INTEGRATED MANAGEMENT OF *FUSARIUM* WILT OF TOMATOES USING FUNGICIDES, ORGANIC MATTER AND NEEM EXTRACT

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (PLANT PATHOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior permission of the author and/or Kenyatta University.

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DECLARATION BY SUPERVISORS

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DEDICATION

This research work is dedicated to my parents and my family members for the support they gave me during the period of study.

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ABBREVIATIONS AND ACRONYMS

ANOVA - Analysis of Variance

AEZ - Agro-Ecological Zones

AVRDC - Asian Vegetable Research and Development Centre

BSD - Biological Soil Disinfestation

CBS - Central Bureau of Statistics

F. o. l. - *Fusarium oxysporum f.* sp. *lycopersici*

HCDA - Horticultural Crops Development Authority

IPM - Integrated Pest Management

KARI - Kenya Agricultural Research Institute

NKCP - Neem Kernel Cake Powder

NSW - New South Wales

PDA - Potato Dextrose Agar

SPSS - Statistical Package for Social Sciences

S.E - Standard Error

ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum f.* sp. *lycopersici (F. o. l.)* is a major tomato production constraint in Kirinyaga district. This study was carried out to investigate the use of organic soil amendments and fungicides/biopesticides singly or in combination for the management of *Fusarium* wilt. The most popular tomato varieties in the region; Rio Grande, Onyx and Cal J were used. The varieties have high productivity, marketability and good keeping qualities. Wilting disease symptom was recorded by all the farmers interviewed. Five isolates of *F. o. l*; isolate 5, 6, 8, 9 and 11 were compared for their infection abilities on Rio Grande, Onyx and Cal J. Findings from greenhouse indicated that there was no significant difference ((P > 0.05) in *Fusarium* wilt percentage incidences on tomato plants as a result of infection by various isolates. However, a higher percentage incidence was recorded in tomato plants infected with isolate 6. Isolate 6 was more infective on Cal J (% incidence 85.00) than on Onyx (62.50%) and Rio Grande (36.00%). Three fungicides; Milraz, Ridomil and Ortiva were used in *in vitro* experiments to determine their inhibition abilities on hyphal growth. Higher inhibition on fungal hyphal growth was recorded in media treated with Ridomil (Mean diameter 26.24 ± 4.14 mm). Hyphae on media incorporated with Ortiva had bigger diameter (37.22 ± 5.12 mm) than Milraz (32.18 ± 4.77) but not significantly different. This finding therefore indicated that, Ridomil highly inhibited *Fusarium* hyphal growth compared to Milraz and Ortiva. A comparison in different rates of Neem Kernel Cake Powder (NKCP) showed that lower disease incidence (42.5 ± 19.8%) and severity (34.3 ± 15.2%) were recorded in plants in pots amended with 10 g NKCP than in pots treated with lower rates of 9 g, 7 g, 5 g and 3 g NKCP. Plants in pots treated with 3 g NKCP had the highest percentage disease incidence (67.5 ± 23.1) and severity (43.2 ± 20.5). Stem diameter and plant heights of the *Fusarium* wilt infected plants treated with 9 g and 10 g Neem extract were bigger than the rest of the treatment although the difference was not significant (p > 0.05). Findings from this experiment showed that, the most effective rate of the Neem cake powder was 10 g/pot. Plants grown in pots treated with "Fungicides + Neem cake powder +Organic matter" had lower disease incidences (mean 40.5%) than those grown in pots treated with other combinations of Fungicides, Neem cake powder and Organic matter. However, the differences in the disease incidences were not significant (P >0.05). Stem diameters and plant heights recorded from different treatments followed a similar trend although there was no significant difference (P>0.05). It was noted that taller plants had the biggest stem diameters, showing a significant relationship between the plant heights and the stem diameters (r = 0.833, P = 0.000). From the results of the study, the use of integrated approach was more effective than single methods in the management of *Fusarium* wilt of tomatoes. The farmers are therefore advised to use a combination of methods in order to effectively control the disease and improve crop performance.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae. Tomato is considered one of the world's most important and popular vegetables (Pritesh *et al.*, 2011). It is the most important tropical vegetable crop wildly used throughout the world (Hadian *et al.*, 2011). Most of the regions in Kenya produce tomatoes which are sold in the local markets. The produce marketed in the bigger cities, such as Nairobi, Mombasa, Nakuru, Kisumu, Eldoret and other major towns are sourced from Kirinyaga District (Mwea area), Meru Central District (Mitunguu area and Isiolo region), Nyeri District, Nakuru District (Bahati and Kabazi areas) and Taita Taveta District (Kirimi Sindi website, 2011). Tomato is one of the most important local market vegetable in Kenya (Kavoi *et al.*, 2004). The crop is mainly grown by small scale farmers in most arable areas in Kenya from the coastal belt to the upper highlands. In 2004, an estimated 75,101 tones of tomato valued at over KSh. 1 billion were produced in Central Province surpassing all the other vegetables in value (KARI, 2005). Tomato fruits are used fresh in salads or cooked as a vegetable, in processed form as tomato paste (puree), tomato sauce, ketchup, juice and can also be dried. Tomato is rich in vitamins A and C and is gaining importance because it contains lycopene a food component known to reduce the incidence of prostrate cancer, heart and age related diseases (AVRDC, 2003). The major varieties grown in Kenya are Cal J, Rio Grande, Rodade, Nema 1200, Nema 1400, Onyx VF2 and Monyala F1 (KARI, 2005; Kirimi Sindi Website, 2011). The tomato is a high-value horticultural crop for the local market. It is an important dietary component, contributing to improved nutrition and livelihood for both rural and urban population (Waiganjo *et al.*, 2006). In Kenya, it is the most popular vegetable crop

after *Brassica* and constitutes the most important source of income for a large population of small scale farmers. Tomato is fairly adaptable, but grows well in warm conditions with optimum temperatures of 15°C -25 °C. High humidity and temperatures reduce fruit set and yields. Very low temperatures delay colour formation and ripening and temperatures above 30°C inhibit fruit set, lycopene development and flavour. Tomato thrives best in low to medium rainfall with supplementary irrigation during the off-season. Wet conditions increase disease attacks and affect fruit ripening. Tomatoes grow well in a wide range of soil types, which are high in organic matter, well-drained and a pH range of 5 - 7.5 (Waiganjo *et al.*, 2006; Hanson *et al.*, 2001). Tomato plants prefer soil that is well drained and heavily amended with organic matter. The soil should have good moisture retaining capacity. Elevation of between 1000 M to 2000 M above sea level is suitable for the tomato growth (Robert, 2005). Many constraints affect productivity and quality of tomato among which diseases play a salient role (Pritesh *et al.*, 2011). The pests that attack tomato crops include aphids, fruit worms, mites, thrips, white flies, cutworms, spider mites and corn borer (Winand *et al.*, 1999). The most common diseases of tomato include early blight, anthracnose, bacterial wilt, bacterial canker, tomato spotted wilt, *Verticillium* wilt and *Fusarium* wilt (Winand *et al.*, 1999). The wilt diseases are caused by bacteria (*Pseudomonas* spp*)* and fungi (*Fusarium* and *Verticillium* spp) (Mardi *et al.*, 2002). Tomato *Fusarium* wilt is considered as one of the most important diseases of tomato both in field and greenhouse – grown tomatoes worldwide (Abdel-Monaim, 2012; Amini *et al.*, 2010; Sheu *et al.*, 2006). *Fusarium oxysporum f.* sp. *lycopersici* (Sacc.) is economically important wilting pathogen of tomato (Hadian *et al.*, 2011). Losses of up to 80% have been observed in tomato due to *Fusarium* wilt. Attempts to control *Fusarium* wilt have experienced limited success. The difficulty in the control is due to emergence of new pathogenic races (Juliano *et al.*, 2005), death

of biocontrol organisms as a result of continuous use of fungicides (Dewaard *et al.,* 1993) and lack of enough fields for long-term crop rotation. *Fusarium oxysporum f.* sp. *lycopersici* is a soil inhabitant and attacks plants through the roots. This study investigated the use of organic soil amendment and fungicides/biopesticides singly and in combination in the management of *Fusarium* wilt in tomato.

1.2 Statement of problem

A major constraint of tomato production in Kirinyaga District is *Fusarium* wilt that causes wilting and eventual death of the affected plants. Majority of the farmers however attempt to control the disease but with limited success. For example, farmers who reported to have been using chemicals in control of wilting also reported wilting problems on their fields. The methods used by these farmers also have financial implications on the farmers.

1.3 Justification

Kirinyaga District is on of the major tomato producing zones supplying Nairobi region with the produce (Kirimi Sindi website, 2011; Kavoi *et al.*, 2004). In the district one of the constraints of tomato production is *Fusarium* wilt caused by *Fusarium oxysporum f.* sp. *lycopersici*. Over the years, farmers have over depended on chemicals for both pest and disease control and these have damaging effects on the natural environment, the agro-ecosystem and to human beings. Use of chemicals has led to build-up of phytotoxicity in the soil. Although integrated approach has been tried, one involving the use of neem, organic matter and fungicides has not been attempted in the study area. This study therefore investigated the use of organic soil amendments and fungicides/bio-pesticides singly and in combination in management of *Fusarium* wilt in tomato.

1.4 Hypotheses

1. Tomatoes grown by farmers in Kirinyaga District do not differ in terms of the variety type.
2. There is no significant difference in the incidence of *Fusarium* wilt in the tomato varieties grown in Kirinyaga District
3. Methods used by farmers in Kirinyaga District to control tomato *Fusarium* wilt do not differ.
4. Isolates of *F. o. l.* from different locations of Kirinyaga District do not differ in their pathogenicity against various tomato varieties grown in Kirinyaga District.
5. Efficacies of Fungicides, Neem extract and Organic matter separately and in combination in control of *Fusarium* wilt of tomato do not differ.

1.5 Objectives

1.5.1 General objective

To effectively manage *Fusarium oxysporum f.* sp. *lycopersici* by integrating fungicides, organic matter and Neem extract for better tomato plant performance.

1.5.2 Specific objectives

1. To document the common tomato varieties grown by farmers in Kirinyaga District.
2. To determine the incidence of *Fusarium* wilt on tomato varieties grown in Kirinyaga District.
3. To document the management strategies against *Fusarium oxysporum f.* sp. *lycopersici* in Kirinyaga District to control *Fusarium* wilt.
4. To determine the pathogenicity of various fungal isolates of *Fusarium oxysporum f.* sp.

lycopersici against various tomato varieties grown in Kirinyaga District

5. To compare the efficacies of Fungicides, Neem extract and Organic matter separately and in combination in management of *Fusarium* wilt of tomatoes.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Fusarium* wilt of tomatoes

Fusarium wilt of tomato caused by *Fusarium oxysporum f.* sp. *lycopersici* was first described in 1985 (Pamela, 1998). The *Fusarium* fungus is present in all important tomato growing regions of the world (Mohammad, 1990). *Fusarium oxysporum f.* sp. *lycopersici* is placed in the order Hyphales and the genus *Fusarium*. The fungus produces three types of asexual spores; microconidia, macroconidia and chlamydospores (Agrios, 1988). In solid culture medium such as potato dextrose agar (PDA), the different isolates of *Fusarium oxysporum* have varying appearances. In general, the aerial mycelium first appears white and then changes to a variety of colours ranging from violet to dark purple depending on the strain. A culture with sporodochia appears cream or orange in colour (Smith *et al.*, 1988). Symptoms of attack first appear as slight vein clearing on the outer portion of the young leaves followed by epinasty of the older leaves (Sally *et al.*, 2006). At the seedling stage, plants may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasy are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves and finally death of the entire plant (Ann, 2002; Agrios, 1988). Browning of the vascular tissue is a strong evidence of the *Fusarium* wilt.

2.2 Disease dissemination

Most *Fusarium oxysporum* exist as saprophyte in soil and organic matter but some are plant pathogenic (Smith *et al.*, 1988). *Fusarium* enters the plant through root tips (Sally *et al.*, 2006; Agrios, 1988). The mycelium grows in the xylem vessels where they cut off water supply

resulting to wilting (Stephen *et al.*, 2003). There is often an association of *Fusarium* wilt and nematode colonization where the nematodes provide potential entry route for the fungus. Infection and disease development in *Fusarium* wilt are favoured by warm soil temperature and low soil moisture (K-state, 2004; Lewis, 2003). *F. oxysporum* occurs, survives, and grows in soils of all types, but sandy soils provide conditions that are most favourable for growth and development (Lowell, 2001; Everett, 1923). *Fusarium* wilt tends to be most severe in sandy soils and generally less of a problem in heavier clay soils. Natural suppression of *Fusarium* wilt disease is known to occur in many soils (Larkin *et al.*, 2002). *Fusarium oxysporum* is primarily spread over short distances by irrigation water and contaminated farm equipment (Stephen *et al.*, 2003). The fungus can also be spread over long distances either in infected transplants or soil (Agrios, 1988). It is also possible that the spores are spread by wind. *Fusarium oxysporum* persist in soil for a long period as chlamydospores in plant residues. Chlamydospore germination is stimulated by host root excretions or contact with pieces of non-colonized plant remains. The fungus can also survive in the fibrous root system of many plants including common weeds such as species of crab grass, mallow and pigweed (Mohammad, 1990).

2.3 Control of *Fusarium* wilt

The control of *Fusarium* wilt of tomatoes is important in maintaining plant vigour and fruit quality and quantity. Documented methods that are used in the control of the disease include cultural, biological, use of resistance, chemical (Pottorf, 2006; Agrios, 1988) and use of natural products (Kimaru *et al.*, 2004). However, each method has got its own strengths and limitations.

2.3.1 Biological control

According to Momol *et al.* (2003), several isolates of nonpathogenic *Fusarium spp* (*F. oxysporum* and *F. solani*) that effectively controlled *Fusarium* wilt in greenhouse tests have been identified. The isolates include CS-20, CS-1, CS-24 and Fo 47 of which was consistently effective when applied at high rate. The mechanism of action of biocontrol by two of these isolates (CS-1 and CS-20) involved induced systemic resistance, and that these isolates effectively reduced disease incidence at low biocontrol inoculum densities and high pathogen densities (Larkin *et al.*, 2002). These results indicated a high potential for development of these isolates as biocontrol agents. Application of a combination of fungus *Gliocladium virens* and bacterium *Burkholderia cepaci, Pseudomonas fluorescens* and *Trichoderma hamatum* significantly reduced disease incidence and significantly increased yield as measured by total weight and number of fruits per plant (Larkin *et al.*, 1998). Tests done by Attitala *et al.* (2001) showed that, after spraying with zoospores of *Phytopthora cryptogea* followed by *Fusarium oxysporum f.* sp. *lycopersici* inoculation, tomato plants show no wilt disease. According to studies done by Akköprü and Demir (2005), arbuscular mycorrhizal fungi (AMF), *G. intraradices*, and some Gram-negative and fluorescent rhizobacteria (RB), *P. fluorescens*, *P. putida* and *Enterobacter cloaceae*, isolated from the rhizoplane of solanaceous plants were effective against the important soil-borne pathogen of tomato, *F. o. l.* Both biocontrol agents (either AMF or RB), which are important members of the rhizosphere population, are very efficient and successful in the inhibition of root rot diseases. According to Monda (2002), bacterial biocontrol agents with promising biocontrol activities against *Fusarium oxysporum f.* sp. *lycopersici* include *Pseudomonas fluorescens, P. Putida, P. chlororaphis, Bacillus subtilis, Streptomyces pulcher, S. corchorusii* and *S. mutabilis*.

2.3.2 Good farm management

Improving the vigour of a plant often helps to increase resistance to pathogen attack (Agrios, 1988). Appropriate choice of fertilizer leads to change in soil pH which may lead to unfavourable conditions for development of the pathogen. Raising soil pH between 6 - 7.5 and fertilizing with nitrogenous fertilizer controlled *Fusarium* wilt of tomato, chrysanthemum and other crops (Kimaru, 1998). Crop rotation with non-solanaceous crops for three years is usually recommended to avoid pest problems common to this group of vegetables. The crop should be rotated with grasses and cereals whenever possible (Sally *et al.,* 2006). The limitation of rotation is that most farmers have small field sizes therefore cannot afford to practice long-term crop rotation. According to studies done in Massachusetts and Oklahoma, staking reduces disease incidence (Hazzard *et al.*, 1996; Patterson, 1990). The more promising control measure of *Fusarium* wilt disease is the use of soil amendments which lead to the reduction or elimination of inoculum of a pathogen in the soil (Owino *et al.*, 1995). Efficacy of soil amendments against soil borne pathogen is attributed to enhanced host nutrition, suppression of common soil borne pests by healthy soils (Jeff, 2009) and heat produced during decomposition (Kimaru *et al.*, 2004). Decomposed organic materials have long been recommended in most agricultural and horticultural systems using organic farming methods and they offer possible control strategy for soil borne diseases (Kimaru *et al.*, 2004). Indications of systemic composts that are disease suppressive have been reported for several vegetables (Logsdon, 1995).

2.3.3 Use of resistance

The most cost-effective and environmentally safe method of control is the use of resistant cultivars whenever they are available. The use of resistant varieties is the best strategy for

disease control (Sheu *et al.,* 2006). According to Pritesh *et al.* (2011), identification and utilization of tomato plant varieties resistant to the disease represents a valid alternative to the use of chemicals. However, breeding for resistance can be very difficult when no dominant gene is known. In addition, new races of pathogens overcoming host resistance can develop (Akköprü *et al.*, 2005; Momol *et al.*, 2003). The advantages of this method include saving the cost of chemical for control of the disease and enhancing cultivation of previously infested fields. In Kenya, some resistant varieties have been introduced and include rutger and marglobe (Larkin *et al.*, 1998).

2.3.4 Chemical control

Agricultural chemicals are commonly used for management of pests and diseases. However, the high frequency of chemical use, non-target effects, development of resistance to many chemicals, pathogens which remain viable for many years and risks to human health and the surrounding environment have stimulated development of alternative methods for disease management. Moreover, pesticides are not available for some diseases, and pesticides generally are more effective against aerial plant pathogens than their soil-borne counterparts (Recycled Organics Unit, 2006). It is also technically difficult to treat large amount of soil, and the range of approved chemicals is declining as active compounds are withdrawn for toxicological and environmental reasons, for example, methyl bromide has been phased out due to its extremely high ozone depleting potential. The current trend to near zero-market tolerance for pesticide residues in fresh leafy vegetables provides an additional motivation to search for non-chemical means to control pests and diseases (Reuveni *et al.*, 2002). *Fusarium* wilt is controlled by disinfecting the soil with methyl bromide, chloropicrin or metham sodium. Systemic fungicides

such as benomyl, thiabendazole and thiophanate have been used to control *Fusarium* wilt. However, sustainable use of fungicides in *Fusarium* wilt management is difficult due to development of resistant isolates and damaging effects on the natural environment, the agro ecosystem and human beings (Pritesh *et al.*, 2011; Dewaard *et al.*, 1993). Excessive use of chemicals results into buildup of phytotoxicity in the soil (Blancard, 1993).

2.3.5 Uses of neem extract

The neem tree belongs to the order sapindales and family meliaceae. The neem tree is a tall, evergreen, hardy fast growing ornamental shade tree. The tree can reach a height of 15 -20 M and 2 – 5 M girth with deep green foliage and masses of honey scented flowers (Jacobson, 1989). Several products are manufactured from neem. These include neem oil, soaps, cosmetics, lubricant, fertilizers and pesticides like neemix, azatin and turplex. The fruit, seed, kernels, twigs, bark and roots have been shown to possess some compounds that are nematicidal, fungicidal and insecticidal (Randhawa and Parma, 1993). Neem extracts possess antimicrobial activity with notable effects on some fungal pathogens (Conventry *et al.*, 2001). Significant levels of control of downy mildew of grape caused by *Plasmopora viticola* have been reported as a protective treatment. Several rust pathogens like wheat rust and bean rust have been reported to be controlled using foliar applications of neem seed oil. Foliar application of aqueous leaf extract inhibited foliar pathogens *Phaeoisariopsis personata* and *Puccinia arachidis* on peanut (Ghewande, 1989). Tests done by Kimaru *et al.*, 2004 revealed that the neem cake powder contains ingredients that have fungistatic effects against *Fusarium* wilt of tomatoes. Similar findings were also reported by Coventry *et al.*, 2001.

2.3.6 Integrated pest management

Integrated Pest Management (IPM) is recognized as an effective approach for increasing agricultural productivity and combating environmental degradation in developing countries (Waiganjo *et al.,* 2006). The Integrated Pest Management seeks to improve the productivity of high–value marketed horticultural crops in the East African region (Kenya, Uganda and Tanzania) (Waiganjo *et al.,* 2006). Practices that can help to build healthy soils include crop rotation, organic matter additions or using high-residue tillage implements. A significant amount of research has been conducted on the suppression of pests and diseases through the application of compost products worldwide. The results have shown that composts can provide natural biological control of soil borne diseases affecting collar and roots as well as plant foliage (Recycled Organics Unit, 2006). The inclusion of green manures and cover crops in a rotation is an excellent way to sponsor fertility, suppress weeds and provide a break in pest cycles (Jeff, 2009). Incorporating several different species of crops in a rotation, along with manures and/or compost, ensures a diversity of organic matter sources. This diversity leads to a more minerally balanced soil and a pool of nutrients which become available slowly over time, reducing leaching, waste and toxicity that can result from immediately-available inorganic fertilizer additions (Jeff, 2009; Recycled Organics Unit, 2006). Ultimately, managing for good soil fertility is extremely important because the soil environment and the surrounding air environment are in reality virtually inseparable, and the establishment of a functional and stable system in one environment can have far-reaching impacts in the other.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study area

Kirinyaga District is one of the seven districts in Central Province of Kenya. It is a high potential area with annual average rainfall ranging from 800 - 2200 mm (Figure 3.2). In most of the areas, the soils are deep and moderate to highly fertile (Figure 3.3). The average annual temperature range is 9.7-21.6°C (Table 3.1). The District covers an area of about 1479 sq. Km. It has a total area of about 112,700 hectares with 95,500 hectares (85%) under agriculture (Jaetzold, 1983). There are two permanent rivers, namely Thiba and Nyamindi, which facilitate the growing of rice and horticultural crops in the lower parts of the District. Among the most important horticultural crops are tomato, French bean, onions, banana, mango, pawpaw and avocado (KARI, 2005). The district is one of the most densely populated in Kenya with a population density of 354 persons/ km^2 and a total population of 528,054 persons (CBS-2009 census data). The total number of households was 154,220 by the year 2007 (CBS-2009 census data). The District has five divisions including Mwea, Ndia, Kirinyaga Central, Gichugu and Mt. Kenya Forest (Waiganjo *et al.,* 2006) (Figure 3.1). The District is divided into Tropical-alpine (TA), Upper Highland (UH), Lower Highland (LH), Upper Midland (UM) and Lower Midland (LM) Agro-ecological Zones as shown in Figure 3.4.

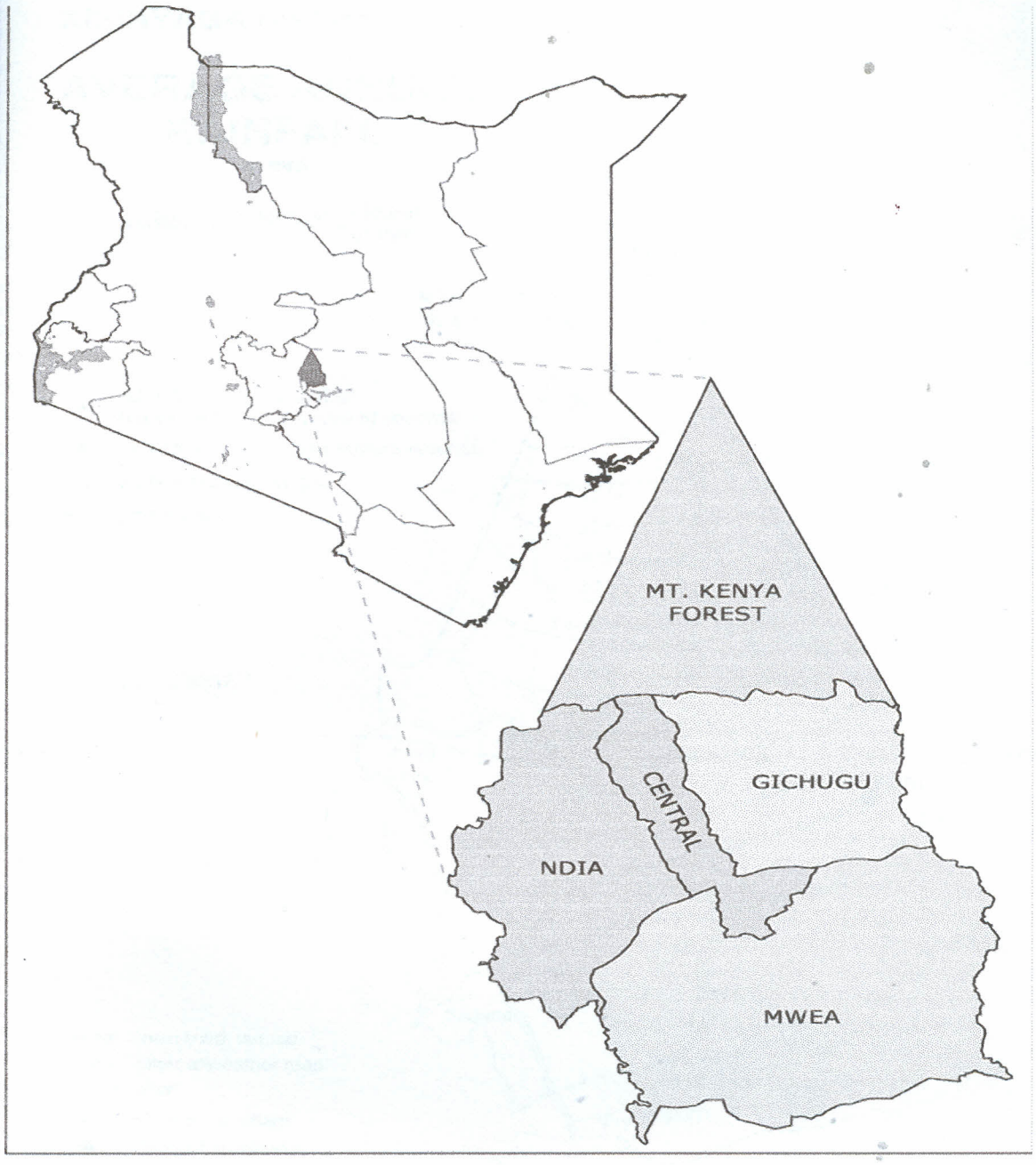

Figure 3.1: Kirinyaga District, Kenya and the Administrative Divisions

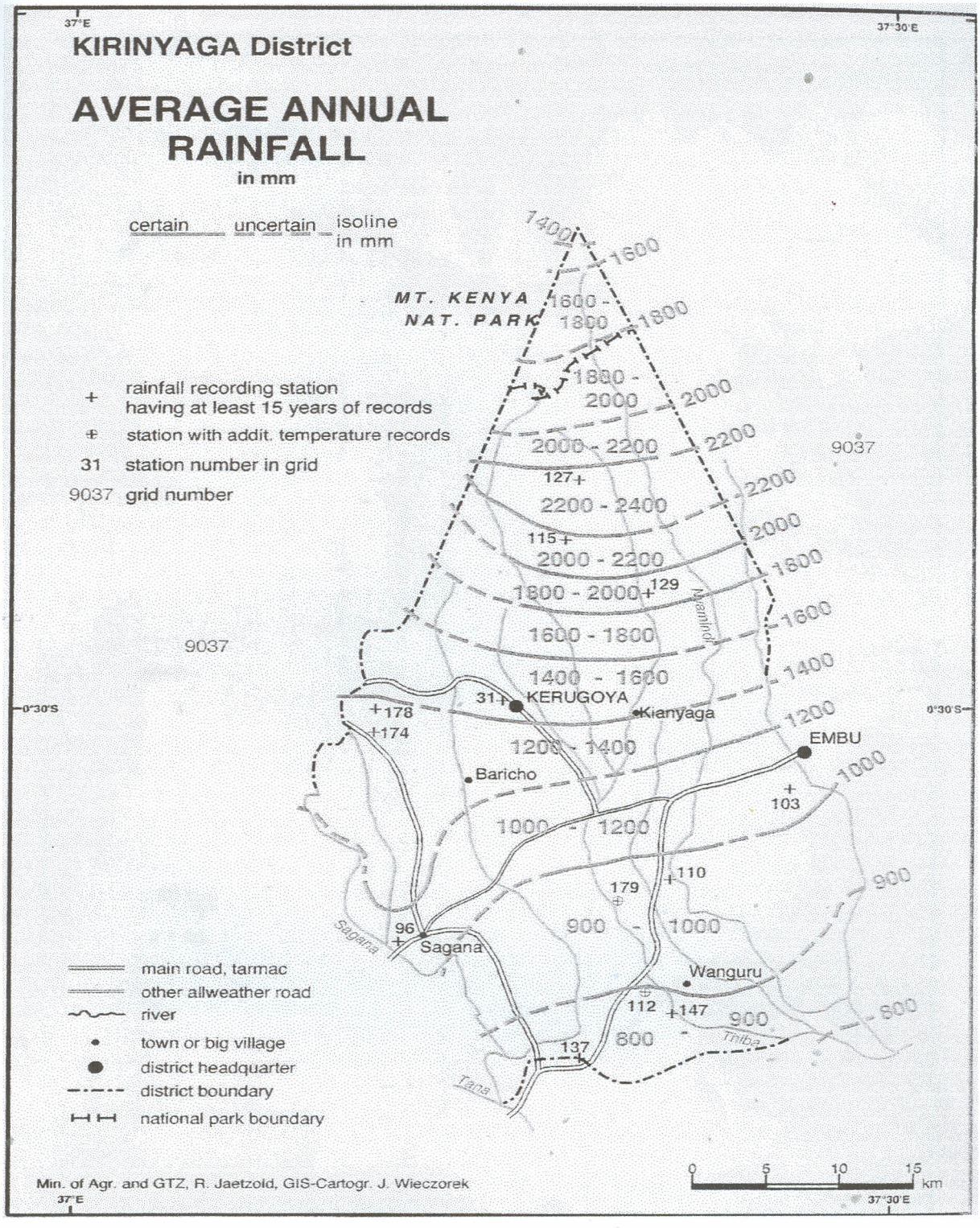

Figure 3.2: Average annual rainfall for Kirinyaga District

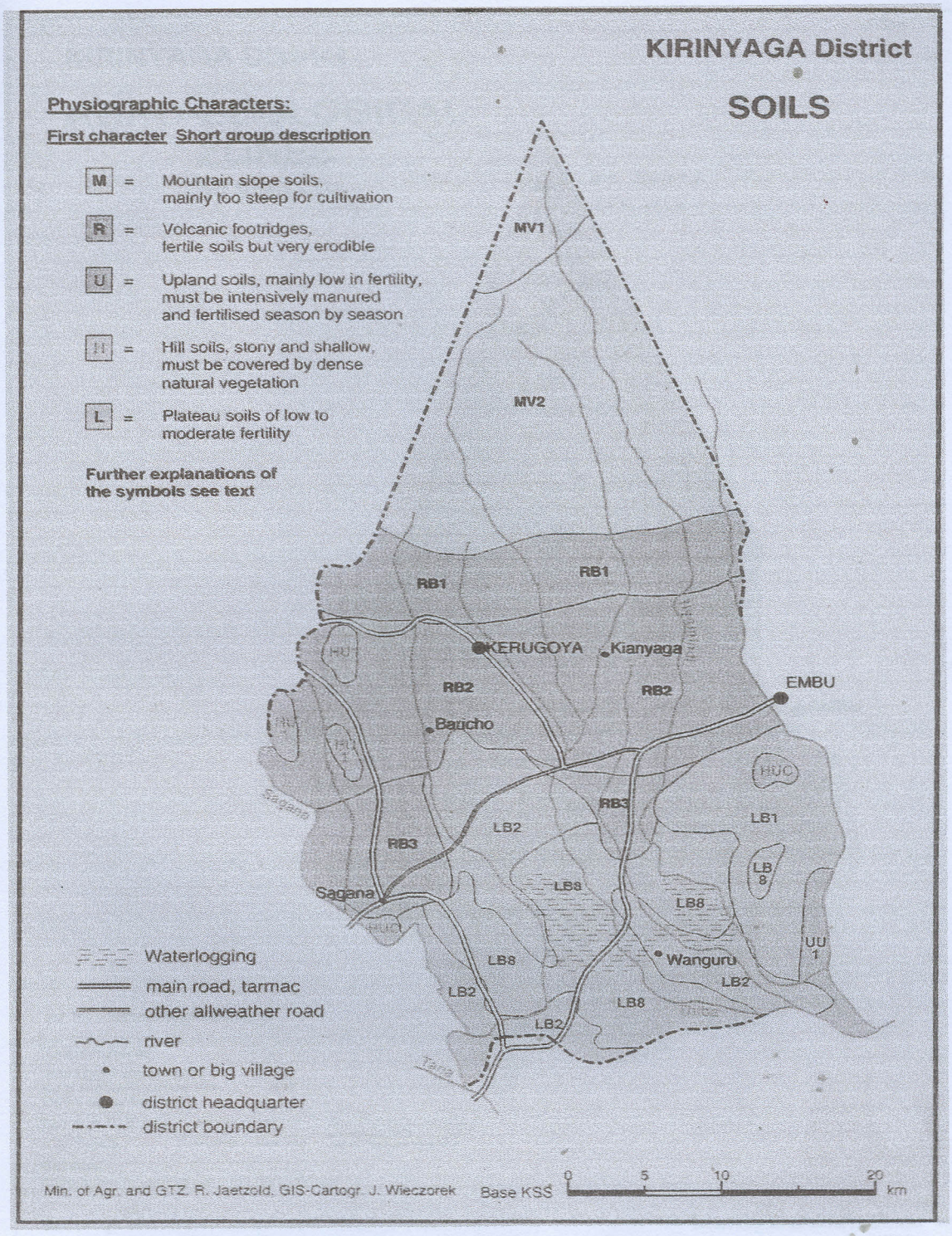

Figure 3.3: Kirinyaga District soils

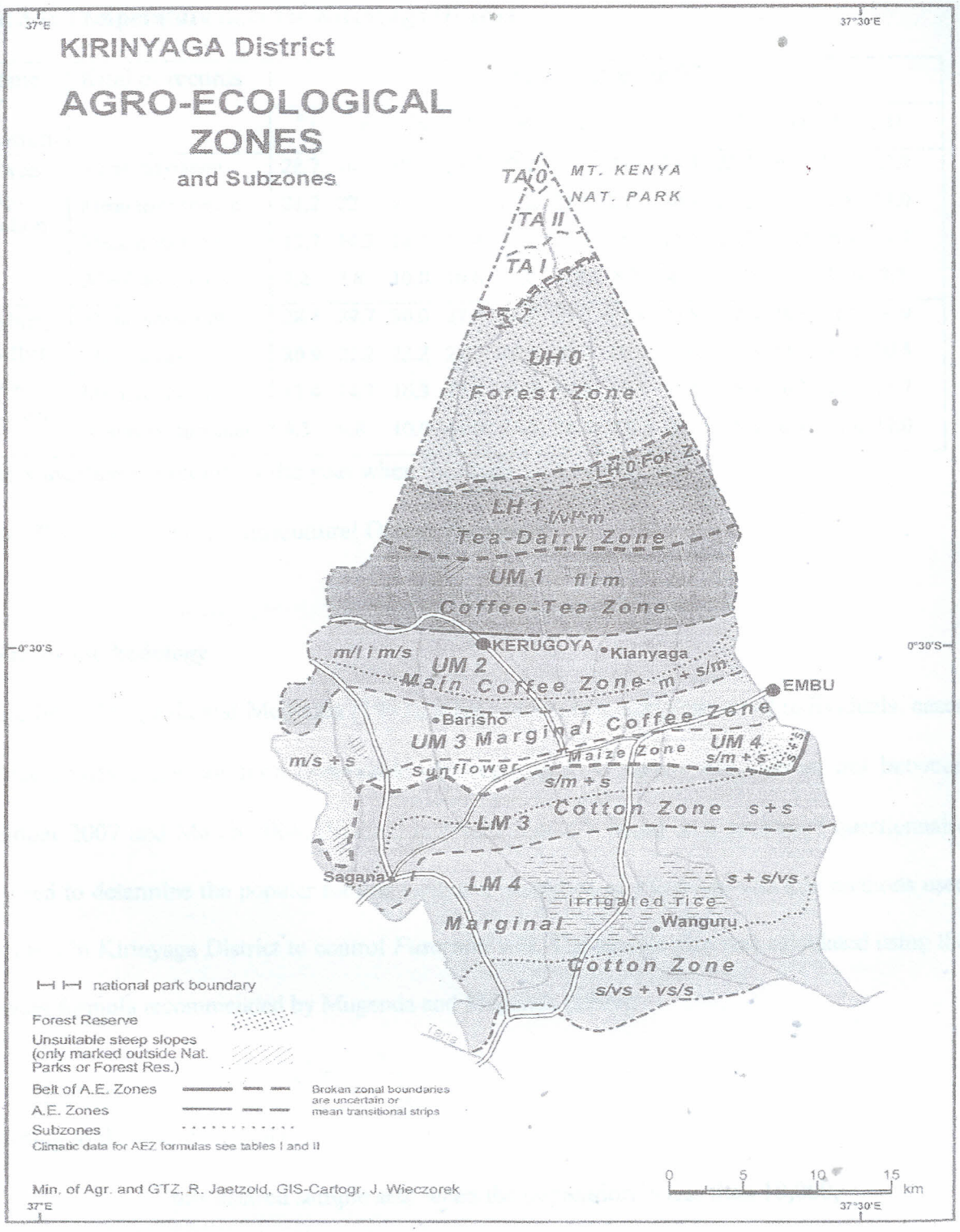

Figure 3.4: Agro-Ecological Zones (AEZ) of Kirinyaga District

Table 3.1: Temperature data for Kirinyaga District

Name of Station	Kind of records	Temperature in °C											
		*J	F	M	A	M	J	J	A	S	O	N	D
Mwea Exp. Station	Mean maximum	28.7	30.3	30.4	28.6	27.2	26.4	25.1	25.4	28.2	29.5	27.5	27.2
	Mean temperature	21.2	22.3	23.4	23.0	22.2	21.0	20.2	20.3	22.0	23.2	22.0	21.0
	Mean minimum	13.7	14.3	16.3	17.4	17.2	15.6	15.2	15.2	15.8	16.9	16.4	14.7
	Absolute minimum	7.2	7.8	10.0	10.6	12.0	9.9	8.3	8.9	8.9	8.9	10.0	9.3
Tebere Cotton Res. Station	Mean maximum	28.4	29.7	30.0	27.9	26.8	25.5	24.3	24.9	27.5	28.8	26.9	26.9
	Mean temperature	20.9	22.2	23.2	22.7	21.9	20.7	19.8	20.0	21.5	22.6	21.5	20.8
	Mean minimum	13.4	14.7	16.3	17.4	16.9	15.8	15.3	15.1	15.5	16.3	16.1	14.7
	Absolute minimum	9.3	9.8	10.0	14.0	13.0	10.5	9.0	9.5	10.5	8.0	11.0	11.0

*Letters indicate the month of the year when the study was carried out

Source: Kirinyaga District Agricultural Office, Kerugoya

3.2 Survey methodology

According to Mugenda and Mugenda (1999), a population is a complete set of individuals, cases or objects with some common observable characteristics. A survey was carried out between December 2007 and March 2008. Personal interview methods based on a structured questionnaire were used to determine the popular tomato cultivars, incidence of *Fusarium* wilt and methods used by farmers in Kirinyaga District to control *Fusarium* wilt. The sample size was calculated using the following formula recommended by Mugenda and Mugenda (1999);

$$nf = \frac{n}{1 - \frac{n}{N}}$$

According to the above formula,

nf= desired sample size when the population is less than 10,000,

n= desired sample when the population is more than 10,000 (384)

N= estimate of the population size.

The survey targeted 150 farmers who had the actual crop in the field, this constituting at least the minimum requirement of 10% sample, out of the accessible population (Mugenda and Mugenda, 1999). However, due to financial constraint the sample size of 114 farmers was interviewed with assistance from extension officers from KARI, Embu.

3.3 Preparation of neem extract

Neem kernel cake powder (NKCP) was obtained from Wonder Herb Products, Nairobi. Distilled water was used to extract the active ingredients from different amounts of NKCP. Amounts of NKCP soaked in water were 12, 20, 28, 36 and 40 grams per litre. The amounts per litre were weighed and soaked overnight in 200 ml of distilled water, strained and sterilized using membrane filter (200 µm). The extract was used to amend Potato Dextrose Agar (PDA).

3.4 PDA amendment

Thirty nine grams of PDA powder were dissolved in 800 ml distilled water and sterilized in the autoclave. The molten PDA was mixed with 200 ml of known concentration of NKCP water extract and poured in Petri dishes. The concentrations of NKCP were 12, 20, 28, 36 and 40 g/l. Measurements of 0.35 g of each of the Fungicides (Ridomil MZ, Milraz and Ortiva) were put in separate conical flasks containing 800 ml of molten PDA, making a concentration which was within the range of concentrations tested by Jash *et al.*, 2004. The PDA and the fungicides were mixed thoroughly and left on the bench for 30 minutes to cool.

3.5 Soil sterilization

Soil for greenhouse tests was sterilized at Kenyatta University. Sterilization was carried by autoclaving the soil sample at 121 °C for three hours.

3.6 Soil infestation

Soil used in greenhouse tests was artificially infested with *Fusarium oxysporum f.* sp. *lycopersici.* Disks (6 mm diameter) of PDA taken from 10 days old culture of *Fusarium oxysporum f.* sp. *lycopersici* using a cork borer were placed in the soil (two disks per planting pot). The infested soil was thoroughly mixed with the inoculum.

3.7 Pathogenicity of *F. o. l.* isolates from different locations of Kirinyaga District

3.7.1 Isolation of *F. o. l.* from different locations of Kirinyaga District

Fusarium oxysporum f. sp. *lycopersici* was isolated from infected tomato plants (*Lycopersicon esculentum*, Mill) collected from farmers' fields in Kirinyaga District. Stems of infected plants were randomly picked from the farms and used for isolation of *F. o. l.* The stems were washed in distilled water to remove any foreign materials. Five-millimetre pieces were cut, dropped in 70% alcohol and then sterilized in 0.5 % sodium hypochlorite solution for three minutes. The pieces were then rinsed in sterile distilled water three times and blot dried. The dried pieces were picked using forceps and placed on the surface of PDA media in Petri dishes. The fungus was allowed to grow for seven days then sub cultured. The pure culture was incubated at 24 °C for 10 days. *Fusarium* species was identified on the basis of morphological characteristics (Nelson *et al.*, 1983).

3.7.2 Susceptibility of tomato cultivars grown in Kirinyaga District to *Fusarium* wilt

Greenhouse tests were carried out to compare the pathogenicity of five isolates from different regions on three most popular tomato cultivars (Rio Grande, Onyx and Cal J) in Kirinyaga District. Test plants were grown in pots containing sterilized soil artificially infested with

Fusarium oxysporum f. sp. *lycopersici* as described in section 3.6. Each pot had five plants and the treatments were replicated five times. Control pots were not inoculated. Disease assessment based on disease incidence, disease severity, plant height and basal diameters was done on 30th, 60th and 80th days after transplanting. Disease incidence was calculated using the following formula recommended by Masood *et al.*, 2010;

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Disease severity was based on the percentage of leaves yellowed and wilted and calculated using the following formula (Anitha *et al.*, 2010; Amini *et al.*, 2010; Masood *et al.*, 2010);

$$\text{Disease severity} = \frac{\text{Proportion of leaves with symptom}}{\text{The total number of leaves}} \times 100$$

3.8 Efficacy of fungicides, neem extract and organic matter in management of *Fusarium* Wilt of tomatoes

3.8.1 *In vitro* experiment 1: Effect of neem extract on mycelial growth of *F. o. l.*

One 5 mm diameter mycelial plug of a 10 day old culture of *F. o. l.* was transferred aseptically to the centre of PDA in Petri dishes amended with 12, 20, 28, 36 and 40g/l of NKCP. Control Petri dishes were not treated. The Petri dishes were incubated at room temperature and the diameter of the fungus measured at two, four, six, eight and 10 days after inoculation. The effect of neem extract was determined by calculating the inhibition percentage as follows (Kawsar *et al.*, 2008, Biratu *et al.*, 1990);

$$\text{Inhibition \%} = \frac{C-T}{C-M} X 100$$

Where C = Diameter (mm) of fungal mycelium on control Petri dishes

T = Diameter (mm) of fungal mycelium on Petri dishes treated with neem extract

M = Initial diameter (mm) of mycelial plug

Five replicates per treatment were used and arranged in a random complete block design.

3.8.2 *In vitro* experiment 2: Effects of three Fungicides (Ridomil MZ, Milraz and Ortiva) on mycelial growth of *F. o. l.*

PDA (18 ml) amended with three Fungicides (Ridomil MZ, Milraz and Ortiva) as detailed in section 3.4 was dispensed into sterile Petri dishes. One mycelial plug from the periphery of 10 days old fungal culture was aseptically transferred onto the centre of each Petri dish containing different Fungicide/PDA mixture. Measurement and calculation was done as in section 3.3.

3.8.3 *In vitro* experiment 3: Effects of a combination of neem extract and Fungicide (Ridomil) on mycelial growth of *F. o. l.*

This experiment tested the effect of synergism of the most efficacious Fungicide from the *in vitro* test 2 and the best concentration of NKCP obtained in the *in vitro* test 1. The Fungicide and NCKP were used in combinations as follows:

$Fungicide^{50} + NKCP^{50}$

$Fungicide^{75} + NKCP^{25}$

$Fungicide^{25} + NKCP^{75}$

The superscripts 25, 50 and 75 indicate 25%, 50% and 75% of the recommended rates of application of fungicide and NKCP respectively as used in the treatments. The combinations were incorporated into the PDA as in *in vitro* test 1. One 5 mm diameter mycelial plug from the periphery of a 10 day old fungal culture was transferred aseptically to the centre of the PDA in a Petri dish. Each treatment was replicated five times in a random complete block design. Inhibition percentage was determined as in *in vitro* test 1.

3.8.4 Greenhouse experiment 1: Effect of NKCP on *Fusarium* wilt caused by *F. o. l.* in tomatoes

The tests were carried out at Kenyatta University to investigate the effect of NKCP on development of *Fusarium* wilt in tomatoes. Eight grams of NPK fertilizer (20:10:10) were thoroughly mixed with soil in each of the planting pots. NKCP was incorporated into the planting pots at the rate of three, five, seven, nine and ten grams per planting pot. Sterilized soil in the planting pots was artificially infested with *F .o. l.* Control planting pots were not treated with NKCP. A complete randomized block design with five replicates was used. Disease assessment was based on percentage incidence, disease severity, shoot length and basal stem diameters. Disease incidence and severity were determined at 30, 60 and 80 days after planting. Shoot length was taken from the first basal node to the tip of the youngest apical bud on the 30^{th}, 60^{th} and 80^{th} days after planting. Stem diameter was taken just below the first basal node in centimetres at the same time the shoot length was taken. This experiment determined the best application rate of NKCP in the soil.

3.8.5 Greenhouse experiment 2: Effect of combinations of NKCP, Organic matter and Fungicides on *Fusarium* wilt of tomatoes

Fungicides used this experiment were applied as per the manufacturer's recommendations. Neem cake powder was applied at a rate of 10 g per planting pot, the rate established in Section 3.8.4 as the most efficacious. Organic matter used for this experimental work was goat manure. Hundred grammes of goat manure was put per planting pot. This test determined the effect of Organic matter, NKCP and Fungicides on *Fusarium* wilt of tomatoes. The most efficacious fungicide identified in *in vitro* test 2 was used and applied as per manufacturers' recommendations. NKCP

was applied in the soil at the best rate determined in greenhouse experiment 1. Soil was artificially infested with *F. o. l.* before transplanting. Seven treatments were made as follows:

(i) Fungicide only

(ii) Fungicide and NKCP

(iii) Fungicide and organic matter

(iv) Organic matter and NKCP

(V) Organic matter only.

(Vi) NKCP only

(Vii) NKCP, Organic matter and Fungicides.

Each of the treatment was replicated five times and arranged in a random complete block design. Test plants grown in uninoculated soil with none of the amendments served as controls. Fungicides were applied as per manufactures' recommendations. Disease assessment was carried out as described for greenhouse experiment 1.

3.9 Re-isolation of *F. oxysporum f.* sp. *lycopersici* from the stem

Re-isolation of *F. o. l.* was done from tomato plants previously inoculated with the fungus in Greenhouse Experiment 2. The stem from which re-isolation was done were cut into five millimetre pieces, sterilized in 0.5% sodium hypochlorite solution for 30 seconds, washed in three changes of distilled water and dried between filter papers. The pieces were placed on potato dextrose agar (PDA) in Petri dishes and incubated for 7 days. Identification of *Fusarium oxysporum f.* sp. *lycopersici* was done on the basis of morphological characteristics (Nelson *et al.*, 1983).

3.10 Data analysis

Research findings were analysed and results presented using line graphs and tables. Social findings on the *Fusarium* wilt management were analysed using Statistical Package for Social Sciences (SPSS) while laboratory and greenhouse findings were analysed using MINITAB statistical package. Analysis of Variance (ANOVA) was used to analyse disease incidence, severity, plant heights and plant basal diameters against treatments in greenhouse tests. Means were sepersted using the Tukey's test.

CHAPTER FOUR

RESULTS

4.1 Tomato varieties grown by farmers in Kirinyaga District

The study established that the tomato varieties grown in Kirinyaga District are mainly, Rio Grande, Onyx and Cal J (Table 4.1). The most popular variety was Rio Grande, grown by 55% (n=63) of the farmers, while Cal J grown by 13% (n=15) of the farmers interviewed was the least popular variety. The least number of farmers (4%, n=4) grew mixed varieties (Table 4.1). Farmers' preference for different tomato varieties was based on marketability, productivity, postharvest keeping quality, early maturity and disease resistance (Table 4.2). Most farmers preferred Rio Grande for marketability (40), high production (54), postharvest keeping quality (12), early maturity (31) and low production cost (11). Cal J was the least preferred variety for marketability (13), disease resistance (1) and low cost of production (2). The mixed varieties were the least preferred for all the targeted parameters (Table 4.2).

Table 4.1: Tomato varieties grown by the sampled farmers in Kirinyaga District

Tomato variety grown by the farmer	Number of farmers N = 114	Percentage 100%
Cal J	15	13
Onyx	32	28
Rio Grande	63	55
Mixed varieties	4	4

Table 4.2: Number of farmers who prefer to grow tomato varieties and the reasons for preference

Tomato varieties	Reasons for preference of the tomato varieties					
	Marketability	High production	Keeping quality	Disease resistance	Early maturity	Low production cost
Cal J	11	13	8	1	4	2
Onyx	26	29	1	28	2	5
Rio Grande	40	54	12	20	31	11
Mixed varieties	3	0	0	1	0	2

4.2 Tomato disease problems encountered by farmers in Kirinyaga District

Majority of farmers sampled in Kirinyaga District mainly cited wilting as the major symptom (74.6%; n=85). Other disease symptoms cited were stunted growth (7.0%; n=8), yellowing of leaves (3.5%; n=4) and falling of leaves (10.5%; n=12) (Table 4.3). Majority of the farmers who reported wilting problems grow Rio Grande (n=46). Other production constraints were reported by 4.4% (n=5) of the farmers (Table 4.3).

Table 4.3: Common disease symptoms experienced by the farmers growing particular tomato varieties

Variety grown by the farmer	Disease symptoms experienced by the sampled farmers				
	Wilting	Stunted growth	Yellowing of leaves	Falling of leaves	Others
Cal J	11	0	0	4	0
Onyx	24	3	0	4	1
Rio Grande	46	5	4	4	4
Mixed variety	4	0	0	0	0
Total number of farmers	**85**	**8**	**4**	**12**	**5**
Percentage number of farmers	**74.6**	**7.0**	**3.5**	**10.5**	**4.4**

4.3 Methods used by farmers to control tomato *Fusarium* wilt in Kirinyaga District

The study established that majority of the farmers attempted to control *Fusarium* wilt. Control methods were mainly chemical used by 87.7% (n=100), crop rotation used by 86.0% (n=98), applying ash in planting used by 61.4% (n=70) and roguing used by 65.8% (n=75) of the farmers sampled (Table 4.4). However, a smaller percentage (13.1%; n=15) of the sampled farmers did not use any measures to control *Fusarium* wilt (Table 4.4).

Table 4. 4: Methods used by sampled farmers to control tomato *Fusarium* wilt

Method of control	Number of farmers	Percentage of farmers
Chemical control	100	87.7
Crop rotation	98	86.0
Applying ash	70	61.4
Roguing	75	65.8
No control	15	13.1

4.4 Susceptibility of Tomato cultivars grown in Kirinyaga District to tomato *Fusarium* Wilt

4.4.1 Disease incidences

The five isolates used had different infection abilities on tomato varieties. There was no significant difference ($P \leq 0.05$) in disease incidences on Onyx and Cal J after inoculation with isolate 5 and isolate 6 (Table 4.5). However, isolate 6 was more virulent on Cal J (85.00%) than on Onyx (62.50%) and Rio Grande (36.00%) (Table 4.5). All the isolates tested had higher disease incidences on Cal J in comparison to Rio Grande and Onyx. Rio Grande recorded the lowest disease incidences after inoculation with the five isolates (Table 4.5). This was significantly lower ($P \leq 0.05$) than in Cal J after inoculation with the five isolates apart from isolate 8. Disease incidences on Onyx and Cal J were not significantly different ($P \leq 0.05$) for isolate 5, 6, 8 and 9 but significantly different ($P \leq 0.05$) for isolate 11 (Table 4.5). However, Cal J recorded higher disease incidences than Onyx for all isolates tested. The lowest disease incidences were recorded after inoculation with isolate 8. These were significantly lower ($P \leq 0.05$) than disease incidences after inoculation with isolate 6 on Cal J (Table 4.5).

Table 4.5: Mean percentage disease incidence per tomato variety recorded 60 days after treatment with *F. o. l.* Isolates

Isolates	[3]Mean percentage disease incidence per tomato variety		
	[2]Rio Grande	Onyx	Cal J
5	15.00[1]d	57.50b	72.50ab
6	36.00c	62.50ab	85.00a
8	7.50d	24.50c	40.50bcd
9	28.75cd	43.25bc	65.00ab
11	18.75cd	30.00cd	72.50ab
Control	0.00e	0.00e	0.00e

[1]Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

[2]Tomato cultivars used in the study

[3]Mean percentage disease incidences per tomato variety after inoculation with *F. o. l.* isolates

4.4.2 Disease severity

Disease severity on individual tomato varieties showed that *Fusarium* wilt was more severe in Cal J than in Onyx and Rio Grande (Table 4.6). There was no significant difference ($P \leq 0.05$) in disease severity as a result of infection with isolate 5 and 6. Isolate 8 caused the lowest disease severity on Rio Grande and Cal J which was significantly lower ($P \leq 0.05$) than in isolate 6 (Table 4.6). Cal J had the highest disease severity for all the isolates tested. Disease severity on Cal J was significantly higher ($P \leq 0.05$) than in Rio Grande and Onyx for isolate 5 and 11 (Table 4.6). Disease severity on Cal J was significantly higher ($P \leq 0.05$) after infection with isolate 5, 6 and 11 than isolate 8 and 9 (Table 4.6).

Table 4.6: Percentage disease severity per tomato variety recorded after 60 days after treatment with *F. o. l.* Isolates

Isolates	[3]Percentage disease severity per tomato variety		
	[2]Rio Grande	Onyx	Cal J
5	12.50[1]b	31.25b	52.75a
6	28.75ab	35.00ab	53.00a
8	3.75c	12.50bc	21.75b
9	23.75b	28.75b	35.00b
11	23.75b	6.50c	42.50a
Control	0.00d	0.00d	0.00d

[1]Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

[2]Tomato cultivars used in the study

[3]Mean percentage disease severity per tomato variety after inoculation with *F. o. l.* Isolates

4.4.3 Plant heights

There was no significant difference ($P \leq 0.05$) in individual plant heights after treatment with isolate 5, 8, 9 and 11 (Table 4.7). Tomato plants treated with isolate 6 were significantly shorter ($P \leq 0.05$) than Onyx and Cal J plants treated with isolate 9. Control plants were also significantly taller ($P \leq 0.05$) than Rio Grande and Onyx plants treated with isolate 6 (Table 4.7). However, all the *F. o. l.* isolates had an effect on the plant heights (Table 4.7).

Table 4.7: Mean heights of tomato varieties (Rio Grande, Onyx and Cal J) after 60 days growth in soil inoculated with different isolates of *F. o. l.*

Isolates	[3]Mean plant heights (cm)		
	[2]Rio Grande	Onyx	Cal J
5	38.83[1]a	44.07a	29.23ab
6	22.93b	20.43b	20.20b
8	45.67a	38.10ab	41.30a
9	38.33ab	39.70a	38.87a
11	45.27a	39.23ab	36.57ab
Control	46.83a	44.50a	44.50a

[1]Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

[2]Tomato cultivars used in the study

[3]Mean heights (cm) on individual plants after inoculation with *F. o. l* isolates

4.4.4 Plant stem diameter

The test showed that all isolates had an effect on plant stem diameter (Table 4.8). There was no significant difference ($P \leq 0.05$) in the stem basal diameters of Rio Grande, Onyx and Cal J after treatment with isolate 5, 9, 8 and 11 (Table 4.8). Onyx and Cal J tomato plants treated with isolate 6 were significantly smaller ($P \leq 0.05$) than all other treated plants and the control plants. Cal J plants treated with isolate 6, 8 and 9 had smaller basal diameters than Rio Grande and Onyx treated the same way though the differences were not significant ($P \leq 0.05$) for isolate 8 and 9. Control plants for the three varieties were taller than the treated plants though there was

no significant difference (P≤ 0.05) apart from Onyx and Cal J tomato plants that were significantly (P≤ 0.05) shorter after treatment with isolate 6 (Table 4.8).

Table 4.8: Mean basal stem diameters of tomato varieties (Rio Grande, Onyx and Cal J) after 60 days growth in soil inoculated with different isolates of *F. o. l.*

Isolates	[3]Mean basal stem diameters (cm) of tomato varieties		
	[2]Rio Grande	Onyx	Cal J
5	0.50[1]a	0.50a	0.50a
6	0.47a	0.35b	0.30b
8	0.57a	0.60a	0.50a
9	0.53a	0.60a	0.50a
11	0.53a	0.50a	0.50a
Control	0.63a	0.60a	0.60a

[1]Mean values in the same column denoted by similar letters are not significantly different at P ≤ 0.05

[2]Tomato cultivars used in the study

[3]Mean basal stem diameters on individual plants after inoculation with *F. o. l* isolates

4.5 Efficacy of fungicides, Neem extract and Organic matter in management of *Fusarium* wilt of tomatoes

4.5.1 Effects of NKCP on mycelial growth of *Fusarium oxysporum f.* sp. *lycopersici*

As the concentration of the neem extract was increased, the size of fungal mycelia decreased (r = -0.863, P = 0.060), though the mycelial diameters were not significantly different except for the treatment rate of 40 g/l which was significantly different from the other treatments (P≤ 0.05). Neem extract concentration of 40 g/l was more inhibitory (88.9%) on mycelial growth than lower rates of 36, 28, 20 and 12 g/l respectively (Table 4.9). Concentration of 12 g/l had the

lowest inhibition (45%) on the mycelial growth (r = -0.697, P = 0.303) though the mycelial sizes were not significantly different (P≤ 0.05) from mycelial sizes in concentration of 20, 28 and 36 g/l. At concentration of 40 g/l, the mycelia sizes were significantly lower (P≤ 0.05) than in all other treatments. The mycelial sizes on the control plates were significantly higher (P≤ 0.05) than in the amended plates (Table 4.9: Figure 4.1). From the regression curve (Figure 4.2), mycelial growth rate for concentration 40 g/ml (y = 15x=13) was 7.99 times lower than the growth rate of the control (y = 1.88x+6.32) This implied that NKCP reduced the mycelial growth.

Table 4.9: Mean mycelial diameter of *F. o. l.* (mm) recorded on Petri dishes treated with different concentrations of Neem Kernel Cake Powder after 11 days

Concentration	[1]Mean mycelial diameter	% Inhibition
12g/litre	31.52b	45.9
20g/litre	30.04b	48.9
28g/litre	28.16b	59.4
36g/litre	26.60b	60.9
40g/litre	11.96c	88.9
Control	58.00a	0.0

[1]Mean mycelial diameter on amended and control plates

Mean mycelial diameters denoted by similar letters are not significantly different (95% CI)

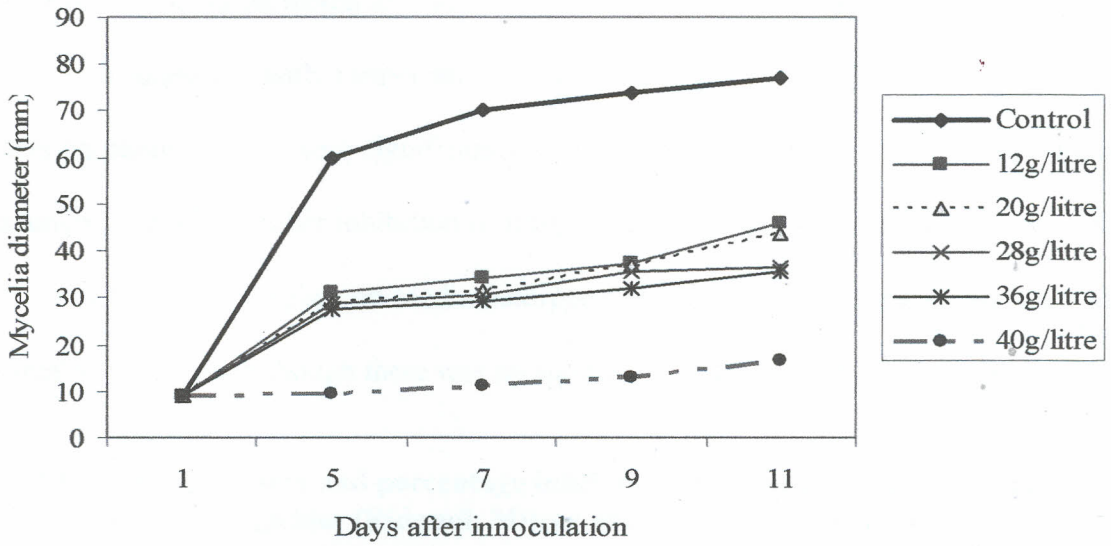

Figure 4.1: Trend in Mycelial growth of *F. o. l.* on media incorporated with different concentrations of Neem Kernel Cake Powder after 11 days

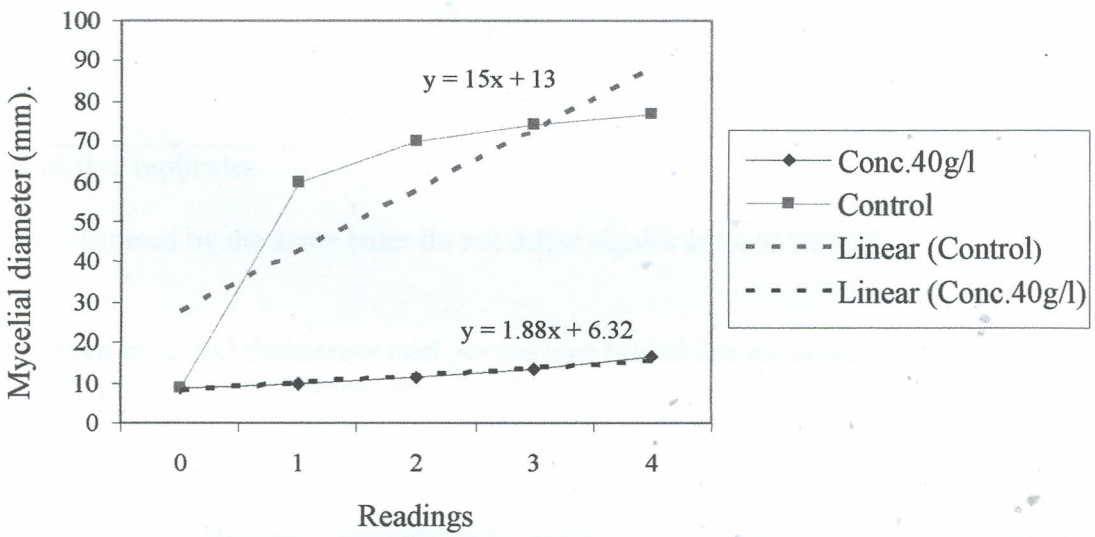

Figure 4.2: Regression curve for mycelial growth on media amended with 40 g NKCP/litre

4.5.2 Effects of three Fungicides (Ridomil MZ, Milraz and Ortiva) on mycelial growth of *F. o. l.*

The mycelial diameters on media amended with Ridomil MZ were significantly lower ($P \leq 0.05$) than in plates amended with Ortiva and in the control plates (Table 4.10). Mean mycelial diameters on control plates were significantly different ($P \leq 0.05$) from Ridomil MZ, Ortiva and Milraz amended plates. Higher inhibition of fungal hyphal growth was recorded in media treated with Ridomil (36.1%). Hyphae on media incorporated with Ortiva had bigger diameter than in media treated with Milraz though there was no significant difference ($P \leq 0.05$) (Table 4.10).

Table 4.10: Mean diameters and percentage inhibition of hyphae on media amended with the fungicides (Ridomil, Milraz and Ortiva) recorded after 23 days

Treatments	[1]Mean size (mm)	% Inhibition
Control	45.55[2]a	0
Ridomil MZ	26.24c	36.1
Milraz	32.18abc	15.4
Ortiva	37.22b	8.9

[1]Mean of five replicates

[2]Figures followed by the same letter do not differ significantly at 95%CI

4.5.3 Mean mycelial diameters and percentage inhibition on media amended with different ratios of Fungicide: NKCP after 22 days

Mycelial diameters in three ratios of Fungicide: NKCP showed no significant difference ($P \leq 0.05$) (Table 4.11). However, mycelial sizes were bigger in control treatment (mean 41.38 mm) than in the other treatments but there was no significant difference ($P \leq 0.05$). The treatment ratio of 25:75 had the highest inhibition (Figure 3) of 20.9% (Table 4.11). Treatment ratios of 50:50 and 75:25 had inhibition of 18.3% and 8.5% respectively (Table 4.11).

Table 4.11: Mean mycelial diameters (mm) and inhibition percentages using different treatment ratios of Fungicide: NKCP recorded after 22 days

Treatment ratio	Mean mycelial diameter (mm)	Inhibition %
25: 75	34.03 ± 7.25	20.9
50:50	34.92 ± 7.77	18.3
75:25	38.38 ± 8.56	8.5
Control	41.38 ± 7.71	0

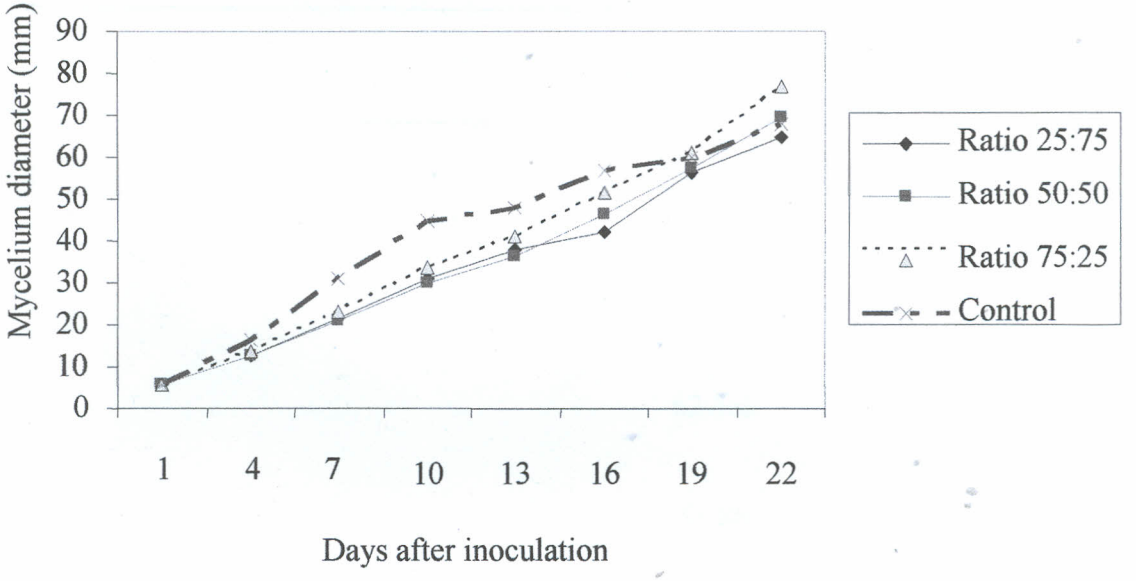

Figure 4.3: Trend in mycelial growth on media amended with different treatment ratios of Fungicide: NKCP recorded in 22 days.

4.5.4 Effects of NKCP on *Fusarium* wilt caused by *F. o. l.* on tomatoes

4.5.4.1 Effects on disease incidence

There was significant difference ($P \leq 0.05$) in disease incidences in various treatments. Plants treated with Neem extract concentration of 10 g/pot recorded the lowest disease incidence (42.5%). This was significantly lower ($P \leq 0.05$) than all other treated plants. There was no significant difference ($P \leq 0.05$) in disease incidences at treatment rates of 7 and 9 g per planting pot. Control plants had the highest disease incidence (71.3%) though not significantly different ($P \leq 0.05$) from plants treated at the rate of 3 g/pot (Table 4.12).

Table 4.12: Percentage disease incidence in tomato plant infected by *F. o. l.* and treated with different rates of Neem kernel cake powder recorded at 30, 60 and 80 days after planting

NKCP rate (g/pot)	% disease incidence
Control	71.3a
3 g	67.5a
5 g	62.5ab
7 g	53.8b
9 g	50.5b
10 g	42.5c

Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

NKCP indicates Neem Kernel Cake Powder

4.5.4.2 Effects on disease severity

Increase in the rate of NKCP application had an effect in reduction of disease severity (Table 4.13). In pots treated with higher rate of 10 g NKCP/pot, the disease severity was significantly lower ($P \leq 0.05$) than in lower rates of 9, 7, 5 and 3 g/pot. Control plants had the highest disease severity (57.5%). This was significantly different ($P \leq 0.05$) from other treatments (Table 4.13). Plants treated with 3 g/pot had the highest disease severity among the NKCP treated plants (Table 4.13). There was no significant difference ($P \leq 0.05$) in disease severity among plants treated at the rates of 7 and 9 g NKCP/pot.

Table 4.13: Percentage disease severity in tomato plant infected by *F. o. l.* and treated with different rates of Neem kernel cake powder recorded at 30, 60 and 80 days after planting

NKCP rate (g/pot)	% Disease severity after treatment with different amounts of NKCP
Control	57.5a
3 g	43.2b
5 g	40.5bc
7 g	35.1c
9 g	35.9c
10 g	34.3d

Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

NKCP indicates Neem Kernel Cake Powder

4.5.4.3 Plant heights

There was significant difference ($P \leq 0.05$) in heights of treated plants (Table 4.14). Plants treated with higher rate of 10 g NKCP/pot had the highest mean plant height (52.70 cm). This was not significantly different ($P \leq 0.05$) from plants treated at the rate of 9 g/pot but significantly different from other treatments. Control plants had the lowest mean height (31.25 cm) which was significantly lower ($P \leq 0.05$) than all other treatments (Table 4.14).

Tale 4.14: Plant heights of tomato plant infected by *F. o. l.* and treated with different rates of Neem kernel cake powder recorded at 30, 60 and 80 days after planting

[2]NKCP extract	Plant Height (cm)
Control	31.25[1]d
3 g	42.88c
5 g	44.50bc
7 g	46.63b
9 g	51.53a
10 g	52.70a

[1]Mean in the same column denoted by similar letters are not significantly different at 95% CI

[2]Treatment rate with NKCP

NKCP indicates Neem Kernel Cake Powder

4.5.4.4 Basal stem diameter

Plants treated with 10 g NKCP/pot had the highest mean stem diameter (0.58 mm). However, this was not significantly different ($P \leq 0.05$) from stem diameters of plants treated with 9, 7 and 5 g NKCP/pot. Mean basal stem diameters in control pots and in treatment of 3 g NKCP/pot were significantly lower ($P \leq 0.05$) than all the other treatments (Table 4.15). There was no significant difference ($P \leq 0.05$) in basal stem diameters of control plants and plants treated with 3 g NKCP/pot (Table 4.15).

Table 4.15: Basal diameter in tomato plant infected by *F. o. l.* and treated with different rates of Neem kernel cake powder recorded at 30, 60 and 80 days after planting

NKCP extract	Stem diameter (cm)
Control	0.43b
3 g	0.48b
5 g	0.56a
7 g	0.49ab
9 g	0.59a
10 g	0.58a

Means in the same column denoted by similar letters are not significantly different at 95% CI

NKCP indicates Neem Kernel Cake Powder

4.5.5 Effects of combinations of NKCP, Organic matter and Fungicides on *Fusarium* wilt disease incidence and severity and, plant height and basal stem diameters

4.5.5.1 Effects on disease incidence

Plants grown in pots treated with "Fungicides + Neem cake powder +Organic matter" had lower disease incidence (mean 40.5%) than all the other treatments. This was significantly different ($P \leq 0.05$) from other treated plants (Table 4.16). Disease incidence on plants treated with NKCP only was not significantly different ($P \leq 0.05$) from the incidences among plants treated with Organic matter only (Table 4.16). Plants treated with *Fusarium* only recorded the highest disease incidence (71.3%). This was significantly different ($P \leq 0.05$) from the other treated plants (Table

4.16). Control plants recorded the lowest disease incidence (0.00%) which was significantly different ($P \leq 0.05$) from all treated plants (Table 4.16).

Table 4.16: Percentage disease incidences on infected plants treated with different combinations of Fungicides, NKCP and Organic matter recorded at 30, 60 and 80 days after planting

Treatments	% Incidence
Fungicide alone	51.3bc
Fungicide + NKCP	46.3c
Fungicide + NKCP + Organic matter	40.5d
Fungicide + Organic matter	48.8c
Fusarium alone	71.3a
NKCP only	59.3b
Organic matter only	58.8b
Organic matter + NKCP	46.3c
Control	0.00e

Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

NKCP indicates Neem Kernel Cake Powder

4.5.5.2 Effects on disease severity

There was a significant difference ($P \leq 0.05$) in the disease severity among the treated plants (Table 4.17). Plants treated with "Fungicides + Neem cake powder +Organic matter" had the lowest disease severity (27.2%). This was significantly different ($P \leq 0.05$) from the other treatments. Plants treated with *Fusarium* only recorded the highest disease severity (57.5%). This was significantly different ($P \leq 0.05$) from other treatments (Table 4.17). Control plants had the lowest severity (0.00%) which was significantly different ($P \leq 0.05$) from other treatments (Table 4.17).

Table 4.17: Percentage disease severity on infected plants treated with different combinations of Fungicides, NKCP and Organic matter recorded at 30, 60 and 80 days after planting

Treatments	% Disease severity
Fungicide alone	48.1ab
Fungicide + NKCP	41.3b
Fungicide + NKCP + Organic matter	27.2d
Fungicide + Organic matter	39.9b
Fusarium alone	57.5a
NKCP only	34.3c
Organic matter only	36.0bc
Organic matter + NKCP	35.2c
Control	0.00e

Mean values in the same column denoted by similar letters are not significantly different at $P \leq 0.05$

NKCP indicates Neem Kernel Cake Powder

4.5.5.3 Stem heights

Plants treated with "Fungicides + Neem cake powder +Organic matter" recorded the highest mean height (51.70 cm) among the treated plants which was significantly different ($P \leq 0.05$) from heights of plants treated with fungicide alone (37.22 cm) and plants treated with *Fusarium* alone (Table 4.18). There was no significant difference ($P \leq 0.05$) in the mean heights of plants treated with *Fusarium* alone and other treated plants apart from those treated with "Fungicides + Neem cake powder +Organic matter" (Table 4.18). There was no significant difference ($P \leq 0.05$) in the plant heights of Control plants and those treated with "Fungicides + Neem cake powder +Organic matter" (Table 4.18).

Table **4.18: Heights of infected tomato plants treated with different combinations of Fungicides, NKCP and Organic matter recorded at 30, 60 and 80 days after planting**

Treatments	Plant heights (cm)
Fungicide alone	37.22b
Fungicide + NKCP	44.43ab
Fungicide + NKCP + Organic matter	51.70a
Fungicide + Organic matter	41.85ab
Fusarium alone	31.25b
NKCP only	42.95ab
Organic matter only	40.70ab
Organic matter + NKCP	42.48 ab
Control	55.00a

Mean values in similar column denoted by the same letters are not significantly different at 95% CI

NKCP indicates Neem Kernel Cake Powder

4.5.5.4 Basal stem diameters

Plants treated with "Fungicides + Neem cake powder +Organic matter" recorded the biggest mean stem diameter (0.64 cm) among the treated plants (Table 4.19). However, this was not significantly different ($P \leq 0.05$) from mean diameters of plants treated with Fungicide alone, Fungicide and Organic matter and Organic matter only (Table 4.19). Plants treated with *Fusarium* alone had the smallest mean stem diameter (0.43 cm) which was significantly lower ($P \leq 0.05$) than in all other treatments apart from "Organic matter + NKCP" (Table 4.19). Control plants had the biggest stem diameter (0.66 cm) but this was not significantly different ($P \leq 0.05$) from plants treated with "Fungicides + Neem cake powder +Organic matter" (Table 4.19).

Table 4.19: Basal stem diameters of infected tomato plants treated with different combinations of Fungicides, NKCP and Organic matter recorded at 30, 60 and 80 days after planting in infested soil

Treatments	Stem diameters (cm)
Fungicide alone	0.56ab
Fungicide + NKCP	0.56b
Fungicide + NKCP + Organic matter	0.64a
Fungicide + Organic matter	0.51ab
Fusarium alone	0.43c
NKCP only	0.55b
Organic matter only	0.56ab
Organic matter + NKCP	0.50bc
Control	0.66a

Mean values in similar column denoted by the same letters are not significantly different at 95% CI

NKCP indicates Neem Kernel Cake Powder

It was noted that taller plants had bigger stem diameters, showing a significant relationship between the plant heights and the stem diameters ($r = 0.833$, $P = 0.000$). Total deaths of the plants were recorded after the study established that all the infected plants had completely died.

Plate 4.1: Tomato wilt symptoms on potted plants inoculated with *F. o. l.*

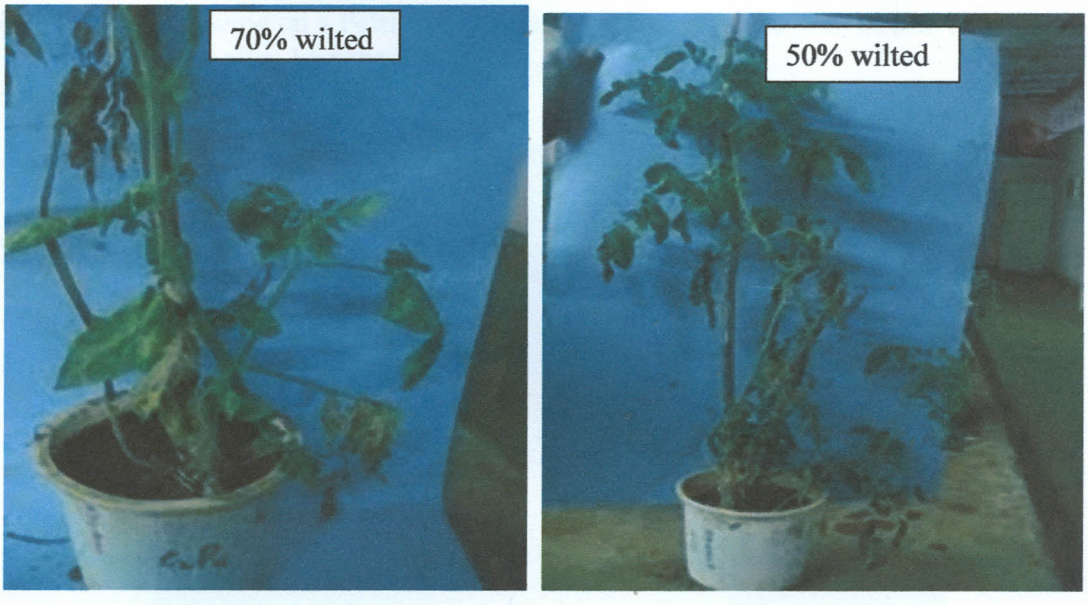

Plate 4.2: Potted tomato plants inoculated with *F. o. l.* showing levels of wilting

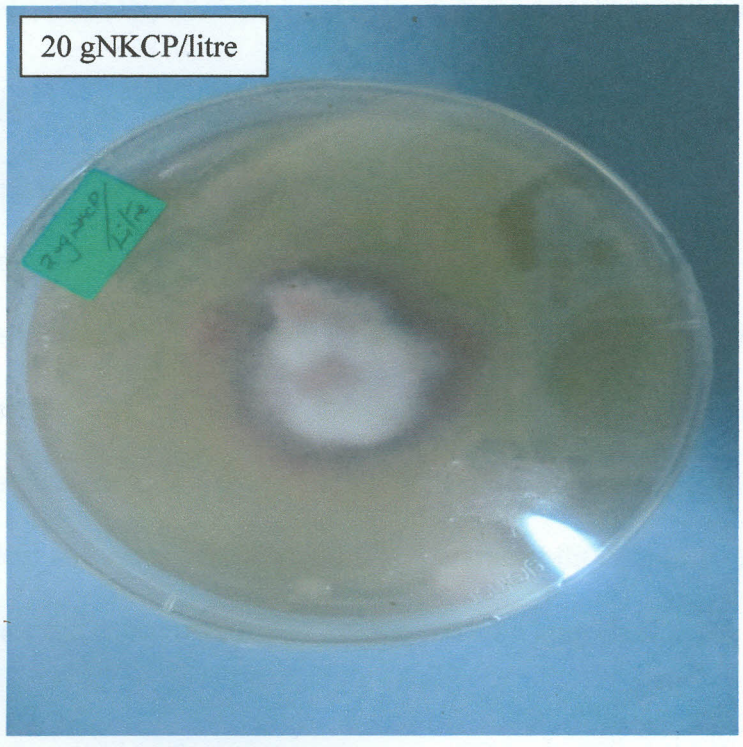

Plate 4.3: *F. o. l.* growing on a Petri dish containing PDA amended with NKCP at a rate of 20 g/litre after 11 days

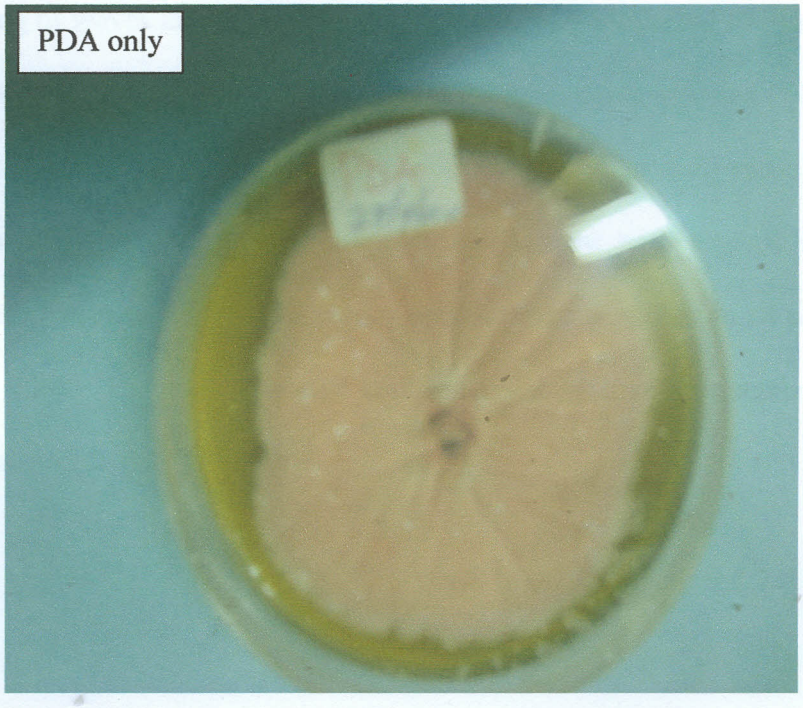

Plate 4.4: *F. o. l.* growing on a Petri dish containing non-amended PDA after 11 days

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Tomato varieties grown by farmers in Kirinyaga District

The study revealed that farmers in Kirinyaga District mainly prefer to grow Rio Grande, Onyx and Cal J tomato varieties. These cultivars are marketable and highly yielding. Although wilting problems are associated with Rio Grande, the variety is still preferred by majority of the farmers (n=63). The preference for this variety was attributed to high marketability, high production, good keeping quality and early maturity. The high marketability could be attributed to the high keeping quality since the consumers prefer tomatoes with a long shelf life.

5.1.2 Tomato disease problems encountered by farmers in Kirinyaga District

The major disease problems encountered by farmers in Kirinyaga District are wilting, stunted growth, yellowing of leaves and leaf defoliation. Wilting was attributed to *Fusarium* wilt disease which was prevalent in the area. The high number of wilting cases reported (74.6%) could be due to warm temperatures that were apparent at period of study favouring spore germination. This could also be attributed to lack of enough soil amendment with organic matter that would increase soil nutrients to improve crop vigour and improve suppression of common soil borne pathogens (Jeff, 2009). The organic matter could increase systemic resistance to *Fusarium* wilt (Recycled Organics Unit, 2006).

5.1.3 Methods used by farmers to control *Fusarium* wilt in Kirinyaga District

The study established that the farmers use different methods to control *Fusarium* wilt. Most of the farmers interviewed (87.7%) use chemical control. Chemicals used by the farmers include Ridomil Gold, Ridomil MZ, Ortiva, Milraz and Ozothine which are applied as foliar sprays. This could be attributed to the availability of the chemicals in the local agro shops and basic awareness of inorganic farming. The pronounced use of chemicals could also be attributed to the fact that the farmers were targeting to control the insect vectors on their fields. Other methods used by the farmers to control *Fusarium* wilt included crop rotation, applying ash and rouging. A small number of farmers (13.1%) did not use any method to control *Fusarium* wilt. This could be attributed to the limited knowledge about the *Fusarium* wilt pathogen and the expense associated with control. Though most farmers apply chemical control measures, *Fusarium* wilt cases were still common in the fields. This could be attributed to inappropriate use of chemicals for the disease control, lack of effective crop rotation to kill the pathogen and inadequate organic soil amendment that would improve crop vigour to resist attack. The use of ash by some farmers could be attributed to lack of enough capital and limited knowledge to undertake integrated *Fusarium* wilt disease control.

5.1.4 Susceptibility of tomato varieties grown in Kirinyaga District to *Fusarium* wilt

The three popular tomato varieties grown in the District had different susceptibility levels to *Fusarium* wilt. Isolate 5 and 6 obtained from Kangai and Mutithi respectively were more pathogenic on Onyx and Cal J than isolate 8, 9 and 11 obtained from Kagio, Kianyaga and Kutus respectively. The differences in pathogenicity could be attributed to mutation of the *Fusarium* wilt pathogen. This could be as a result of different levels of pesticide (Ridomil Gold, Ridomil

MZ, Ortiva, Ozothine and Milraz) use in different regions leading to differential mutation levels among the isolates (Juliano *et al.*, 2005). Among the varieties, Cal J had the highest incidence and severity as result of infection with different isolates. The differences in susceptibility among the varieties could be as a result of different inherent genetic resistance to the *Fusarium* wilt pathogen (Pritesh *et al.,* 2011; Larkin *et al.*, 2002). The isolates also had varied effects on stem heights and diameters. Isolate 6 had the greatest effects in reducing the plant heights and basal diameters. Other isolates had varied effects in reduction of plant stems and basal diameters on different tomato varieties. Isolate 6 significantly ($P \leq 0.05$) reduced the basal diameters of Onyx and Cal J plants. This could be as a result of genetic differences among the isolates leading to different pathogenicity (Juliano *et al.*, 2005).

5.1.5 Efficacies of Fungicides, Neem extract and Organic Matter in Management of *Fusarium* wilt of tomatoes

5.1.5.1 Effects of NKCP on mycelial growth of *F. o. l*

The study revealed that, as the concentration of NKCP extract was increased, the mean mycelial diameter decreased. This could be attributed to a variety of chemical constituents such as nimolicinol, isolimolicinolide, azadirachtin, azadirachtol, nimlinone, nimbocinol, nimbocinone and nimocin contained in neem (Tewari, 1992) which could be fungistatic (Dubey *et al.*, 2009). The higher the amount of NKCP per litre, the higher the inhibition level. Control plates treated with fungus alone had the highest mycelial diameter which confirms that NKCP has antifungal compounds that inhibited mycelial growth.

5.1.5.2 Effects of three Fungicides (Ridomil, Milraz and Ortiva) on mycelial growth

Plates treated with Ridomil recorded the smallest mycelial diameter. This could be as a result of the interaction of Metalaxyl and Mancozeb contained in Ridomil MZ making it more effective than Ortiva and Milraz (Samoucha *et al.,* 1984). Milraz is a broad spectrum fungicide that contains Propineb and Cymoxanil while Ortiva contains Azoxystrobin which is also a broad spectrum fungicide. The lower performance of Ortiva could be as a result of lack of a combination of active ingredients as in Milraz and Ridomil (Francis *et al.*, 2001).

5.1.5.3 Effects of combination of Neem extract and the Fungicide (Ridomil) on mycelial growth of *F. o. l.* on Petri dishes

The high inhibition to mycelial growth could be attributed to high levels of Azadirachtin provided by NKCP supplemented with little quantities of active ingredients in Ridomil (metalaxyl 40g/kg + mancozeb 640g/kg). As the concentration of NKCP decreased, inhibition levels reduced probably due to a reduction in fungicidal compounds provided by NKCP. The study therefore revealed that NKCP contains fungicidal compounds that are effective against *Fusarium* wilt fungus in probably higher amounts compared to active ingredients in Ridomil as a percentage. Dubey and Kumar (2003) reported Azadirachtin to be as good as fungicide Bavistin and Mancozeb in activity.

5.1.5.4 Effects of NKCP on *Fusarium* wilt caused by *F. o. l.* in tomatoes

The significantly ($P \leq 0.05$) higher performance of tomato plants grown in soil amended with NKCP compared to those grown in non-amended soil (control) indicated that NKCP suppressed pathogenic effects of *F. o. l.* Neem contains a variety of chemical constituents such as nimolicinol, isolimolicinolide, azadirachtin, azadirachtol, nimlinone, nimbocinol, nimbocinone,

nimocin, salannin (Conventry *et al.* 2001; Tewari, 1992). Dubey and Kumar (2003) reported the fungicidal effects of Azadirachtin are as good as the fungicides bavistin and mancozeb. These chemicals present in plant cause deleterious effect on the microorganisms. The suppressive effects could be attributed to production of fungistatic substances such as Azadirachtin that are taken up by tomato plants improving host resistance (Kimaru *et al.*, 2004). The neem seedcake contains higher levels of nitrogen, phosphorus, potassium, calcium and magnesium which possibly improved the crop vigour and consequently resistance to *Fusarium* wilt (Ros *et al.*, 2005; Kimaru *et al.*, 2004). The poor disease development could therefore be associated with enhanced performance of tomato plants due to high nitrogen nutrition from the neem seedcake.

5.1.5.5 Effects of a combination of NKCP, Organic matter and Fungicides on *Fusarium* Wilt of tomatoes

There was a significantly ($P \leq 0.05$) increased performance of tomato plants as a result of integration of measures in management of *Fusarium* wilt. Plants treated with "Fungicides + Neem cake powder +Organic matter" had higher performance than all other treated plants. This could be attributed to the interactive effects of Organic matter, provided by NKCP and Organic manure, and Fungicides. The Fungicide used (Ridomil) contains "metalaxyl 40g/kg + mancozeb 640g/kg" and is both a contact and a systemic fungicide. Neem extract has both antifungal and nutritional effects when applied (Kimaru *et al.*, 2004). It contains Azadirachtin which is an antifungal and also provides plant nutrients improving crop vigour and resistance to *Fusarium* wilt. The use of organic matter improves plant nutrients in the soil therefore crop performance and resistance to soil borne pests and diseases (Ros *et al.*, 2005). Organic compounds are also known to reduce toxicity of poisonous compounds in the soil that build up as a result of continuous use of Fungicides (Jeff, 2009). This leads to the improvement of conditions for living

organisms in the soil, some of which serve as biocontrol to *Fusarium* species in the soil (Recycled Organics Unit, 2006). According to Noriaki *et al.* (2006), organic matter releases acetic acid and/or butyric acid that suppress survival of *Fusarium oxysporum f.* sp. *lycopersici.*

5.2 CONCLUSIONS

The study established that the popular tomato cultivars in Kirinyaga District are Cal J, Onyx and Rio Grande in order of their increasing popularity. The study also established that the incidences of *Fusarium* wilt were common on all the three varieties with most of the farmers using varied methods to control the disease. Chemical control was preferred by most of the farmers possibly because the chemicals are readily available in the local agro shops. The study also revealed that *F. o. l.* isolates from different regions had different infection abilities on the three tomato cultivars with Cal J being more susceptible than Onyx and Rio Grande for all the five isolates tested. This suggests that there is a possibility of existence of *F. o. l.* races. Greenhouse tests on *Fusarium* Wilt management established that, integrated management of the disease using fungicides, organic matter and neem cake powder was more effective that individual methods. This therefore suggests a positive interaction of fungicides, organic matter and neem kernel cake powder against *Fusarium* wilt.

5.3 RECOMMENDATIONS

5.3.1 For the farmer

Since *Fusarium* wilt disease incidence is high in most of the farms in Kirinyaga District, the study recommends that the farmers practice integrated management of *Fusarium* wilt in tomatoes involving Neem cake powder, Organic soil amendments and Fungicides. Use of resistant tomato

varieties and integrated approach in management of *Fusarium* wilt would greatly reduce disease incidences and severity and also improve crop vigour.

5.3.2 For policy makers and researchers

The study recommends tests of other organic materials against *Fusarium* wilt. These materials could be plant parts obtained from indigenous plants and types of manures that are locally available and cheap to the farmers. There is also a need for the Ministry of Agriculture in collaboration with HCDA to educate the farmers to create awareness about *Fusarium* wilt disease and how to effectively control the disease using the Integrated Approach. Similar studies are also necessary in other areas where *Fusarium* wilt is prevalent. The study also recommends further studies to establish the races of *F. o. l.* that could be present in the study area and consequently the tomato varieties that are resistant to the races.

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Appendix: Questionnaire

Topic: The tomato varieties, incidences of *Fusarium* wilt of tomatoes and measures undertaken to control *Fusarium* wilt of tomatoes

Dear sir / madam,

I am Mugo Dishon Njiru pursuing a Master of Science Degree in plant pathology at Kenyatta University in the school of Pure and Applied Sciences. Kindly help me fill the questions to enable me conduct my research work.

Thank you in advance for your co-operation.

Section A: Background information

1. Date of interview.....
2. Name of interviewee.....
3. Your province.....
4. District.....
5. Location.....Farm code.....
6. Respondent's age (Yrs)
   1. 0-10 ☐
   2. 11-20 ☐
   3. 21 -30 ☐
   4. 31-40 ☐
   5. above 40 ☐
7. Gender
   1. Male ☐
   2. Female ☐
8. Education background
   1. Not educated ☐
   2. Primary level ☐
   3. Secondary level ☐
   4. College/university ☐
9. Do you own the land or you have leased?
   1. Own ☐

2. Leased ☐

Section B: Specific information

1. (a) Do you grow tomatoes?

   1. Yes ☐
   2. No ☐

   (b) If yes, how many stems do you have on your farm? _____

   (c) How do you water your plants? 1. By rain ☐

   2. Irrigation ☐

2. (a) Which varieties of tomato do you grow on your farm? _____

   _____

   (b) Why do you prefer the varieties? _____

   _____

3. What are the common problems encountered in growing of tomatoes?

   1. Diseases ☐
   2. Insect pests ☐
   3. Nematodes ☐
   4. Soil fertility ☐
   5. Theft ☐
   6. Others ☐ specify _____

4. What are the common tomato disease symptoms you experience in growing of tomatoes?

   1. Wilting ☐
   2. Retarding ☐
   3. Yellowing ☐
   4. Falling of leaves ☐
   5. Others ☐ specify _____

5. How do you treat the disease symptoms named in Question 4 above? _____

   _____

   _____

6. Is this treatment effective?

1. Yes ☐
2. No ☐

7. State other problems encountered in tomato production _____

_____