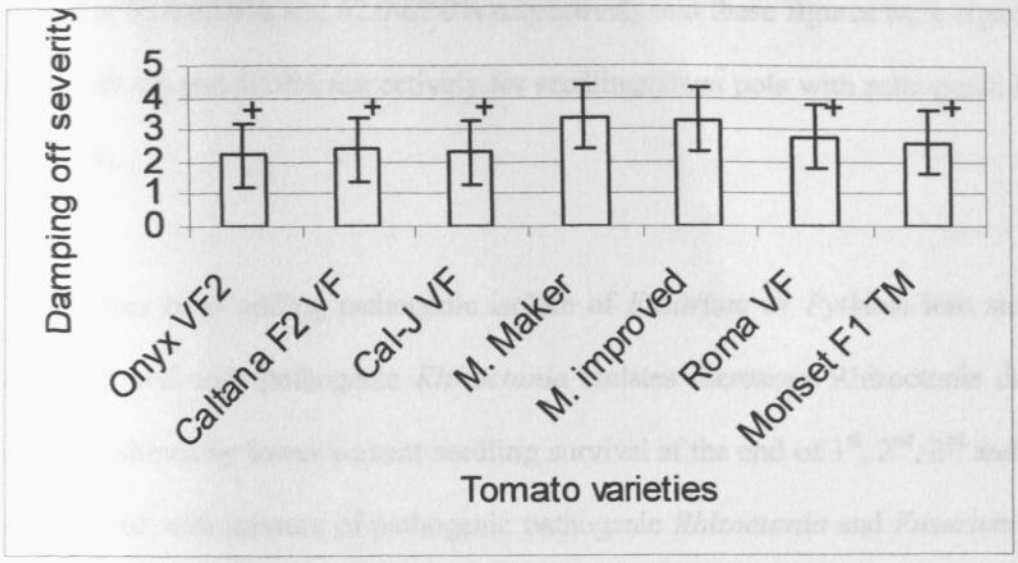

Figure 3: Rhizoctonia damping off severity from various tomato varieties

Vertical bars topped with asterisks are not significantly ($P \leq 0.05$) different from each other disease severity. M. Maker=Money Maker; M. improved=Marglobe improved.

5.4.2. Synergism between *Rhizoctonia solani* and the other soilborne pathogens on Rhizoctonia damping off of tomato

Adding nonpathogenic isolates of multinucleate and binucleate *Rhizoctonia* into sterilised soil mix infested with pathogenic multinucleate *Rhizoctonia* isolates reduced the damping off on tomato as shown by higher percent seedling survival at the end of 1st, 2nd, 3rd and 4th weeks from pots with the isolates mix compared to those with pathogenic *Rhizoctonia* alone (Table 19). At the end of 1st week the percentage survival of tomato seedlings from treatments with pathogenic multinucleate *R. solani* , nonpathogenic isolates of multinucleate and binucleate *Rhizoctonia* were 38.5%, 44.0% and 44.2 % and these were not significantly ($P \leq 0.05$) different from each other but were significantly different from the uninfested control with 45.0% survival. However at the end of 2nd and 3rd weeks the treatments with nonpathogenic isolates of multinucleate and binucleate *Rhizoctonia* had significantly ($P \leq 0.05$) higher percent seedling

survival of 82.0-85.0% and 62.0-67.0% respectively and these figures were significantly higher than the 69.4% and 52.0% respectively for seedlings from pots with pathogenic *R. solani* alone (Table 19).

On the other hand adding pathogenic isolate of *Fusarium* or *Pythium* into sterilized potting media infested with pathogenic *Rhizoctonia* isolates increased Rhizoctonia damping off on tomato as shown by lower percent seedling survival at the end of 1st, 2nd, 3rd and 4th week from pots infested with mixture of pathogenic pathogenic *Rhizoctonia* and *Fusarium* or *Pythium* as compared to those with pathogenic *Rhizoctonia* alone. At the end of the 1st week, all the treatments recorded percent survival of 28.0-45.0% and were not significantly different from the untreated control except where *Pythium* was present. At the end of 2nd and 3rd weeks the treatments with pathogenic isolates of *Fusarium* and *Pythium* had significantly ($P \leq 0.05$) lower percent seedling survival of 56.7 -55.0 % and 47.0 - 43.0.0% respectively and these figures were significantly lower than the 69.4% and 52.0% respectively for seedlings from pots with pathogenic *R. solani* alone (Table 19)

By the end of the 4th week, the uninfested treatment had the highest mean percent survival of 95.5% and this was significantly ($P \leq 0.05$) different from other treatments. This was followed by the interaction between pathogenic *R. solani* + binucleate *Rhizoctonia* with mean of 62.0%, pathogenic *R. solani* + nonpathogenic *R. solani* with 60.0%, pathogenic *R. solani* alone with 49.4%, pathogenic *R. solani* + pathogenic *Fusarium* with 44.5% and pathogenic *R. solani* + pathogenic *Pythium* with 41.0% (Table 19). On individual treatments, the mean percent survival of tomato seedlings from treatments with *R. solani* alone was 52.3%. Adding nonpathogenic *R. solani* or binucleate *R. solani* to pots infested with pathogenic *R. solani*

increased the mean percent survival to higher than 60.0% but this was not significantlty different from each other. This indicated biological control of Rhizoctonia damping off of tomato by nonpathogenic *R. solani* or binucleate *R. solani*. On the other hand the presence of *Fusarium* or *Pythium* isolates in pots infested with pathogenic *R. solani* decreased the percent survival of seedlings to below 50.0% respectively indicating positive synergism in causing the damping off disease. The treatments with *Fusarium* and *Pythium* were not significantly ($P \leq 0.05$) different from each other (Table 19). Adding the *Pseudomonas* bacteria isolate to sterile soil mix infested with pathogenic *Rhizoctonia solani* and a mixer of all isolates did not cause a significant ($P \leq 0.05$) difference on tomato seedling survival at the end of 1st- 4th weeks as compared to treatments with pathogenic *R. solani* alone.

Table 19: Mean percent seedling survival of *R. solani* infected tomato from various soilbone pathogen interactions

Treatment	Weeks after planting			
	1	2	3	4
PRS	38.5	69.4	52.0	49.4
PRS+NRS	44.0	82.0	61.0	60.0
PR+BRS	44.2	85.0	67.0	62.0
PRS+PF	37.4	56.7	47.0	44.5
PRS+P	28.0	55.0	43.0	41.0
PRS + Pba	40.0	65.7	54.0	52.0
PRS + MRS	39.0	73.1	51.6	49.5
Uninfested	45.0	98.0	96.0	95.5
Mean	39.5	74.4	61.0	58.7
LSD ($P \leq 0.05$)	6.5	17.0	19.3	19.8
CV (%)	16.4	22.8	31.6	33.8

PRS=pathogenic *R. solai* alone; PRS+NRS=pathogenic *R. solani*+Nonpathogenic multinucleate *R. solani*; PRS + BRS= pathogenic *R. solani* + Binucleate *R. solanii*; PRS+ PF= pathogenic *R. solani* + Pathogenic *Fusarium* isolates; PRS+ P= pathogenic *R. solani* + Pathogenic *Pythium* isolate; PRS+Pba= pathogenic *R. solani* + nonpathogenic *Pseudomonas* isolates; PRS+MRS= pathogenic *R. solani* + Mixer of all isolates; Untreated=uninfested control.

At the end of 4th week, the damping off severity score was 3.2 for plants with pathogenic *Rhizoctonia* isolates alone and not treated with the nonpathogenic multinucleate *Rhizoctonia,* binucleate *Rhizoctonia, Fusarium, Pythium* or P*seudomonas* isolates (Figure 4). Adding nonpathogenic multinucleate *R. solani* or binucleate *Rhizoctonia* depressed the damping off severity score to 3.0 and 2.9 respectively thus indicating biocontrol in causing the damping off of tomato. The treatments with nonpathogenic multinucleate *R. solani* or binucleate *Rhizoctonia* were not significantly different from each other. On the other hand presence of *Pythium* or *Fusarium* in *R. solani* infested pots increased the damping off severity score to 3.4 and 3.5 respectively, this being indicative of positive synergism. The treatments with *Fusarium* and *Pythium* were not significantly different from each other (Figure 4). *Pseudomonas* or a mix of all isolates when combined with pathogenic *R. solani* produced high damping off severity rating that was no significantly ($P \leq 0.05$) different from the pathogenic *R. solani* alone.

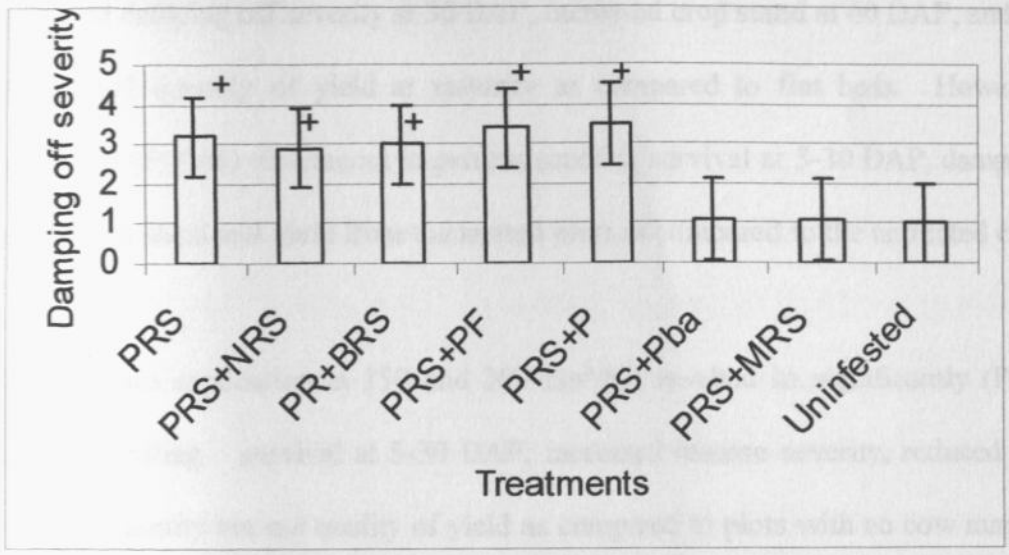

Figure 4: Rhizoctonia damping off of tomato severity from various soilborne pathogen interactions

Vertical bars topped with asterisks are significantly (P≤0.05) different from the uninfested control disease severity. RS=pathogenic *R. solai* alone; PRS+NRS=pathogenic *R. solani*+Nonpathogenic multinucleate *R. solani*; PRS + BRS= pathogenic *R. solani* + Binucleate *R. solanii*; PRS+ PF= pathogenic *R. solani* + Pathogenic *Fusarium* isolates; PRS+ P= pathogenic *R. solani* + Pathogenic *Pythium* isolate; PRS+Pba= pathogenic *R. solani* + nonpathogenic *Pseudomonas* isolates; PRS+MRS= pathogenic *R. solani* + Mixer of all isolates; Untreated=uninfested control.

5.4.3. Efficacy of cultural methods in management of Rhizoctonia damping off of tomato

The various control practices at different levels tested had a significant effect on percent seedling survival at 5-30 DAP, damping off severity at 30 DAP, crop stand at 60 DAP and yield quality and quantity at maturity in a field inoculated with pathogenic strains of *R. solani*. There were low levels of the disease in uninfested control (Table 20). Planting tomato on raised beds increased, though not significantly (P≤0.05) percent seedling survival at 5-30 DAP,

decreased damping off severity at 30 DAP, increased crop stand at 60 DAP, and increased both quality and quantity of yield at maturity as compared to flat beds. However there were significant ($P \leq 0.05$) differences in percent seedling survival at 5-30 DAP, damping off severity rating, crop stand and yield from the treated plots as compared to the untreated control.

Cow manure application at 150 and 300 cm^3/hill resulted in significantly ($P \leq 0.05$) reduced percent seedling survival at 5-30 DAP, increased disease severity, reduced crop stand and lowered quantity but not quality of yield as compared to plots with no cow manure. Increasing manure application from 150 to 300 cm^3/hill resulted to decrease, though not always significantly ($P \leq 0.05$) different in percent survival of seedling, increase in damping off severity, decrease in crop stand and decrease in yield. All the treatments were significantly different from the untreated control on seedling survival, disease rating, crop stand and yield (Table 20).

Watering the plots normally or daily increased infection by *Rhizoctonia* as indicated by lower percent seedling survival, higher damping off, lower crop stand and lower quality and quantity of fruit yield. Further increase in irrigation interval to altrnate and finally after 2 days resulted in lower damping off severity (infection) but also in poor germination, smaller bush and blossom end rot of fruits; all which greatly depressed quality and quantity of yield (Table 20). Transplanting 21 days old seedlings from a *Rhizoctonia* free nursery to *Rhizoctonia* infested field resulted in significant ($P \leq 0.05$) increase on percent tomato seedling survival at 25 and 30 DAP, decrease in damping off severity at 30 DAP, increased in percent crop stand at 60 DAP and higher quality and quantity of yield as compared to direct seeding in a *Rhizoctonia* infested

field. All the treatments were significantly different from the untreated control according to F=LSD at P≤0.05 (Table 20).

Combining raised beds + cow manure application at 150 cm^3 and 300 cm^3 resulted in significantly (P≤0.05) reduced percent seedling survival at 5-30 DAP, increased damping off severity rating, decreased crop stand and lowered yield as compared to the uninfested control. Increasing manure application on raised beds from 150 cm^3 to 300 cm^3 resulted in reduced but not not significantly (P≤0.05) percent seedling survival, increased damping off severity, lowered crop stand and yield. All the treatments produced significantly lower percent seedling survival, higher damping off severity, lower crop stand and lower yield than the untreated control (Table 20).

Combining cow manure application + watering at normal, daily, alternate and finally 2 days interval resulted in corresponding decrease in percent seedling survival at 5-30 DAP, increase in disease severity, decrease in crop stand and decrease in both quality and quantity of yield than when any of them was applied singly. Combining cow manure application at 150 cm^3 with watering at daily interval, alternate and finally 2 days interval produced lower seedling survival, lower disease score, higher crop stand and higher yield than combining cow manure application at 300 cm^3 and their corresponding levels of watering interval. Thus though varying cow manure application from 150 to 300 cm3 /hill produced lower percent seedling survival, lower disease severity, lower crop stand and lower yield, the effects were not significant till manure application was combined with watering regime. All the treatments produced significantly lower percent seedling survival, higher disease score, lower crop stand and lower yield than the untreated control (Table 20).

Combining raised bed with watering daily, alternate and finally 2 days interval resulted to correspondingly decrease in percent seedling survival, increase in damping off severity, decrease in crop stand and decrease in both quality and quantity of yield as compared to the uninfested control. At 5 and 10 DAP, raised bed + watering daily produced significantly higher percent seedling survival than raised bed + watering at alternate days. However percent seedling survival from raised bed + watering daily was not significantly ($P \leq 0.05$) different from raised bed + watering alternate days at 15-30DAP. Raised bed +watering at 2 days produced significantly lower percent seedling survival, higher damping off severity, lower crop stand and lower yield than raised bed + water daily or raised bed + watering at alternate days . Raised bed + watering daily produced higher but not significantly different seedling survival than raised bed + watering at alternate days. However, raised bed + watering daily produced significantly higher crop stand and yield than raised bed + watering at alternate days. All the treatments produced significantly ($P \leq 0.05$) lower percent seedling survival, higher damping off severity, lower crop stand and lower yield than the untreated control (Table 20).

Combining planting on raised beds + cow manure application + varying watering interval resulted in significantly ($P \leq 0.05$) lower percent seedling survival at 5-30 DAP, higher damping off severity, lower crop stand and lower quality and quantity of yield as compared to the uinifested control. Planting on raised beds with cow manure at $150cm^3$ and $300cm^3$ and watering daily, alternate days and finally at 2 days produced lower seedling survival, lower damping off severity, lower crop stand and lower yield than combining raised bed with cow manure at 300 cm3 and their corresponding level of watering regime. Increasing cow manure application from 150 to 300 cm3 on raised bed alone could not produce significant ($P \leq 0.05$) change in percent seedling survival, disease rating crop stand or yield. However, when daily

watering, alternate or 2 days interval were applied to raised bed + cow manure at 150 or raised bed+ cow manure at 300 cm3 there was a significant change in percent seedling survival, damping off severity rating or crop stand and yield. All the treatments produced significantly lower percent seedling survival, higher damping off severity score, lower crop stand and lower quantity of yield than the untreated control (Table 20).

Fumigation with Basamid granular produced the highest germination for the 3 varieties and protected the plant 100% against *Rhizoctonia solani* infection in the field. Fumigated plots produced significantly ($P \leq 0.05$) higher percent seedling survival at 5-30 DAP, lower damping off severity (100% free of disease disease), higher crop stand and higher quality and quantity of yield as compared to the infested nonfumigated plots and the uninfested control (Table 20). Planting tomato seeds dressed with Gaucho MT 390 FS on *Rhizoctonia* infested field resulted in significantly ($P \leq 0.05$) higher percent seedling survival at 5-30 DAP, lower damping off severity, higher crop stand and higher quantility and quantity of yield as compared to untreated seeds. Chemical seed dressing with Captan 1200EC resulted to insignificant ($P \leq 0.05$) changes on percent seedling survival at 5- 30 DAP, damping off severity, crop stand and yield as compared to untreated seeds (Table 20).

Table 20: Percent seedling survival, damping off severity, percent crop stand, quality and quantity of yield of *R. solani* infected tomato from various levels of cultural control methods

TREATMENT	5 DAP	10 DAP	15 DAP	20 DAP	25 DAP	30 DAP	Disease severity	Crop stand 60 DAP	Fruit quality GI	GII	GIII	Yield (Ton/ha)
Raised beds	45.1	80.9	82.1	78.6	75.0	68.5	2.7	66.3	26.5	48.6	28.8	57.5
Flat beds	40.7	72.3	73.1	68.5	66.2	64.3	3.0	61.5	27.0	44.2	28.4	49.3
Cow manure 150cm^3	35.2	67.1	66.8	62.3	59.0	57.0	3.3	53.6	39.8	41.0	19.1	44.6
Cow manure 300cm^3	37.0	64.0	61.9	59.0	55.0	53.7	3.5	50.2	44.0	45.9	10.0	44.0
Watering normally	40.0	81.6	83.9	70.2	65.4	62.0	3.3	60.0	33.3	46.8	19.8	52.3
Watering daily	34.3	52.9	66.0	64.7	63.0	58.4	2.5	54.7	21.2	46.7	32.0	50.5
Watering alternate days	18.9	30.4	48.0	58.3	56.0	50.0	2.1	47.8	10.2	32.8	57.0	43.0
Watering every 2 days	0.03	7.0	33.7	30.0	29.9	27.6	1.4	28.2	2.7	15.0	82.1	21.9
Transplanting	0.0	0.0	0.0	0.0	85.0	78.0	1.8	78.7	26.6	30.3	43.0	60.0
Raised beds+ cow manure 150cm^3	43.0	73.8	71.0	66.0	60.3	56.6	3.1	53.4	40.0	42.5	17.4	42.9
Raised beds+ cow manure at 300cm^3	40.1	61.2	68.0	63.4	54.0	51.0	3.4	48.1	45.2	38.5	16.5	41.6
Cow manure 150cm^3+ watering daily	27.0	55.6	69.5	60.7	64.0	63.0	3.1	47.7	20.1	48.2	31.6	44.0
Cow manure150cm^3+ watering alternate days	8.5	31.4	55.1	54.0	48.6	45.4	2.6	40.0	18.3	46.4	35.2	37.2
Cow manure 150cm^3+ watering 2 days	3.3	20.0	29.9	25.2	23.8	21.6	1.7	17.4	1.5	28.6	69.8	19.4
Cow manure 300cm^3+ watering daily	24.2	40.3	57.0	45.8	50.5	48.1	3.0	43.2	24.3	35.7	40.4	42.0
Cow manure 300cm^3+ watering alternate days	13.7	33.4	47.0	54.5	49.6	42.0	2.3	38.2	15.0	46.2	38.6	30.3
Cow manure at 300cm^3+ watering every 2 days	0.03	15.1	20.6	23.4	19.0	16.3	1.9	14.4	2.2	22.7	75.0	16.0
Raised beds+ watering daily	41. 2	76.1	70.9	62.0	59.0	57.8	2.5	52.4	10.7	47.0	50.1	40.3

Table 20. Continued

Raised beds+ watering alternate days	23.8	50.0	64.2	54.8	50.6	48.3	2.1	46.5	7.5	34.5	58.0	46.3
Raised beds+ watering every 2 days	0.03	19.0	27.0	26.2	25.3	24.0	1.3	23.0	0.03	9.9	90.0	17.7
Raised beds+ cow manure 150cm^3+ watering daily	25.9	66.4	64.0	61.6	58.7	55.0	2.9	44.4	25.7	41.8	32.3	40.0
Raised beds+ cow manure 150cm^3+ watering alternate days	13.3	36.1	60.9	53.1	50.4	45.6	1.9	38.6	10.0	37.1	52.7	33.0
Raised beds+ cow manure 150cm^3+watering 2 days	3.6	12.0	26.3	28.0	23.0	21.3	1.6	19.5	6.6	17.7	76.2	20.2
Raised beds+ cow manure 300cm^3+watering daily	18.0	51.4	68.7	60.2	56.0	50.0	2.8	39.7	34.2	36.4	29.3	38.4
Raised beds+ cow manure 300cm^3+watering alternate days	10.5	31.6	50.0	48.0	42.2	37.9	2.2	29.9	11.9	32.4	55.7	31.2
Raised beds + cow manure 300cm^3 + watering 2 days	0.03	11.0	26.0	21.5	19.4	17.1	1.7	15.0	2.7	22.1	5.0	18.0
Basamid	53.0	81.4	95.0	95.0	95.0	93.0	1.0	90.5	65.2	30.4	13.3	69.9
Gaucho 380 FS TM	52.4	70.0	83.2	83.2	81.5	81.5	1.6	79.0	41.3	33.1	25.6	62.0
Captan 1200EC	25.6	67.3	71.7	66.6	61.0	60.0	2.7	53.0	27.7	47.4	24.8	50.6
Uninfested	48.0	74.0	88.1	86.0	86.0	84.0	1.2	82.0	27.0	38.1	34.9	66.0
Mean	23.4	47.8	57.7	54.4	54.4	51.3	2.3	47.1	22.3	36.3	42.1	50.0
LSD (P≤0.05)	14.7	15.0	26.2	15.4	14.0	13.8	0.4	12.3	14.0	10.0	12.2	9.4
CV (%)	28.0	35.3	31.5	27.2	36.0	38.0	47.0	34.6	70.0	27.4	65.1	32.0

GI-GIII=Tomato fruit grades 1-3 respectively; Ton/ha= Tomato fruit yield in tons per hectare.

The combined efficacy of the various levels of cultural control metods either singly or in combination when compared with the coneventional disease control using fumigation with Basamid and chemical seed dressing varied on percent survival of *R. solani* infected tomato seedlings at 5 -30 DAP, damping off severity at 30 DAP, percent crop stand at 60 DAP and quality and quantity of fruit yield at maturity (Tables 21, 22, 23 and figure 5, 6 rsspectively).

Chemical fumigation with Basamid consistently produced higher seedling survival at 5-30DAP than all the cultural methods and the chemical seed dressing (Table 21). Temperature rose to a maximum of 50^0C under the plastic mulch within 3 weeks during chemical fumigation with Basamid before planting. Transplanting produced high percent seedling survival of more than 70.0% at 25 and 30 DAP. At 25 DAP, percent seedling survival from transplanted plots were not significantly different from those plots treated with Basamid. However at 30 DAP, transplanting produced significantly ($P \leq 0.05$) lower percent seedling survival than fumigation with Basamid but higher than other treatments.

At 5-30 DAP raised bed, cow manure application, and raised bed + cow manure application, and chemical seed dressing produced high percent seedling survival and were not significantly ($P \leq 0.05$) different from each other. These treatments were not also significantly different from the plots with Basamid at 5 and 10 DAP but from 15-30 DAP they produced significantly lower seedling survival than the basamid treated. Raised beds + cow manure application + varying watering regime, cow manure application + varying watering regime and varying watering regime consistently produced low percent seedling survival and not significantly different from each other at 5-25 DAP (Table 21).

By 30 DAP, chemical seed dressing, raised bed, cow manure application and raised bed + manure application had high percent seedling survival of more than 60.0% but these were not significantly ($P \leq 0.05$) different from each other. Varying watering interval and varying watering interval + cow manure application had a moderate percent seedling survival of less than 60.0% whilst raised beds + varying watering interval and raised beds + cow manure application + varying watering interval had low cumulative percent seedling survival of less than 50.0% and were significantly different from each other at $P \leq 0.05$ (Table 21).

Table 21: Percent seedling survival of *R. solani* infected tomato from various cultural control methods

Treatment	Days after planting					
	5	10	15	20	25	30
Planting on raised beds	46.2	79.2	79.1	76.4	73.0	70.2
Cow manure application	41.8	72.1	73.4	68.8	67.3	64.4
Varying watering interval	30.3	55.0	63.2	61.2	61.0	56.4
Transplanting	0.0	0.0	0.0	0.0	85.0	78.0
Raised beds + cow manure	44.7	71.7	76.7	73.8	68.1	65.1
Raised beds + varying watering interval	30.0	44.8	51.7	49.7	47.1	44.0
Varying watering interval + cow manure	31.0	55.5	62.0	56.6	57.2	54.0
Raised beds + cow manure + varying watering interval	29.4	49.6	56.9	51.7	48.2	45.2
Basamid	53.0	81.4	95.0	95.0	95.0	93.0
Mean	39.5	62.4	71.4	68.3	67.9	64.9
LSD ($P \leq 0.05$)	9.4	13.4	14.0	15.0	15.4	14.6
CV (%)	23.7	20.8	19.6	21.9	22.6	24.1

Manure application, and raised beds + cow manure had high damping off severity rating of 3.4 and 3.3 respectively and not significantly ($P \leq 0.05$) different from each other (Figure 5). Planting on raised beds, cow manure + varying watering interval, and raised beds + varying watering interval + cow manure application produced moderate damping off severity of 2.2-2.7 and were not significantly ($P \leq 0.05$) different from each other. Transplanting, chemical seed

dressing, varying watering interval and raised beds + varying watering interval produced low damping off severity score of 1.8-2.2 and were not significantly ($P \leq 0.05$) different from each other. Chemical fumigation with Basamid had the lowest disease rating of 1.0 and was significantly different from the rest of the control methods tested (Figure 5).

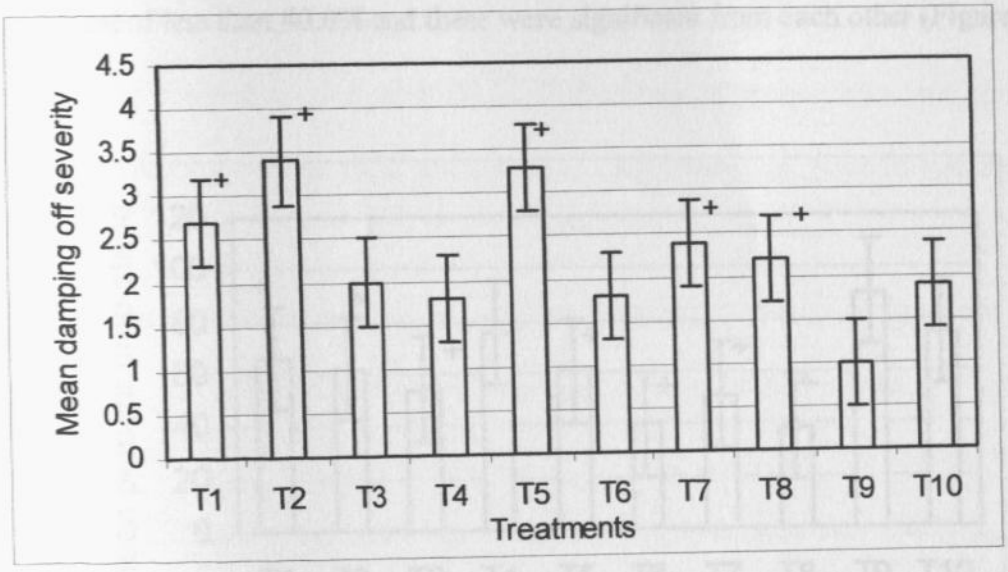

Figure 5: Rhizoctonia damping off of tomato severity from various cultural control methods Vertical bars topped with asterisks are significantly ($P \leq 0.05$) different from the treatments with the Basamid and chemical seed dressing as standard checks disease severity. T1= Raised beds, T2= Cow manure application, T3= Varying watering interval, T4= Transplanting, T5= Taised beds + cow manure, T6= Raised beds + varying watering interval, T7= Varying watering interval + cow manure, T8= Raised beds + cow manure + varying watering interval, T9= Basamid, T10= Chemical seed dressing.

Chemical fumigation with Basamid had the highest crop stand of 93.0% at 60 DAP and was significantly different from all the other treatments (Figure 6). Chemical seed dressing, transplanting and raised beds, cow manure application and raised beds+cow manure application

recorded had percent crop stand of more than 60.0% and these were not significantly (P≤0.05) different from each other. Effect of varying watering interval and varying watering interval + cow manure application had average crop stand of more than 50.0% and not significantly different from each other whilst raised beds + cow manure application+ varying watering interval and raised beds + cow manure application + varying watering interval had low percent crop stand of less than 40.0% and these were significant from each other (Figure 6).

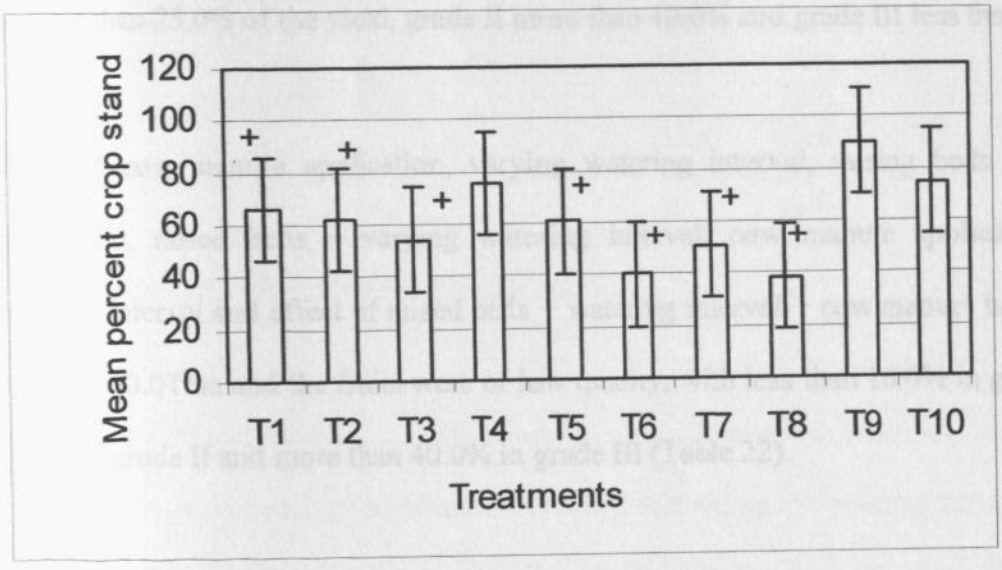

Figure 6: Percent crop stand of *R. solani* infected tomato from various cultural control Methods

Vertical bars topped with asterisks are significantly (P≤0.05) different from the treatments with the Basamid and chemical seed dressing as standard checks percent crop stand. T1= Raised beds, T2= Cow manure application, T3= Varying watering interval, T4= Transplanting, T5= Taised beds + cow manure, T6= Raised beds + varying watering interval, T7= Varying watering interval + cow manure, T8= Raised beds + cow manure + varying watering interval, T9= Basamid, T10= Chemical seed dressing.

The various control strategies had significant effect on the quality and quantity of the marketable tomato fruits produced. Chemical fumigation with Basamid not only produced significantly highest yield per hectare of 78.2 tons but also the quality was higher than the rest of the control strategies. The quality of tomato fruits produced from plots treated with Basamid were high and comprised 65.2% in grade I, 30.4% in grade II and 13.3% in grade III (Table 22). Effect of Transplanting, planting on raised bed and chemical seed treatment had high tomato fruit yield of more than 50.0T/ha with averagely good quality with grade I comprising of more than 25.0% of the yield, grade II more than 40.0% and grade III less than 30.0%.

Effect of cow manure application, varying watering interval, raising beds + cow manure application, raised beds + varying watering interval, cow manure application + varying watering interval and effect of raised beds + watering interval + cow manure had low yields of less than 50.0T/ha and the fruits were of low quality, with less than 16.0% in grade I, less than 40.0% in grade II and more than 40.0% in grade III (Table 22).

Table 22: Quality and quantity of fruit yield of *R. solani* infected tomato from various cultural control methods

Treatment	Percent fruit grade			Fruit yield
	G1	GII	GIII	(Ton/ha)
Planting on raised beds	26.5	48.6	24.8	57.5
Cow manure application	14.6	41.9	43.5	44.3
Varying watering interval	11.4	31.5	57.0	38.5
Transplanting	26.6	30.3	43.0	60.0
Raised beds + cow manure	16.9	40.3	16.5	42.3
Raised beds + varying watering interval	6.1	28.5	65.4	39.0
Watering interval + cowmanure	14.6	38.0	48.4	31.5
Raised beds + cow manure + Varying watering interval	15.2	31.3	53.5	30.1
Basamid	65.2	30.4	13.3	78.2
Chemical seed dressing	41.3	33.1	25.6	62.0
Mean	23.4	35.4	39.1	50.7
LSD (P≤0.05)	18.2	6.7	19.4	10.6
CV%	57.5	18.9	53.7	20.9

Ton/ha=Yield of tomato fruit in tons per hectare

The interaction of various control practices and *Rhizoctonia* pathogen had a significant effect on percent seedling survival at 30 DAP, dampinmg off severity rating, percent crop stand at flowering and yield at maturity (Table 23). In all treatments, there was direct relation between percent crop stand and yield (Ton/ha) but not always in other parameters. Based on the overall damping off disease parameters, fumigation with Basamid produced significantly (P≤0.05) the higher disease control with percent survival and crop stand of 93.0% and 90.5% respectively, lowest disease score of 1.0 and highest yield of 78.2 tons/ha.

Transplanting, chemical seed dressing, raised beds, cow manure application, raised beds + manure produced moderate mean percent seedling survival and crop stand of more than 60.0%, disease severity of 1.8-3.3, and yield of more than 50.0 tons/ha. Varying watering interval, varying watering interval + cow manure, raised beds + varying watering interval, raised beds + cow manure + varying watering interval produced low mean percent survival and crop stand of less than 60.0%, damping off severity of 2.2-2.4, and yield of less than 40.0 ton/ha. In most cases except where there was cow manure application, treatments with higher percent seedling survival also had lower disease rating, higher crop stand and consequently higher mean yield.

Table 23: Rhizoctonia damping off of tomato disease parameters from various cultural control methods

Treatment	Percent survival 30 DAP	Disease severity	Crop stand at flowering	Yield (Ton/ha)
Planting on raised beds	70.2	2.7	66.3	57.5
Cow manure application	64.4	3.4	62.0	44.3
Varying watering interval	56.4	2.0	54.3	38.5
Transplanting	78.0	1.8	75.4	60.0
Raised beds + cow manure	65.1	3.3	60.7	42.3
Raised beds + varying watering interval	44.0	1.8	39.9	39.0
Varying watering interval+ cow manure	54.0	2.4	51.1	31.5
Raised beds + cow manure + Varying watering interval	45.2	2.2	38.7	30.1
Basamid	93.0	1.0	90.5	78.2
Chemical seed dressing	76.8	1.9	75.0	62.0
Grand mean	64.9	2.3	61.4	50.7
LSD ((0.05)	15.6	0.7	16.3	10.6
CV%	24.1	32.5	26.5	20.9

Ton/ha=Yield of tomato fruit in tons per hectare

5.5: Discussion.

5.5.1. Effect of host resistance on Rhizoctonia damping off of tomato

Lack of resistance to Rhizoctonia damping off of tomato in the commercial tomato varieties implies a real challenge in the management of the disease in Kenya. Lack of resistance in tomato to local strains of *R. solani* has been reported elsewhere. Danesha *et al* (1999) reported that out of the 10 tomato cultivars that were grown commercially in East Tennessee, only 2 were intermediately resistant to the local strains of *R. solani*.

In the current study, the effect of *R. solani* on tomato seedling survival was greatest between 1st and 3rd weeks after planting then reaching to a maximum by the fourth week, with marginal decrease in the 5th week. This implies that the most vulnerable stage of tomato seedlings to *Rhizoctonia solani* infection is the first three weeks after planting. This is in agreement with findings by Danesha *et al* (1999) and Koike *et al* (1996) who reported seedling damping off as the most severe stage of infection by *R. solani* on tomato and occurs mostly between emergence and 30 days after planting.

The differential susceptibility of the seedlings with advance in age could be due to resistance development resulting from lignification of the lower stem making it difficult for the pathogen to penetrate. Direct penetration is one way *Rhizoctonia* invades the host plant. Lignification of lesion borders in *Rhizoctonia* infected seedlings as a mechanism of resistance to the pathogen with age of the host has been reported by other researchers. Stockwell *et al* (1987) reported that hypocotyl tissue of beans, *Phaselous vulgaris* var. Red Kidney was highly susceptible to *R. solani* isolate P.B. during the first three weeks and showed resistance thereafter. The changes in resistance was associated with elongation and maturation of the hypocotyls and

concomitant changes in the pectic substances and calcium content of cell wall (Bateman *et al.*, 1965). Lesion borders formed on the bean hypocotyls infected with *R. solani* were surrounded by autoflorescent cell wall resistant to maceration by cell wall degrading enzymes. These lesion border cell walls stained positively for lignin and phenols. However the lesion borders were macerated by a mixture of cellulase and where macerated after delignification, but not after extraction of calcium or fat and waxes. Peroxidase and polyphenols were found to be active in young lesions in advance of fungal hyphae suggesting that lignification of cell walls may be an important factor in limiting lesion expansion in stem canker of beans after exposure to *R. solani*.

Lignification of host cell wall as a mechanism of resistance to *Rhizoctonia* invasion is further supported by various reports. Cardoso *et al* (1994) and Haran *et al* (1996) used molecular techniques to show that the expression of the cellwall degrading enzymes during mycoparasitism of *Rhizoctonia solani* by *Trichoderma harzianum* was regulated in a very specific and finely tuned manner that was affected by the host. As the antagonistic interaction proceeded, chitinase decreased and endochitinase gradually increased. The differential expression of *T. harzianum* chitinase influenced the overall antagonistic ability of the fungus against the host as it resulted to a decrease in damping off incidences on tomato and beans under the greenhouse conditions when *T. harzianum* was added to sterilized soil mixture infested with *R. solani* planted with tomato or beans. *Trichoderma spp* produced chitinase and glucanase when grown or parasatized on live mycelium of *R. solani* and *S. rolfsii* (Elad *et al.*, 1982, 1983).

5.5.2. Synergism between *Rhizoctonia solani* and other soilborne pathogens on the Rhizoctonia damping off of tomato

The antagonism observed between pathogenic *R. solani* and nonpathogenic multinucleate and binucleate *R. solani* in reducing the incidence and severity of Rhizoctonia damping off of tomato under the greenhouse conditions indicated a potential for the biocontrol of the disease by microbial antagonism. The decrease in damping off incidence and severity could be due to antagonism resulting from various biocontrol mechanisms. On the other hand presence of pathogenic isolates of *Fusaium* and *Pythium* in pathogenic *R. solani* infested potting soil increased the Rhizoctonia damping off of tomato disease incidence and severity indicating the importance of controlling these pathogens where *R. solani* occur.

The positive synergism between *R. solani* strains and pathogenic isolates of *Fusarium* and *Pythium* in causing damping off of tomato could be due to that these pathogens facilitate entry of the host plant by *Rhizoctonia* due to the wounds they create which act as invasion avenues. Agrios (1988) reported that root knot nematodes can predispose the host to *Fusaarium, Rhizoctonia* and *Pseudomonas* pathogens through the wounds created or by causing the break up of the host resistance. Effect of *Pythium* in reducing percent survival of tomato seedlings when added to the pathogenic *Rhizoctonia* infested soil planted with tomato was greater than that of *Fusarium* probably due to the fact that *Pythium* in its own right is a major cause of damping off of tomato (Herdina *et al.*, 1995).

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Since all these pathogens exists in the same environment, this could be the sensitive balance of nature that keeps the population of the pathogenic isolates of *R. solani* at low levels above which they can wipe out the host plants. A combination of all the isolates under

experimentation had no effect on percent seedling survival as compared to the pots with the pathogenic *Rhizoctonia* alone. This could be due to antagonistic effects resulting from such a combination. However under natural conditions it may not be possible to get such a combination of isolates in one production location.

Several research data are available on the influence of other pathogenic and nonpathogenic soilborne microorganisms on the damping off diseases caused by *Rhizoctonia solani* and other pathogens. Sneh *et al* (1996) reported involvement of nonpathogenic isolates of *Rhizoctonia solani* np-R in biological control of *R. solani*. Cardoso *et al* (1987) reported biocontrol of Rhizoctonia root rot of snap beans by 4 binucleate *Rhizoctonia* isolates as shown by significant ($P \leq 0.05$) reduction in disease incidence and severity under greenhouse and field conditions.

Asaka *et al* (1996) reported an isolate of *Bacillus subtilis* RB14 which produced antibiotic Iturin A and surfurin that controlled *Rhizoctonia* in in vitro and suppressed damping off of tomato under greenhouse conditions. Krishnamurthy *et al* (1996) reported control of *Rhizoctonia solani*, the rice sheath blight pathogen by *T. viride* and *T. harzianum.* Hennis *et al* (1968) showed that 8 out of 10 strains of *Bacillus subtilis* and 1 out of 8 strains of *B. licheniformis* inhibited growth and reduced sclerotia formation in *Rhizoctonia*. Durman *et al* (1999) reported that 4 out of 5 isolates of *Trichoderma* controlled *R. solani* in the laboratory and reduced growth and survival of sclerotia in the greenhouse conditions. Reduction of Rhizoctonia root rot severity in beans has been demonstrated with *Trichoderma* species, binucleate *Rhizoctonia*-like fungi, *Gliocladium* species, *Laetisaria arvalis*, as biocontrol agents (Papavizas, 1985). However the findings of this study differ from that of Defago *et al*

(1990) who reported suppression of black rot of tobacco and other root diseases by strains of *Pseudomonas flourescens*.

Also presence of *Rhizoctonia* isolates can influence root rot diseases caused by other pathogens. Nelson *et al* (1981) and Peterson *et al* (1960) reported that presence of *Rhizoctonia* isolates increased incidence and severity of *Fusarium* wilt of tomato at flowering. Symptoms of *Fusarium* wilt on tomato which includes drooping of petals, yellowing and wilting of leaves followed by plant death increased with the addition of *R. solani* (Nelson, 1981). A distinct internal symptom which is brownish discoloration of the vascular tissue caused by exudation of phenols as a reaction to the presence of the pathogen also became more prominent with presence of *R. solani* (Peterson 1960). This could be due to *R. solani* predisposing the plant to infection by *Fusarium* through creating wounds that enhances entry by the latter.

5.5.3. Effect of various cultural methods on Rhizoctonia damping off of tomato

All the cultural practices tested singly or in combination, had direct or indirect impact on Rhizoctonia damping off incidence and severity thus affecting disease level, crop stand and yield. These practices influenced the disease levels by influencing the level of biological activity and P^H as with cow manure, drainage, compaction; structure as with raised beds, cow manure, and level of resistance to the disease as with transplanting; all of which influence development and vigour (Abawi *et al.,* 1990, Burke *et al.,* 1991). Consequently growing tomato in raised beds and long watering interval will reduce diseases that are favoured by high moisture such as those caused by *Rhizoctonia spp* and *Pythium spp* (Abawi *et al.,* 1990, Burke *et al.,*1991). Perhaps planting on raised beds resulted in better drainage

hence reducing infection by *R. solani* and increased yield as compared to planting on flat beds. However, although increasing watering interval to alternate and finally to after 2 days greately reduced soil moisture, it also severely reduced germination and emergence, resulting in poor crop stand and yield.

The results of this study are in agreement with research data from University of California, Agricultural and natural resource (2005) which reported that planting in high and well drained beds, waiting to plant till temperatures are 15 – 20^0C generally reduced soil moisture levels hence controlled diseases caused by *Rhizoctonia*, *Pythium*, and *Phytopthora* in cucurbits. Burke *et al* (1991) reported management of Rhizoctonia diseases in cucurbits by shallow planting and irrigating upto 15cm. Soil moisture not only affect *Rhizoctonia* multiplication and dispersal but also affect seed germination and emergence, plant growth and vigour and thus yield (Abawi *et al.*, 1990). Thus although the lowest damping off disease was recorded from crops watered at 2 days interval, also the lowest yield was from same treatment with manure at 300cm^3. Thus the gains brought by reduced moisture in lowering disease level were consequently cancelled by poor germination and lower vigour, low crop stand, smaller bush, smaller fruits and blossom end rot on fruits due to water stress.

Cow manure application at 150 and 300 cm^3 /hill increased Rhizoctonia infection in tomato and reduced yield as compared to no manure. *Rhizoctonia* is a nonspecialized soilborne fungi that rapidly colonises decomposing soil organic matter hence the natural population of the fungi in the soil in form of sclerotia and mycelia associated with decomposed soil organic matter (Abawi et al., 1990; Carol and Dona, 1986). However most of the fruits were of high grade, comprising mostly of grade I and II from plots with cow manure as opposed to those

without. This could be due to the fact that cow manure acts as a fertilizer in its own right and also improves soil structure and water holding capacity. Most farmers in Kenya use cow manure in place of fertilizer but in *Rhizoctonia* infested fields, the benefit from soil fertilization by manure are clearly overtaken by the fact that manure promote *Rhizoctonia* infection.

When using organic amendments, their level of decomposition should be considered in *Rhizoctonia* infested soils. Fully decomposed manure such as cow manure will promote *Rhizoctonia* infection whereas fresh organic matter will reduce the disease (Maker *et al*., 1965; Papavizas *et al*., 1963; Maning *et al*., 1969) probably due to high amount of carbon relative to nitrogen (C:N ratio=40:1) present in cow manurethat act as a source of biological energy. Hointik *et al* 1966) examined that animal manure is rich in cellulosic material that acts as a source of biological energy thus making the manure support various types of microflora. For green manure, some will promote *Rhizoctonia* infection while others suppress the pathogen (Maker *et al*., 1965; Papavizas *et al*., 1980; Abawi *et al* 1990; Carol and Dona, 1986). Miller *et al* (1966, 1968) and Kirmani *et al* (1975) found that the C: N ratio of organic amendments influences the effect of the various amendments on pathogenesis and disease development with most of the soilbone pathogens including root-knot nematodes being favoured when more nitrogen is available. Decomposition of plant residues involves utilization of nitrogen and carbon present in them by microorganism for their growth hence increase in their populations which in turn can bring about biocontrol through such processes as competiton, antibiosis and mycoparasitism. Organic matter decomposition product such as ammonia, carbon dioxide among others may also play a role in pathogen decline (Maker *et al*., 1965; Papavizas *et al*., 1963 ; Byrdson, 1970). Eno *et al* (1966) examined that the anhydrous and hydrous form of

ammonia released by various forms of organic residues and at different conditions can differentially influence the growth of various microorganisms. Several research data is available on the influence of organic amendments of varying C:N ration on soilborne diseases. Maker *et al* (1965) reported that crop residues of alfalfa (C:N=40.5:2:7), lettuce (C:N=40:1) and cotton gin (C:N=40:1) trash increased root rot severity of Pinto beans caused by *Rhizoctonia solani* and *Fusarium solani phaseoli* whereas residues of barley (C:N=48:0.5), sorghum (C:N=40:0.5), soybean (C:N=42:6) and Sudan grass (C:N=40:1.5) reduced it.

Raising tomato seedlings in *Rhizoctonia* free nursery and taking them to the *Rhizoctonia* infested field after 21 DAP significantly decreased damping off diease perhaps because the seedlings are exposed to the inoculum when resistance has already set in due to lignification of cell walls as compared to the direct seeding. Similar information is available on the differential susceptibility of seedlings to Rhizoctonia damping off with age (Stockwel *et al.*, 1987; Cardoso *et al.*, 1994; Haran *et al.*, 1996; Elad *et al.*, 1982, 1983 and Bateman *et al.*, 1965). Most farmers transplant at 18-22 days old seedlings. Too old seedlings, over 28 days old can have a negative impact on yield and also result in longer production cycle by at least 2 weeks or more due to transplanting shock. However, transplanting creates wounds at the root tip which could increase peneration by *Rhizoctonia* but this disadvantage could be overtaken by the gains from resistance in the seedlings. This facilitation of entry by *Rhizoctonia* probably explains why there was relatively higher level of infection in transplanted seedlings as compared to direct seeded ones after 30 DAP as shown by smaller increment in cumulative survival at 30 DAP, and crop stand at 60 DAP in the transplanted plots compared to the direct seeded ones.

Abawi *et al* (1990) and Damping off Wikipedia (2008) reported similar finding when they found that stressful conditions such as injury increases seedling infection by damping off pathogens. However all these shortcomings of transplanting were clearly overtaken by the large gains from increase in crop stand, and hence yield from transplanted plots as compared to those from direct seeded plots in *Rhizoctonia* infested field. For all the cultural practices, transplanting produced the highest yield and was significantly different from chemical seed dressing. About 80.0% of the tomato growers in Kenya use transplanting instead of direct seeding to save on costs of raising seedlings in the nursery before taking them to the field. However, even in transplanting seeds are first direct seeded in the nursery, meaning it is not a complete solution to *Rhizoctonia* infection should nurseries be infested. The problem of transplanting shock can be avoided by use of seed propagation trays commonly referred to as speedling trays although the technology is more expensive expensive than using the nursery.

Effect of the seed dressing with fungicides in suppressing *Rhizoctonia* infection depended on active ingredient of the chemical. Gaucho MT 390 FS which contains pencycuron 50g/L, thiram 107g/L and imidacloprid 233g/L produced good disease control whereas Captan 800EC which contains captafol-carbendazim was poor. Summer (1987) reported differential control of *R. solani* isolates in the greenhouse by pencycuron. Captan controls damping off caused by many pathogens but not *Rhizoctonia* (Royal Horticultural Society, 2009). The differential efficacy of various seed dressers against *Rhizoctonia* isolates indicates that a combination of active ingredients or seed dressing with other tatics such as raised beds and others are required for the disease control.

Fumigation with Basamid, active ingredient methyl isothiocyanate had excellent results in the control of Rhizoctonia damping off of tomato and resulted in the highest yield perhaps because both chemical soil fumigation and soil solarisation achieved by polythene mulching of fumigated plots were involved. Both strategies control Rhizoctonia diseases in their own rights. Although plastic mulching was applied after application of Basamid to prevent escape of the fumigant, the length of time and temperature rise of a maximum of 50^0C for 3 weeks under plastic cover was enough to achieve soil solarization effect. Ben-yepet (1988) reported that solarization in combination with metham sodium was more effective in controlling *Verticillium dahliae* and *Fusarium oxysporum fsp vasinfectum* than when either of them was used singly. Basamid has been successfully used as an alternative to methyl bromide in the control of *R. solani* and other soilborne pathogens in tomato production (Cook *et al.*, 1994).

Basamid breaks down into methyl isothiocyanate (MIT), the primary bioactive agent, after its application in moist soil (Ohr, H.D *et al.*, 1996). MIT controls a broad spectrum of soilborne pests, its relatively safe to the user and a non-ozone depletor (Ohr, H.D *et al.*, 1996). However, it must be applied in large quantities which is difficult to handle, it depends on irrigation for its activation after application, it is readily leached which increases the potential for groundwater contamination, it has a short residual activity and the biological vacuum created by such a high efficacy could cause rapid reinfection especially where water or seeds are contaminated with the pathogen (Nolling, 1991; Ohr, H.D, *et al.*, 1996). There were few differences between various control methods in uninfested soil probably due to low levels of pathogenic *Rhizoctonia* strains and other root rot pathogens such as *Pythium* naturally occurring in the field.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1: General discussion

The outcome of the survey on the occurrence and distribution of Rhizoctonia damping off of tomato in major production regions of Kenya indicated that, environmental and crop production factors have greatly contributed to the build up of *R. solani* inoculum in most production areas. The wide host range of the pathogen across the major popular tomato varieties and other important vegetables such as okra, eggplant, beans and kales means that it is a real threat to the horticultural industry in Kenya. Involvement of several soilborne pathogens in causing the Rhizoctonia damping off diseae complex was implied by the isolation of several other fungal and bacteria isolates from *Rhizoctonia* infected tomato plants and rhizosphere soil.

There are several reasons for understanding the number and variability of *Rhizoctonia solani* strains that infect tomato and other vegetables in a certain production locality, their virulence on young seedlings and adult plants. Cultural practices such as crop rotation sequence and length of rotation may differentially affect survival of *Rhizoctonia* strains. Breeding for resistance, evaluation for the success of various control strategies and evaluation of the fungicide resistance must take into account the genotypes of the pathogen involved (Danesha *et al.*, 1999; Carol and Dona 1986; Koike *et al.*, 1996; Zhonghau *et al.*, 2005).

DNA diagnostic procedure does not differentiate between pathogenic and nonpathogenic strains as complete matching between virulence and genotype is not always achieved since phenotype/pathogenicity is influenced by other factors such as temperature, moisture and

physiological age of the host (Onkar *et al.*, 1986; Abawi *et al.*, 1990). In addition, a bioassay provides more information on the host/pathogen interaction by measuring both virulence and numbers. However, the DNA assay can accurately and reliably predict the expected levels of damage caused by *R. solani* on tomato by tracking the progess of the pathogen population dynamics throughout the growing season.

During the survey on the occurrence and distribution of Rhizoctonia damping off in Kenya, it was found that, there exist a direct relationship between the number of *R. solani* isolates and the damping off incidence and severity. However, more research is needed to relate the levels of *R. solani* strains detected prior to planting with the severity of damping off disease. Practical and effective management of root rot diseases by using a single control measure has always been difficult and will probably continue to be so in the future especially in areas where several root rot pathogens may be involved (Abawi *et al.*, 1990, Nolling *et al.*, 1991). Accordingly, it is more appropriate to combine the most effective and practical control measures available when managing Rhizoctonia damping off in vegetables. During this study, good disease control by biological and cultural methods was documented.

The biocontrol of pathogenic *R. solani* and the Rhizoctonia damping off of tomato it causes under the greenhouse conditions by nonpathogenic multinucleate and binucleate *R. solani* indicates the possibility of the disease management by microbial antagonism. On the other hand, the positive synergism between pathogenic *R. solani* and pathogenic *Fusarium*, and *Pythium* in causing Rhizoctonia damping off complex of tomato implied that effective management of the disease must involve development of a full package that controls not only

all the pathogenic strains of *R. solani* that are prevalent in a given growing location but also a concomitant control of *Fusarium* and *Pythium* pathogens present.

Manipulating cultural practices such as transplanting, soil moisture regime and cow manure application resulted in good control of Rhizoctonia damping off of tomato under field Conditions, and in most cases the control achieved compared favourably to the disease control by conventional methods such as seed dressing and chemical fumigation with Basamid. Combining two or more cultural practices that individually suppresses *Rhizoctonia* on their own had a positive synergism/multiplier effect resulting in greater disease control whereas combining those that promote the disease when used individually resulted to a higher disease levels. Furthermore chemical fumigation with basamid that produced the highest level of disease control involves integrating fumigation with MIT and solarisation. During application of Basamid, the soil temperature rose to a maximum of 50^0C under the plstic mulch for a period of 3 weeks. Such high temperature for the long duration was adequate to solarize the soil (Katan, 1976, 181). Combining a method that promote the disease with the one that suppress it, the overall effect depended on the efficacy of each of the methods individually.

Lack off adapted resistant tomato cultivars among the popular germplasm evaluated is a major drawback in the control of the disease in Kenya. Various seed dressing chemicals such as Captan and Gaucho 390 FS MT are available in the market but their effectiveness is questionable; probably due to multiple genera and species of root rot pathogens that occur simultaneously in most production locations and biodegradation after continued use. Currently Basamid is used by a few farmers in Kenya, especially in nurseries to manage several soilborne pathogens. The limitation in the use of Basamid by farmers interviewed was

mainly because it is expensive, toxic, requires technical know-how and the high efficacy is not consistent among the various genera of soilborne pathogens that exist in most production locations.

6.2: Conclusion

The detailed information on the occurrence and distribution of Rhizoctonia damping off of tomato in major production regions of Kenya and the influence of various production and environmental factors on disease development will have a bearing on future research on the biology of the disease and control methods.

The immediate benefit of the genotyping of *R.solani* causing damping off of tomato by microsatellite technique is in the application of the detection procedure to monitor the effectiveness of various disease control methods in the field. The sensitivity of the technique means that the precise location and diversity of the fungus at various production locations can be determined to improve the assessment of the experiments. Therefore, the new control procedures recommended in this study can be assessed more quickly and accurately, which would ultimately mean faster adoption by the farming community. Application of molecular technique will be an important tool for studies in population biology, disease development in response to control strategies, strain identification and evolution of the fungus. The use of molecular technology in combination with conventional plant pathology methods in plant disease diagnosis and pathogen detection has the potential to increase our understanding of the biology of plant patogens and in particular, soilborne ones. The result of this study demonstrates this potential by the application of microsatellite technique for genotyping of *R. solani* isolates causing damping off of tomato and other vegetables in Kenya.

Concerted efforts will be needed to implement the adoption of various nonchemical alternatives documented in this study either singly or in combination with other methods of the disease management such as crop rotation and fungicide treatments for effective control of the complex of soilborne problems and pests that currently limit tomato production in Kenya. In the past there was no urgent need to implement such systems, particularly those based on epidemiological concepts as there was always alternative land or crop to move to.

Since no single method documented in this study will offer complete control of Rhizoctonia damping off of tomato, the present challenge to phytopathologist is to develop integrated sustainable methods in which different combinations of methods are used to manage the disease in tomato and other vegetables. Short term approaches will probably be to focus on the currently registered replacement chemicals such as Basamid, seed treatment with Gaucho MT FS 390 either singly or in combination with other non-chemical methods recommended in this study. It is imperative that tomato growers spread their risks in an integrated pest management approach by employing a number of different tactics to reduce damage by *R. solani* to below economic levels rather than relying exclusively on any single approach discussed in this study.

6.3: Recommendations

1. Periodical survey on the occurrence and distribution of *R. solani* and other soilborne pathogens in various production locations shoukd be undertaken to provide a first hand informations on economical importance of the disease and its epidemiology. This should be used to influence decision making on evaluation of control methods.

2. Multidisciplinary research will be needed to accurately characterize the various pathogenic strains of *Rhizoctonia solani* and other soilborne pathogens involved in causing damping off of tomato and other vegetables in Kenya and study the success of various disease management strategies. This should involve employing such modern techniques as nucleic acid analysis with more loci and larger samples being analysed to increase precision of the results.

3. There is immediate need to establish a predictive diagnostic service for routine characterization and race typing of *R. solani* in all production locations. Such a service will assist to capture new biotypes and help in development of genes for resistance to Rhizoctonia damping off of tomato. The adoption of the DNA based detection procedure by researchers studying applied aspects of the disease and setting up and evaluating trials will involve an initial cost but thereafter would become part of the routine operating budget.

4. More tomato varieties should be screened for resistance to local strains of *R. solani.* Breeding programmes should aim at incorporating both polygenic resistance to ensure effective and durable resistance. Although none of the varieties screened in this study

was found to be resistant to the disease, the moderately resistant varieties should be used in integration with other control methods to improve the disease control .

5. Screening of other popular vegetables such as okra, eggplants, beans and kales for resistance to Rhizoctonia damping off disease should be done to produce alternative crops for rotation with tomato.

6. Further study need to be done to establish the mechanism of interaction between pathogenic *R. solani* isolates and other soilborne pathogens documented in this study in influencing the damping off disease. Such studies should extend to more potential soilborne fungi and bacteria, and seek the possibility of using the promising candidates as a substitute or additive to fungicides in control of the disease.

7. Since majority of the farmers interviewed had no adequate information on the disease, there is immediate need to educate and train them on the disease diagnosis, identification, epidemiological factors and the new control measures recommended in this study.

8. Field trials for applicability of the promising cultural strategies recommended in this study on disease control should be initiated in various production locations.

9. The national extension service should effectively ensure prompt information and technology transfer. To organize, structure or simply to achieve new IPM information

for rapid retrieval or dissemination will require the development of computerized data bases and decision support software. This should include district, provincial and national levels of organization. New educational programs and special training seminars on new procedures for plant disease diagnosis, soil analysis, and pest identification will need to be demonstrated to country extension agents, farm advisers, agricultural consultants and large scale farm crop protection managers to ensure adoption.

10. To achieve these goals, adequate funds, equipments and personnel to support research, information and technology transfer activities such as computers, publication costs and travel must be made available. Site locations for cropping, efficacy, and geographical adaptability testing and grower review should be provided. To achieve these goals will require a strong linkage between researchers and extension personnel as well as growers.

11. More intensive trials should be conducted on Basamid to optimize its usage.

12. More biological orgaisms such as *Trichoderma* and *Bacillus spp* should be screened for antagonism against *R. solani.*

13. More research need to be conducted on use of other potentially viable cultural methods such as crop rotation and phytosanitary methods, and screening of organic amendments of varying C: N ratio such as various types of green manure, compost and animal manure in the disease control.

CHAPTER SEVEN

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CHAPTER EIGHT

APPENDICES

Appendix 1.0: Questionaire: Farmer x Rhizoctonia damping off disease

1. Field Number:.....
2. What variety of tomato do you plant on this farm (Greatest to least area)?

   Var. 1 .....

   Var. 2 .....

   Var. 3 .....

   Var.4 .....

   Var.5 .....

3. Where do you usually obtain planting material from?

   .....

4. Do you raise seedlings in the nursery or direct plant seeds in the field? .....

   .....

5. If nursery system is used, do you fumigate them frequently? .....
6. Do you use boma manure (fumigated or not) in your nursery or field? .....

   .....

7. What other vegetables do you grow in your farm? .....

   .....

8. Interviewer shows tomato plant with Rhizoctonia root and stem rot symptoms, and asks them what

   Causes the disease. If there are no disease plants present, show them a picture.

   (a) Can the producer recognize the disease? Yes..... (01), No ..... (02)

   (b) What do you call the disease (local name) .....

(c) What causes/spreads it? .....

(d) Does the disease occur in the nursery and or field? .....

.....

9. Are other vegetables affected by the disease? ..... Name and rank them .....

.....

10. Is there a problem in your farm? Yes .....(01), No .....(02), Don't know .....(03).

11. Do these problems appear every year? Yes .....(01), No .....(02)

12. What months is this disease severe?

.....

13. List the methods you use to control the disease problem in order of importance (1 = the most important): Give scores for their effectiveness in controlling the problem (1) very effective;

(2) Partly effective; (3) Not effective; (4) Damaging.

Method	Effectiveness
1.	.....
2.	.....
3.	.....
4.	.....
5.	.....

14. Do you ever plant these (diseased) plants? Yes ..... (01), No ..... (02)

15. Do you ever pull these (diseased) plants out? Yes ……..(01), No …….. (02)

If yes, When? ……

16. Are some varieties more severely damaged than others? Yes …….(01), No …….(02)

17. (a). If you have noticed differences , why do you grow the ones that are more diseased? ……..

(b). Why do you think some varieties are more diseased than others? ……

18. Do you have any really good resistant varieties? Yes …….. (01), No …….. (02)

If yes, give names

……

19. Which other good traits does the variety have?

……

……

……

20. Would you be interested in receiving a new variety? Yes …… (01), No …… (02)

If yes, what characteristics would you want in the new variety? (List them in order of importance).

a) ……

b) ……

c) ……

d) ……

e) ……

21. What mode of planting do you use in the field? (01) Ridges ……, (02) flat ground …….. , (03) Moulds …….. , (04) Others ……

22. Do you plant under rainfed or irrigation?

.....

23. What are the characteristics of the varieties that you grow? .....

.....

24. What is your cropping system? (a) Monocrop ..... (b) intercrop .....

Appendix 2.0: Mean Rhizoctonia damping off of tomato prevalence, incidence, severity and number of pathogenic R. *solani* isolates in various agro-ecological zones

Ecological zone	Number of fields sampled	infected isolates	Disease prevalence	incidence	Disease Severity	Total
UM2	22	5	22.7	1.9	1.1	2
UM3	66	33	25.0	5.8	1.3	14
UM4	73	48	32.9	15.7	1.5	24
UM5	61	39	32.0	14.8	1.6	16
Mean			28.2	9.5	1.4	0.25
LSD(P≤0.05)			5.1	10.2	13.4	9.1
CV%			8.0	70.8	46.1	66.5

Appendix 3.0: Mean Rhizoctonia damping off of tomato prevalence, incidence, severity and number of pathogenic *R. solani* isolates in various seasons

Season	Number of fields Sampled	Infected	Disease Prevalence	Incidence	Severity	Total isolates
Wet	137	100	32.9	14.0	1.5	38
Dry	85	45	17.7	4. 5	1.2	18
Mean			25.3	9.3	1.4	0.25
LSD (P≤0.05)			5.1	6.7	0.2	6.5
CV (%)			18.0	70.8	46.1	45.0

Apendix 4.0: Mean Rhizoctonia damping off of tomato prevalence, incidence, severity and pathogenic *R. solani* isolates in various provinces, districts and agro-ecological zones

Province	District	Agro-ecological zone	prevalence	Disease Incidence	Severity	Number of isolates
Central	Kirinyaga	Um2	26.0	12.2	1.1	1
Central	Kirinyaga	Um3	31.4	4.7	1.4	5
Central	Kirinyaga	UM4	33.3	18.0	1.6	8
Central	Kiambu	Um3	19.6	3.9	1.2	2
Central	Kiambu	Um4	27.7	12.6	1.3	2
Central	Maragua	UM3	26.2	7.8	1.4	2
Central	Maragua	Um4	42.0	16.8	1.7	5
Central	Nyeri	UM4	24.5	19.3	1.8	1
Eastern	Makueni	UM4	36.5	18.2	1.5	1
Eastern	Makueni	LM3	35.4	19.1	1.7	14
Eastern	Embu	Um3	18.3	7.3	1.3	3
Eastern	Embu	UM4	40.2	17.8	1.6	4
Eastern	M. Central	Um3	26.4	5.8	1.3	1
Eastern	M. Central	UM4	17.2	15.7	1.3	2
Eastern	M. Central	LM3	26.2	10.0	1.5	1
R.Valley	Nakuru	LM3	34.6	14.7	1.6	1
R.Valley	Kajiando	Um2	8.8	1.6	1.1	1
R.Valley	Kajiando	Um3	21.5	5.3	1.2	1
R.Valley	Kajiando	UM4	15.8	1.5	1.2	1
Mean/Province			119.7	28.4	3.4	14.0
LSD (P≤0.05)/Province			18.4	14.0	0.6	8.0
CV (%)/Province			63.7	71.0	77.5	98.0
Mean/District			53.2	12.6	1.5	6.2
LSD (≤0.05)/District			20.0	7.3	0.3	4.0
CV (%)/District			34.2	57.4	22.1	44.0
Mean/AEZ			28.2	9.5	1.4	0.25
LSD (P≤0.05) /AEZ			5.1	10.2	13.4	9.1
CV (%)/AEZ			18.0	70.8	46.1	66.5

Key: Um2, Um3, UM4, LM3- Agro-ecological zones Um2, Um3, UM4 and LM3 respectively.

M. central=Meru central

R. Valleey=Rift valley

AEZ= Agro-ecological zone

Apendix 5.0: *Rhizoctonia solani* clusters in various agro-ecological zones, administritative boundaries and altitude

Isolate Code	Cluster	AEZ	Provinve	District	Division	Location/Village	Altitude (M)
R1	1	UM4	Central	kirinyaga	Gichugu	Gichugu Townshi	1341
R2	2	LM3	Eastern	Makueni	Makindu	Makindu Township	730
R3	2	UM4	Central	Kirinyaga	Gichugu	Gichugu Townshi	1341
R4	4	UM4	Central	Kirinyaga	Gichugu	Gichugu Township	1341
R5	4	UM4	Eastern	Embu	Mwea	Kimbimbi	1474
R6	1	UM3	Eastern	Embu	Runyenjes	Kyeni	1533
R7	3	UM2	R.Valley	Nakuru	Subukia	Kihoto	1722
R8	4	UM3	Eastern	Embu	Runyenjes	Kyeni	1533
R9	2	UM3	Central	Kirinyyaga	Kagio	Kagio Market	1525
R10	4	UM3	Central	Kirinyaga	Kagio	Kagio Market	1525
R11	7	LM3	Eastern	Makueni	Makindu	Makindu Township	730
R12	6	UM3	Central	Kiambu	Kikuyu	Muguga	1421
R13	4	LM3	Eastern	Makueni	Kibwezi	UoN-Farm	701
R14	6	LM3	Eastern	Makueni	Kibwezi	UoN-Farm	701
R15	3	LM3	Eastern	Makueni	Kibwezi	UoN-Farm	701
R16	4	UM4	Central	Kirinyaga	Mwea	Kimbimbi	1433
R17	1	UM3	Cenral	Kiambu	Kikuyu	Muguga	1421
R18	5	UM4	Central	Maragua	Kigumo	Sabasaba	1430
R19	7	UM4	Central	Kirinyaga	Mwea	Kimbimbi	1433
R20	5	UM4	Central	Kirinyaga	Mwea	Kimbimbi	1433
R21	1	UM4	Eastern	Makueni	Emali	Muoni	784
R22	3	UM3	Eastern	Embu	Runyenjes	Kyeni	1533
R23	5	UM4	Central	Kirinyaga	Mwea	Kimbimbi	1433
R24	2	UM4	Central	Kirinyaga	Mwea	Kimbimbi	1433
R25	4	UM4	Central	Maragua	Kigumo	Sabasaba	1430
R26	3	UM4	Central	Kiambu	Kikuyu	Thogoto	1377
R27	1	UM4	Central	Maragua	Kigumo	Sabasaba	1430
R28	5	UM4	R.valley	Kajiando	Magadi	Ngurumani	1050
R29	5	UM4	Central	Maragua	Kigumo	Sabasaba	1430
R30	1	UM3	Central	Kirinyaga	Kagio	Kagio Township	1533
R31	5	LM3	Eastern	M. Central	Nkubu	Nkubu Township	1630
R32	3	LM3	Eastern	Makueni	Kambuu	Darayani	693
R33	4	UM3	Central	Maragua	Kiharu	Maragua Bridge	1515
R34	2	UM4	Eastern	Embu	Mwea	Kimbimbi	1474
R35	3	UM4	Eastern	M.central	Mitunguu	Mitunguu irr. scheme	1333
R36	7	UM3	Central	Kirinyaga	Kerugoya	Kerugoya Township	1565
R37	1	UM3	R.Valley	Kajiando	Kiseriani	Upper Matasia	1400
R38	4	UM3	Central	Kirinyaga	Kerugoya	Kerugoya Township	1565
R39	4	UM3	Central	Maragua	Kiharu	Maragua Brige	1515
R40	4	UM4	Eastern	M.central	Mitunguu	Mitunguu irr.scheme	1333
R41	1	UM2	Central	Kirinyaga	Kerugoya	Kerugoya Township	1605
R42	5	UM4	Eastern	Embu	Mwea	Kimbimbi	1474

Appendix 5.0. Continued

Isolate Code	Cluster	AEZ	Provinve	District	Division	Location/Village	Altitude (M)
R43	5	UM4	Eastern	Embu	Mwea	Kimbimbi	1474
R44	4	LM3	Eastern	Makueni	Kibwezi	Kyachai	701
R45	4	LM3	Eastern	Makueni	Kibwezi	Kyachai	701
R46	4	LM3	Eastern	Makueni	Kibwezi	Kyachai	701
R47	5	UM4	Central	Maragua	Kigumo	Sabasaba	1430
R48	5	LM3	Eastern	Makueni	Kibwezi	UoN-Farm	701
R49	4	LM3	R.Valley	Nakuru	Naivasha	Lake Naivasha	1312
R50	5	UM4	R.Valley	Kajiando	Lake magadi	Ngurumani	1050
R51	6	LM3	Eastern	Makueni	Mtito Andei	Kathekani	671
R52	3	LM3	Eastern	Makueni	Mtito Andei	Kathekani	671

Key: Um2, Um3, UM4, LM3- Agro-ecological zones Um2, Um3, UM4 and LM3 respectively

M. Central=Meru central

R. Valleey= Rift valley

AEZ= Agro-ecological zone

Appendix 6.0: The range of the F-Statistics (FST)

0 (no genetic divergence) to 1 (fixation for alternate alleles in different subpopulations)

F_{ST}	Genetic differentiation
0 to 0.05	small
0.05 to 0.15	moderate
0.15 to 0.25	large
>0.25	very large

The statistical indexes involved (Gauch, H.G., Jr. 1982) measure:

FIS = the deficiency or excess of average heterozygotes in each population.

FST = the degree of gene differentiation among populations in terms of allele frequencies.

FIT = the deficiency or excess of average heterozygotes in a group of populations.

Appendix 7.0: Co-phenetic technique procedure

This technique involves quantifying the distortion due to the grouping method used. It builds a new similarity or distance matrix, the 'co-phenetic matrix', directly from the dendrogram. Validation is calculated by means of a correlation coefficient between similarity or distance data from the original matrix and those from the new co-phenetic matrix. Whether the original distances are maintained are assessed after the grouping exercise.

If the correlation value is high, we can conclude that the dendrogram does indeed reflect the distances in the original matrix and that therefore there is no distortion due to the grouping method.

Appendix 8.0: Primers screening protocol

A set of 8 polymorphic primers (3 orange, 3 green and 2 yellow) were assembled and tested to ascertain wether they would amplify in a repeatable manner. This initial screening was done on agarose gel (cheaper and faster). The protocol below was used:

1) 5 isolates were ran against the 8 primers: Some primers amplify=X1; Some did not amplify=Y1.
2) The non-amplifying (Y1) kept aside.
3) Those that amplified (X1) were ran against the 56 isolates: Some amplified=X2; Some did not amplify=Y2.
4) The initial non-amplifying in no.2 above (Y1) were ran against all the isolates to ascertain if they amplify or not: Some amplified=Y3; Some did not amplify= those discarded. The final result was a certain number of primers that amplified some or all of the isolates. Each of the primer that amplified was ran against all the 56 samples to generate a data matrix for analysis.

NB: First the procedure was optimized (DNA concentration and reaction temperature).

Appendix 9.0: Media, reagents/buffers and solutions preparation protocol

Appendix 9.1: Preparation of RNase

100mg of Rnase were dissolved in a 100ml of 10mM Tris-7.5, 15mMNacl and heated in boiling water for 15 minute and allowed to cool slowly to room temperature, then dispensed into 1ml aliquots and stored at-20 °C while working stock was stored at 4 °C.

Appendix 9.2: 1. 5% Agarosae + 5% ethidium bromide preparation protocol

Preparation of agarose will follow the procedure described by Hoisington *et al* (1994).

1.5g agarose was dissolved in 100ml distilled water. Some extra water was added incase of evaporation and heated in microwave for 5-10minutes and then cooled in a waterbath to 50°C

1) The gel was poured in the gel mould with combs well placed.

2) The combs were removed and the gel put in the gel tank with running electrophoresis buffer (TBE).

3) Five microlitre of loading dye (Bromopheno blue) was put into each of the 57 tubes to make the DNA sink well into the wells.

4) The dye was mixed with the PCR product by placing on a bench centrifuge at 1300-1500 rpm for 30 seconds and 5µl loaded to the wells.

5) One mililitre of DNA marker was strategically placed into the gel

6) The tank was closed and put it at 100V for 30 seconds.

7) The gel was stained with 5% ethedium bromide (Sambrook et al 1987). 5% ethidium bromide was made by 5ml in 95ml distilled water.

Appendix 9.3: 5% polyacrylamide gel preparation protocol

1) Glass plates, spacers and combs were washed with warm deionized water and air dried.

2) The following components were combined in a 100ml deionised water in beaker to prepare a 25 ml of gel solution for a 36-cm run.

Reagents	Amount
6M Urea (Amresco ®)	9.0g
40% bis-acrylamide (Bionard®)	3.12ml
Deionised Water (dH_2O)	12.5ml
Mixed bed ion exchange resin (Amberilite® beads)	0.2g

1. The solution was stirred until all the Urea crystals dissolved.
2. The acrylamide solution was filtered through a 0.2 cellulose nitrate filter, degased for 3 minutes and transfered to a 100ml graduated cylinder.
3. 2.5ml of filtered 10X TBE buffer was added and the volume adjusted with dH_2O to 25ml.
4. 25µl of freshly made 10% APS solution was added to the gel solution, being careful not to introduce air bubbles and the mix swirled.
5. Gently 17.5µl TEMED (distilled) was added to the gel solution, being careful not to introduce air bubbles.
6. The gel was casted onto thoroughly cleaned glass that had already been mounted in the gel cassette, and a gel casting comb inserted and clamped down with plate clamps.
7. The gel was allowed to polymerize for 2 hours to make gel thickness of 0.2mm

Appendix 9.4: Sample preparation for loading onto the 5% Polyacrylamide gel

The samples for loading were prepared as follows:

1. 1.5 µl of the PCR products was mixed with 1.9µl of a mixture of the following items, to make a total of 2.4 µl of sample for loading.

Reagent	Amount
Deionized formamide	1.3 µl
Gene Ruler 100^{TM} bp plus internal sized standard	0.3 µl
Loading Buffer	0.3 µl

Formmamide, size standard, and loading buffer were premixed for all samples.

2. The samples were heated at 95°C for 2 minutes to denature the DNA, and then placed snap-cooled on ice until ready to load.

Appendix 9.5: Preparation of the electrophoresis buffer (TBE)

The electrophoresis buffer was Tris-borate-EDTA (TBE) at P^H 8.3

To make 1 litre X10 stock solution ;

Reagent	Amount
Tris base	108.0g
Boric acid	55.0g
Na2 EDTAi $2H_2O$	8.3g

The working solution (1X TBE) was 89mM Tris base, 98mM Boric acid, 2mM EDTA; P^H approximately 8.3 at ambient temperature.

To prepare the 1X TBE working solution :

1) 120ml of X10 TBE stock solution was added to a large graduated cylinder.

1) This was diluted to a total volume of 1200ml using deionized water (dH_20).

Appendix 9.6: 3.9% Oxoid Potato dextrose agar (PDA) prepation protocol

Reagent	Amount
Potato	200g
Dextrose	20g
Agar	15g
Distilled water	1000ml

After sterilization by heating in an autoclave at 121^{0}C, 15 psi 15 minutes, the medium was cooled down to 50^{o}C, after which lactic acid was added at the rate of 1.0ml for 1000ml PDA to make APDA and then poured into plates (20ml/plate). Potato nutrient broth was made by mixing 500ml potato extract, 20g sucrose, 15g agar, 1ml trace metal solution and 500ml distilled water, and the Adjust P^H to 6.7.

After sterilization by heating in an autoclave at 121^{0}C, 15 psi 15 minute, the medium was cooled down to 50^{o}C and poured into plates (20ml/plate).

Appendix 9.7: Nutrient agar prepation protocol

Reagent	Percentage
Peptone	0.5
Beef extract	0.3
Agar	1.5
P^H adjusted to neutral at 25^{0}C.	

After sterilization by heating in an autoclave at 121^{0}C, 15 psi 15 minute, the medium was cooled down to 50^{o}C and poured into plates (20ml/plate). Nutrient broth contains no agar and was made by dissolving 8 g of the powder in 1 L of purified water and P^H adjusted to 6.8 ± 0.2 at 25^{0}C.

Appendix 9.8: Potato sucrose agar (PSA) prepation protocol

Reagent	Amount
Potato extract	500ml
Sucrose	20.0g
Agar	15.0g
Trace metal solution	1.0ml
Distilled water	500ml
Adjust P^H to 6.7	

After sterilization by heating in an autoclave at 121^0C, 15 psi 15 minute, the medium was cooled down to 50^oC and poured into plates (20ml/plate).

Appendix 10.0: Loading samples and starting the electrophoresis run

1) The AB1 prism instrument was prepared by mounting the gel casette onto the rear heat transfer plate, and attaching the upper chamber. The upper and lower electrophoresis chambers were filled with with X1 TBE buffer (each with about 600ml). Front heat- transfer plate, water lines and ground wire were attached, and the electrophoresis cables attached to connection in the chamber. All the wells were flushed with running buffer.

2) Then the sample sheet was filled out, file ran and the data collection software started.

3) The plates and gel were checked for new fluorescence. The gel was pre-ran for about 20 minutes to equilibrate the temperature and to make sure all concentrations and components of the electrophoresis system were working properly.

4) All the wells in the gel were flushed with running buffer (1X TBE) using a needle and syringe to clean out particles on the gel surface that may interfere with loading and running of the samples. Any bubbles under the bottom edge of the gel plates in the lower buffer chamber were removed by using a clean needle and syringe, with needle the bent at an angle to guide the bubbles out.

5) After heating (95^0C) the samples to denature the DNA and snap cooled in ice to 4^0C, the entire 2.4 µl of the loading mixture prepared in appendix 6.0 was loaded. The samples were loaded using a shark's tooth comb, and since they are to run side by side, the samples were loaded in alternate wells since the automatic lane tracker in the analysis software needs to have discrete spaces between the samples to identify the lanes properly. To achieve this, the odd-numbered samples were loaded first, then pre-ran for 2 minutes, the remaining wells rinsed and loaded with even-numbered samples, then the gel ran for 2 hours.

Appendix 11.0: Allele frequency by locus for the whole population of *Rhizoctonia solani*

Loci	Allele	Frequency
RB23	183	0.3333
	186	0.4479
	192	0.2083
	194	0.0104
RE 14	160	0.1591
	175	0.1477
	177	0.1136
	180	0.1705
	181	0.1591
	189	0.0795
	190	0.1136
	195	0.0104
RE 102	130	0.1562
	133	0.1667
	135	0.1979
	137	0.1562
	143	0.1875
	145	0.1354
BC5	140	0.2083
	142	0.0521
	155	0.0417
	156	0.0625
	158	0.0729
	161	0.0625
	162	0.0729
	164	0.0521

Appendix 11. BC5 Continued

	168	0.0625
	169	0.1042
	172	0.0625
	174	0.0729
	178	0.0729
AF513014	188	0.1923
	191	0.0769
	193	0.0962
	199	0.0481
	202	0.0288
	204	0.0385
	205	0.0288
	208	0.0192
	211	0.0288
	213	0.0577
	214	0.0481
	215	0.0192
	216	0.0192
	219	0.0192
	222	0.0385
	225	0.0481
	229	0.0385
	230	0.0288
	234	0.0385
	236	0.0481
	238	0.0385
AY212027	285	0.0581
	288	0.1170
	290	0.1702
	291	0.1809
	293	0.1596
	295	0.1809
	297	0.1064
LROR	1410	0.1250
	1415	0.1406
	1480	0.0938
	1520	0.1875
	1523	0.1719
	1795	0.1094
	1800	0.1719

Appendix 12.0: Summary of Genic Variation Statistics for all Loci

Locus	Sample Size	na*	ne*
RB 23	96	4.0000	2.8149
RE 14	88	8.0000	7.3057
RE 102	96	6.0000	5.9077
BC 5	96	13.0000	10.1722
AF513014	104	21.0000	13.0000
AY212027	94	7.0000	6.5744
LROR	64	7.0000	6.6494
Mean	91	9.4286	7.4892
St. Dev		5.7982	3.2530

* na = Observed number of alleles

* ne = Effective number of alleles [Kimura and Crow (1964)]

Appendix 13.0: Summary of Heterozygosity Statistics for all Loci

Locus	Sample Size	Obs_Het	Exp_Het*	Nei**	Ave_Het
RB 23	96	0.7708	0.6515	0.6447	0.6052
RE 14	88	0.8182	0.8730	0.8631	0.6300
RE 102	96	0.9167	0.8395	0.8307	0.7662
BC 5	96	0.7500	0.9112	0.9017	0.7740
AF513014	104	0.8269	0.9320	0.9231	0.8281
AY212027	94	1.0000	0.8570	0.8479	0.8134
LROR	64	0.7812	0.8631	0.8496	0.7703
Mean	91	0.8377	0.8468	0.8373	0.7410
St. Dev		0.0898	0.0919	0.0909	0.0877

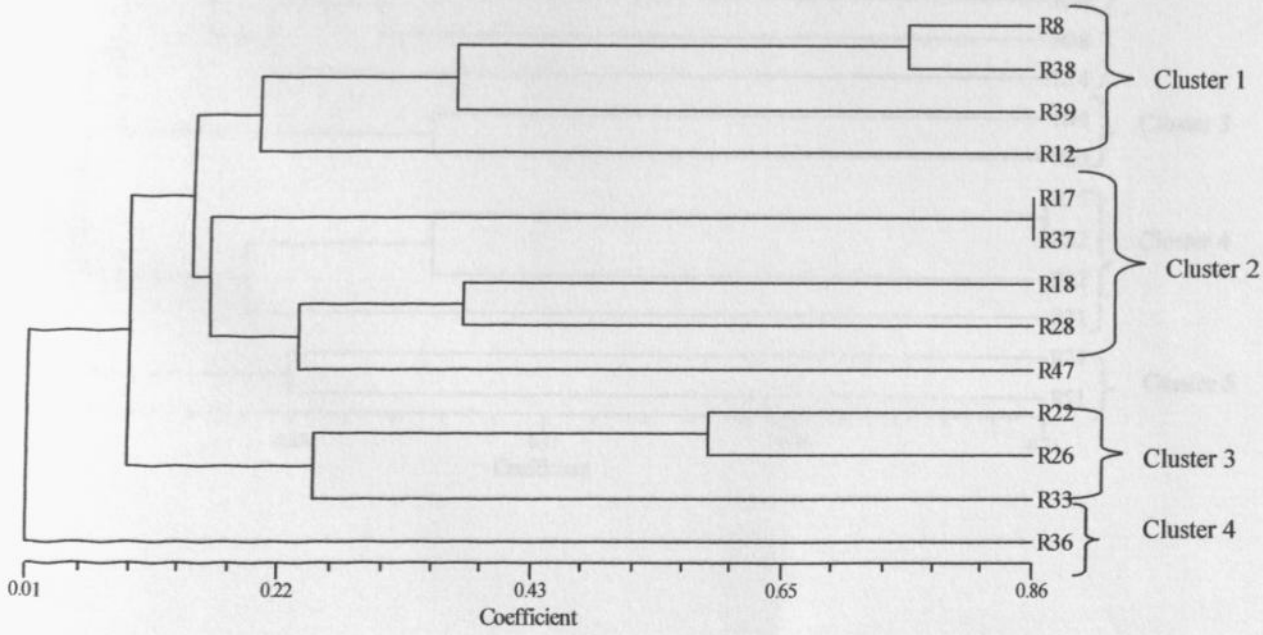
Appendix 14.0: Molecular dendrograms

Appendix 14.1. Molecular clustering of *Rhizoctonia solani* isolates from agro-ecological

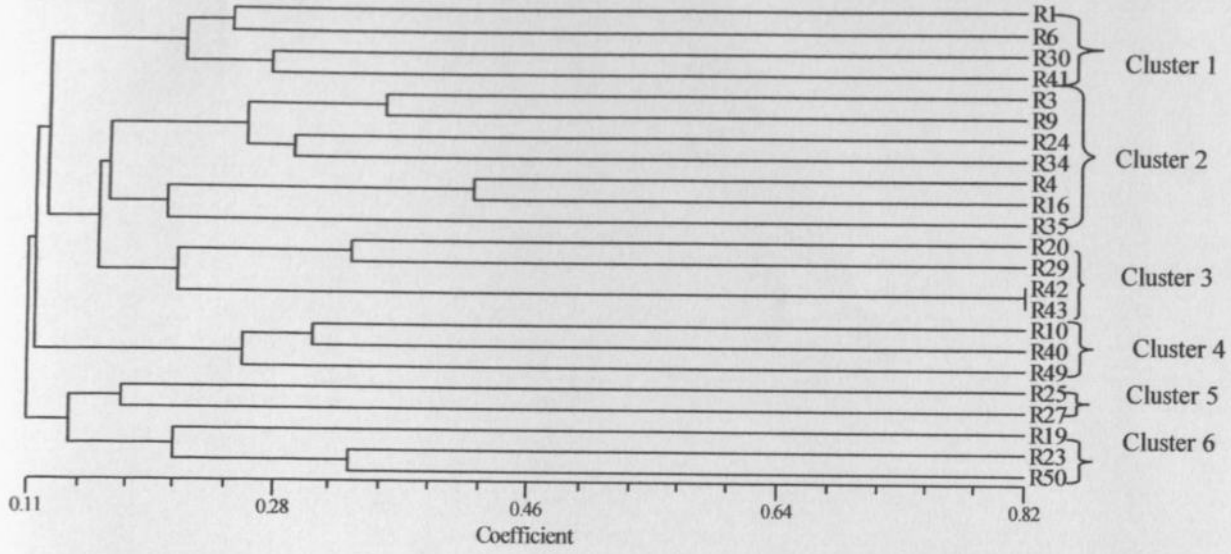
ZoneUM2

Appendix 14.2: Molecular clustering of *Rhizoctonia solani* isolates from agro-ecological

zone UM3



Appendix 14.3: Molecular clustering of *Rhizoctonia solani* isolates from agro-ecological zone UM4



Appendix 14.4: Molecular clustering of *Rhizoctonia solani* isolates from agro-ecological zone LM3