MANAGEMENT OF ROOT-KNOT NEMATODES AND *Fusarium* WILT OF TOMATO BY PRE-TREATMENT OF SEEDLINGS WITH CHEMICAL AND BIOLOGICAL AGENTS

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2014
DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university.

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DEDICATION

I would like to dedicate this work to my parents without whom I would not have come this far, my husband and children for the encouragement and enduring my absence.
ACKNOWLEDGEMENT

I want to thank the Almighty God for his faithfulness and provision through the course programme. There are times when I felt like giving up but his word ‘I can do all things through him who strengthens me’ kept on encouraging me. I acknowledge my supervisors Prof. J. W. Kimenju, and Prof. J.W. Muthomi for their tireless guidance and moral support throughout my studies. I also pass my sincere appreciation to Directors of Agriculture, the late Tom Bonyo and Dr Johnson Irungu and the management and staff of Ministry of Agriculture, Horticulture Division for encouraging me to study and the confidence they had in me. Special thanks go to Amiran Kenya Limited, Juanco Limited and Simlaw Kenya Seed Co. for the support they gave in my research through provision of all the required input.

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ABSTRACT

Tomato (*Solanum lycopersicum*) is one of the most important vegetables grown in Kenya. It is grown for the domestic market under both rainfed and irrigated conditions. Due to the high demand for tomato in the domestic market and for processing, farmers have extensively adopted high yielding varieties and modern technologies like greenhouse production to ensure year round production and increase production. Intensive tomato cultivation in Kenya has resulted in build-up of soil borne diseases, especially root-knot nematodes and *Fusarium* wilt particularly in greenhouses where monoculture and limited crop rotation are practiced. The control of root-knot nematodes and *Fusarium* wilt is basically by use of nematicides and fungicides which are toxic and expensive for the small scale farmers. Therefore, this study was conducted to determine the efficacy of the use of chemical and biological agents in management of root-knot nematodes and *Fusarium* wilt in tomato under intensive cultivation. Greenhouse experiments were conducted over two cropping cycles. Tomato seedlings were planted in sterilized growth media inoculated with root-knot nematode, *Fusarium*, or a combination of the two. The products were Marshal® a chemical agent, Neemraj® (Azadiractin) a neem-based product and Pl plus® (*Paecilomyces lilacinus*) a microbial-based product against root-knot nematodes; Phospht® a fungicide, Root guard® (*Trichorderma, Bacillus, Aspergillus, Chatomium, Escherichia, Azotobacter spp*), and Neemraj® against *Fusarium* wilt; Root guard®, Neemraj® and Pl plus® applied either singly or in combination against the dual infection inoculation of seedling with root-knot nematode and *Fusarium* wilt Fungus. The experiment was laid out in a randomised complete design.

Data on plant height, plant dry matter, number of galls per plant, number of nematode juveniles in potting media, and the number of plants showing *Fusarium* wilt symptoms was determined. The results showed that the effectiveness of the biological products in reducing
root-knot nematodes and *Fusarium* wilt infection was comparable to that of the chemical agents. The neem-based product Neemraj® was more effective than the microbial-based product Pl plus® in reducing severity of root-knot nematodes infection. Neemraj® reduced the number of galls by up to 77% and the number of juveniles by 94%. Microbial based product (Root guard®) was the most effective against *Fusarium* wilt and it reduced the severity of the disease by 100%. In dual inoculations with root-knot nematode and *Fusarium* wilt, the biological products increased shoot biomass and plant height resulting in a reduction of the number of galls, nematode juveniles number of plants with wilt symptoms and stem vascular discoloration. Combined application of Root guard® and Pl plus®, Pl plus® and Neemraj® or Root guard® and Neemraj® reduced the number of galls per plant and number of juveniles by up to 93% while severity of vascular discoloration due to *Fusarium* infection was reduced by up to 100%.

The study indicated that pre-treatment of seedlings with biological and botanical agents is effective in managing root-knot nematode and *Fusarium* wilt in tomato. The combined application of these products have a synergistic effect and improves their efficacy against the root – knot nematode - *Fusarium* disease complex than when the products are applied alone.
CHAPTER 1: INTRODUCTION

1.1 Importance of tomato

Tomato (*Solanum lycopersicum*) is an annual sub-tropical fruit used as a vegetable and is one of the most important vegetables grown in Kenya (MOA, 2002). The crop is among the most important horticultural crops and is grown on over 4 million hectares of land worldwide (FAO, 2003). Tomato is an important source of vitamin A (900 IU), C (23 mg) and B2 (0.04 mg), and minerals such as K (244 mg), Fe (0.5 mg) and P (27 mg) per 100 g sample (Anita and Rabeeth, 2009). It is also an excellent source of lycopene, which is the pigment that makes tomatoes red and has been linked to the prevention of many types of cancer, heart disease and premature aging (Cerkauskas, 2005; Wamache, 2005). Its fruits are used in salads or cooked as a vegetable, processed into tomato paste, sauce and puree (MOA, 2002).

Tomato in Kenya is grown for the domestic market under both rainfed and irrigated conditions (MOA 2010). Lately due to the high demand and especially during the low seasons farmers have extensively adopted high yielding varieties and modern technologies like greenhouse production to ensure year round production (MOA, 2010). The crop is grown for both fresh market and processing industries as there is an increasing demand for processing (Mungai et al., 2000; MOA, 2003). Due to this high demand for tomato, the area and production of tomato has been on the increase from an area of 17,230 ha and a production of 354,356 tons in the year 2009 to 18,178 ha, with a production of 407,374 tons in the year 2011 as shown in Table 1 (MOA, 2012). The most widely grown varieties include Cal J, Fortune maker, Rio-grande, Roma VF, Anna F1, Bravo, Chonto, Marglobe, Aleta, Ornix, Nuru, and Money Maker among others. The major tomato growing counties are
Kirinyaga, Taita Taveta and Bungoma. Tomato production have been facing challenges due to pests and diseases, high cost of farm inputs and in marketing (Waiganjo et al., 2006)

Table 1: Production of Tomato in Selected Counties in Kenya, 2010-2011

<table>
<thead>
<tr>
<th>County</th>
<th>2010 Area(Ha)</th>
<th>2010 Quantity(MT)</th>
<th>2010 Value (Mi)Ksh.</th>
<th>2011 Area(Ha)</th>
<th>2011 Quantity(MT)</th>
<th>2011 Value (Mi)Ksh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taita</td>
<td>1,690</td>
<td>70,328</td>
<td>1,508</td>
<td>1,702</td>
<td>70,743</td>
<td>1,768</td>
</tr>
<tr>
<td>Migori</td>
<td>628</td>
<td>12,300</td>
<td>538</td>
<td>897</td>
<td>17,210</td>
<td>1,154</td>
</tr>
<tr>
<td>Kajiado</td>
<td>1,024</td>
<td>25,706</td>
<td>574</td>
<td>947</td>
<td>43,085</td>
<td>1,041</td>
</tr>
<tr>
<td>Kirinyaga</td>
<td>1,890</td>
<td>43,612</td>
<td>927</td>
<td>1,979</td>
<td>44,555</td>
<td>945</td>
</tr>
<tr>
<td>Homa bay</td>
<td>618</td>
<td>7,681</td>
<td>247</td>
<td>731</td>
<td>13,806</td>
<td>743</td>
</tr>
<tr>
<td>Meru</td>
<td>761</td>
<td>19,304</td>
<td>579</td>
<td>1,169</td>
<td>18,982</td>
<td>694</td>
</tr>
<tr>
<td>Kisii</td>
<td>542</td>
<td>12,571</td>
<td>437</td>
<td>521</td>
<td>11,473</td>
<td>456</td>
</tr>
<tr>
<td>Bungoma</td>
<td>837</td>
<td>11,793</td>
<td>340</td>
<td>885</td>
<td>15,802</td>
<td>452</td>
</tr>
<tr>
<td>Bomet</td>
<td>218</td>
<td>1,410</td>
<td>55</td>
<td>312</td>
<td>22,000</td>
<td>438</td>
</tr>
<tr>
<td>Transzoia</td>
<td>289</td>
<td>8,624</td>
<td>110</td>
<td>470</td>
<td>10,620</td>
<td>367</td>
</tr>
<tr>
<td>Totals</td>
<td>17,52</td>
<td>378,756</td>
<td>10,442</td>
<td>18,178</td>
<td>407,374</td>
<td>12,354</td>
</tr>
</tbody>
</table>

Source: MOA, 2012

1.2 Problem Statement and Justification

Soil-borne diseases and pests present major challenges to the production of horticultural crops, and other crops grown in open fields and under greenhouses in Kenya. Some of the major soil-borne phytopathogens affecting tomato include; *Ralstonia solanacearum* (bacterial wilt), fungi of the genera; *Pythium* (damping off), *Phytophthora* (rots), *Rhizoctonia*, *Verticillium* (wilt) and *Fusarium*, (wilt and rot). Soil-borne pests include cable-worms and nematodes mostly the *Meloidogyne* spp. (root- knot nematodes).
In Kenya root-knot nematodes are widely spread in all tomato growing areas and due to their effects on the crop they are a concern to both smallholder farmers and commercial producers involved in intensive vegetable production (MOA, 2003). Losses in yields range from 28% to 68% (Adesiyan et al., 1990). The Small-scale farmers fail to recognize nematodes because they are found in the soil and their above ground symptoms can be mistaken for other diseases, nutrient deficiencies and climatic changes especially drought. Their microscopic size makes it more difficult to identify them and farmers are required to dig out the crop to check on the presence of root- knots which is not a common practice. Only about 2.5% of the tomato growers in Kenya recognize nematodes as a production constraint while others associate the failure in tomato yields to diseases (Ouko and Ndun’gu, 2001).

*Fusarium* wilt can cause over 30 to 40% yield loss in tomatoes (Anita and Rabeeth, 2009). Studies done in western Kenya identified *Fusarium* wilt of tomato as one of the major diseases of tomato (Nafula, 2008). In western province the disease has been reported in Busia, Vihiga and Kakamega (Schwartz, 2011), while in central province it was reported in Nairobi, Muranga and Kiambu and also in Embu in Eastern Kenya respectively (Obongoya et.al. 2010). Kenyan farmers do not easily recognize and diagnosis soil borne pests and diseases especially root- knot nematodes and *Fusarium* wilt and this often results in use of inappropriate chemicals (Gowen, 2005; Kariuki et al, 2010), resulting into the increase of the pathogens (Nafula, 2008). This has necessitated farmers into rampant use of chemicals especially in the intensive cultivation of vegetables and ornamental plants (Heitefuss, 1989).

In Kenya, the use of nematicides, fungicides, biological controls, cultural practices and pest resistant varieties to reduce crop losses in management of root- knot nematodes and
Fusarium wilt have been used on a small-scale and often irregularly due to low level of awareness (Kimenju et al., 2008). According to Gowen (2005), only 20% of vegetable producers are using nematicides when growing tomatoes. Nematicides are expensive and not readily available to small scale farmers. Nevertheless preplant soil fumigants such as methyl bromide (bromomethane) that have a broad spectrum of activity have been used extensively to protect high-value crops from soil borne pathogens (Ishikawa et al., 2005) but due to their hazardous effect on the environment and human beings they have been banned. Nematicides have been used to control nematodes with remarkable results but they have been found to be toxic, as well as fungicidal drenches used against Fusarium wilt include; carbendazim, Rindomil®, Ortiva® (Waiganjo et al, 2006). Nematicides (class I), insecticides and fungicides (class II and III) respectively are toxic resulting into environmental hazards and reduced economic benefits.

This calls for management practices that will ensure that no danger arise to the natural ecosystem and target crop and can be used in integrated pest management programmes (IPM). The management practice should also be cheaper and readily available to the farmers.

1.3 Objectives

The overall objective of this study was to Pre-treat seedlings using biological and chemical options for the control of root-knot nematode and Fusarium wilt in tomatoes.

The specific objectives were;

i. To determine the effect of pre-treating tomato seedlings with biological and chemicals agents in the control of root-knot nematodes.
ii. To determine the effect of pre-treating tomato seedlings with biological and chemicals in the control of *Fusarium* wilt.

iii. To determine the synergistic potential of combining botanical and biological agents in the control of root- knot nematodes and *Fusarium* wilt.

### 1.4 Hypothesis

i. Pre-treatment of tomato seedlings in the management of root- knot nematodes will suppress infestation of tomato plants by root- knot nematodes.

ii. Pre-treatment of tomato seedlings in the management of *Fusarium* wilt will suppress infection of tomato plants by *Fusarium* wilt disease of tomatoes.

iii. Synergistic effect of botanical and biological agents will suppress infestation of tomatoes by root- knot nematodes and infection by *Fusarium* spp.
CHAPTER 2: LITERATURE REVIEW

2.1 Tomato production in Kenya

Tomato is one of the most important local market vegetable in Kenya. The crop is mainly grown by small scale farmers in most arable areas in Kenya (Muthoni and Njogu, 2011). It is an important crop for smallholder growers and a major source of livelihood and employment for a majority of the farmers who cannot afford huge capital to invest in other cash crops (Kariuki et al., 2010). The total area under tomatoes in the year 2011 was 18,178 Ha with a production of 407,374 M tons. The main counties that produced the bulk of tomatoes were Taita Taveta (14.3%), Migori (9.3%), Kajiado (8.4%), Kirinyaga (7.6%), Homa Bay (6.0%) and Meru (5.6%). Other Counties with untapped potential include Turkana, Isiolo, Tharaka-Nithi and Tana-River (MOA, 2011).

In Kenya, tomatoes are grown either under irrigation or during the rainy season, and of late, in greenhouses. The main forms of irrigation practiced are furrow and sprinkler irrigation, and most recent drip irrigation (Waiganjo et al., 2006). The crop is fairly adaptable and grows well in warm conditions. The optimum diurnal temperatures are 20-27°C day time and 15-17°C at night. Production of tomato in the Kenyan highlands under field conditions has been difficult due to low temperatures (16 to 19°C) and high humidity, greenhouse production is therefore encouraged since it offers warmer conditions that promote faster growth of tomatoes for year-round supply (Kirimi et al., 2012). In the semi-arid regions of Kenya, the crop has the advantage of high temperature and reduced humidity that leads to both high fruit set and yields (MOA, 2003). Production of tomato during the dry season is by irrigation (Kirimi et al., 2012). Erratic irrigation on the other hand may cause cracking and splitting of the fruit skin. Un-even levels of water application, combined with inadequate calcium and potassium in the soil may lead to physiological disorders like blossom end rot (MOA, 2003).
Tomato can be grown in many soil types ranging from sandy loam to clay-loam soils that are rich in organic matter. The ideal soil pH range is 6 to 6.5, higher or lower pH can cause mineral deficiencies or toxicities.

2.2 Constraints in tomato production

The production of tomatoes is faced by various challenges which include high labor costs, high cost of farm inputs, and marketing challenges, nutritional disorders, weeds and pests and diseases and high post-harvest losses (Waiganjo et al., 2006; MOA 2011). Farmers have complained that the cost of farm inputs and labour in tomato production are very high, leaving small profit margins to the farmers (Waiganjo et al., 2006). The labour used on tomato production is either hired or family labour. The labour costs on tomato are mainly for land preparation, weeding, staking/training and harvesting. Labour availability and costs is a major constrains to farmers especially during harvesting (Waiganjo et al., 2006).

Tomato marketing has posed as a major problem faced by farmers. Prices for tomato vary throughout the year depending on the supply and demand. The tomatoes are channelled from the farm-gate to the whole sale markets or retail markets either directly or through middlemen/brokers. The retailers include groceries, supermarkets especially in the urban areas and open air markets in both urban and rural areas. The major problem faced by the farmers is exploitation by the brokers or middle men. Post-harvest losses are high, mainly due to the high perishability of the crop, tomato rejection during sale mainly due to glut leading to poor market prices, pests and diseases, unmarketable small sizes of fruits and other causes including blossom end-rot, lack of market, and non-preference of fruit size or variety (Waiganjo et al., 2006).
Tomato can have several nutritional disorders caused by micronutrient; their symptoms may occur on leaves, stems, or fruit. Among the micronutrient nitrogen (N) is the most limiting nutrient to crop production (Pionke et al., 1990). Like many vegetables, tomato is often heavily fertilized. Large amounts of nitrogen are often lost to leaching below the root-zone of vegetable crops (Pionke et al., 1990). Nitrogen fertilization, along with early season weed control, allows rapid crop establishment and growth, which is critical for the crop to suppress late-emergence weeds (Itulya et al., 1997). Nitrogen deficiency causes restriction in growth rate and uniform chlorosis on the oldest leaves, while excess nitrogen may cause poorly coloured, puffy fruits. Phosphorous (P) deficiency causes purple colouration of the tomato plant. The plant is dwarfed with stiff, often upright leaves that are light green to yellow in the upper side and purple on the underside. Potassium (K) deficiency is characterized by marginal necrosis of older leaves. The necrosis is preceded by scattered small, chlorotic areas near the leaf margin, which enlarge, coalesce and finally become necrotic. While calcium (Ca) deficiency manifests itself as blossom- end rot of the tomato fruit (MOA, 2003).

The major tomato production diseases reported in Kenya include bacterial wilt, early and late blight, leaf curl, tomato spotted wilt virus, leaf spot, powdery mildew, and *Fusarium* wilt (Varela et al., 2003; Waiganjo et al., 2006). Insect pests and other arthropods such as spider mites, thrips, white flies and African bollworm and root- knot nematodes are also a major production constraint in tomato production (Hanson et al., 2001). Severity of these diseases depends on prevailing environmental conditions, type of irrigation system used, the pathogen strains and the tomato varieties grown. Farmers have been using pesticides to control pests and diseases and the frequency of pesticide application varies from one to forty times per season, depending on the pre harvest interval and target pest (Waiganjo et al., 2006). The
recommended control measures include use of chemicals, crop rotations and other cultural practices, and use of disease resistant cultivars coupled with field sanitation, these management practices when used together can be instrumental in alleviating disease constraints in production of tomatoes in Kenya. The fungal diseases identified are controlled using fungicides such as Milraz®, Ridomil®, and Bordeaux mixture while bacterial wilt is managed by rogueing the affected plants and applying lime on the infected holes to prevent the disease from spreading to healthy plants. Pests can be controlled with insecticides such as Karate®, Diazinon® and Bulldock® (Kirimi et al., 2011), while nematicides have been used for control of nematodes. Resistant varieties are limited by the fact that most Kenyan farmers consider yield and pest resistance before they can choose a variety for planting and according to Musyoki et al., (2006), market demand and shelf life are the strongest criteria for varietal selection. Crop rotation on the other hand is limited by skills on selection of crops, the declining land sizes and the marketability of the alternate crop.

2.3 Root- knot nematode in vegetable production

Nematodes are tiny, worm-like, multicellular animals. The number of nematode species is estimated at half a million, many of which are free-living types found in the oceans, in freshwater habitats, and in soils. Plant parasitic species form a smaller group (Dufour et al., 2003). Nematode populations are generally denser and more prevalent in the world’s warmer regions, where longer growing seasons extend feeding periods and increase reproductive rates (Dropkin, 1980). Unlike other pathogens, nematodes are more challenging to control because they live in the soil and cannot be easily seen by farmers (Mai, 1977). They are only noticed when the population is widespread and yield is very low. Root- knot nematodes are one of the major pathogens of tomato worldwide, causing stunted growth and poor fruit production (Sikora and Fernandez, 2005).
2.3.1 Causal agent of root-knots

Root-knot nematodes belong to polyphagous group of highly adapted obligate plant pathogens that have wide host range, infecting more than 5,500 plant species (Trudgill and Blok, 2001). Root-knots in tomatoes are caused by nematodes particularly *Meloidogyne incognita, M. Javanica, M. Hapla, M. arenaria* (Rahma, 2003; Waiganjo *et. al.*, 2006).

2.3.2 Biology of root-knot nematodes, *Meloidogyne* spp.

The females lay eggs on plant tissue or soil, hatching of *Meloidogyne* eggs is temperature driven and occurs without requiring stimulus from plant roots however, root diffusates may sometimes stimulate hatching (Karssen and Moens, 2006). The root-knot nematode has four larval stages, the Juveniles of *Meloidogyne* hatch from eggs as vermiform second stage juveniles (*J*₂), the first moult having occurred within the egg (Rahman, 2003). Newly hatched juveniles have a short free-living stage in the soil, and in the rhizosphere of the host plant. They may re-invade the host plant of their parent or migrate through the soil to a new host. The juveniles enter the plant through the root tips and feed on the plant cells, the surrounding root tissue gives rise to a gall in which the developing juvenile is embedded. After further feeding the juvenile undergo morphological changes and moult three times and eventually become adults. The life cycle of root-knot nematodes is 4 to 8 weeks, depending on temperatures and an adult female may produce up to 2000 eggs (Noling, 2010). Females can continue egg laying after harvest of aerial parts of the plant, the survival stage between crops is generally within the egg being protected by a gelatinous matrix.
2.3.3 Host range of root- knot nematodes

Root-knot nematodes have a very wide host range and growers who have a root-knot nematode problem may find it difficult to control the nematode and its damage through crop rotation due to this wide host range and the fact that species like *M. incognita*, *M. arenaria* race 1, and *M. javanica* which affect alternate hosts are usually found in the same fields. There is also a high degree of specialisation in addition to differences in pathogenicity on specific crop (Mitkowski and Abawi, 2003).

2.3.4 Factors favouring nematode survival

Important environmental factors that influence development of *Meloidogyne* are moist well aerated sandy soils and relatively warm temperatures. Nematodes are most serious on light, sandy soils and in furrow irrigated areas where there is continuous growing of susceptible crops (no rotation), infected volunteer plants, weeds in the fields, farm yard manure and loam soil. The degree of infestation will depend on the nematode species involved, interaction between the nematode and other soil pathogens, and the susceptibility of the cultivar (Talwana et al., 2003). The life cycle of root- knot nematodes is dependent on temperature, and a new generation can arise within 25 days, but under less favorable conditions, the time may be prolonged to 30 to 40 days. Depending on the species plant penetration by root-knot nematodes can occur between 10 and 35°C while at temperatures lower than 14.2°C or higher than 31.7°C, nematodes do not lay eggs (Khanna et al., 2006).

2.3.5 Mode of spread of root- knot nematode

Root - knot nematodes are soil inhabitants and are only able to move not more than a meter through the soil within their lifetime. Their movement from one field to another or within the same field is aided by any process that moves soil or plant tissue and has the ability to disperse plant nematodes these include; farm equipment, muddy shoes contaminated with
nematode infested soil, water during floods and irrigation. The movement of nematode infected plants, seeds, and bulbs can also disperse nematodes internationally if plant quarantine is not well observed (Lambert and Bekal. 2002). The ability of nematodes to form environmentally resistant stages makes their dissemination easy since dried nematodes can be blown by the wind or plant debris over large geographical regions, migrating birds can also carry nematodes (Noel, 1992).

2.3.6 Symptoms of root-knot nematode infestation.

Plants infested with root-knot nematodes may show slow or stunted growth (Noling, 2010). In the field they are distributed in oval patches or elongated patches depending on the direction of cultivation. The leaves show wilting, yellowing or chlorosis and there is premature dropping of fruits and flowers and malformed fruits, infected plants are likely to wilt earlier and excessively under temperature or moisture stress (Mulrooney, 2012). Infestations may also occur without causing any above-ground symptoms, the typical damage caused through feeding by root-knot nematode are different sized galls formed on the plant roots (Budai et al., 2005) or knots on roots from where the nematodes derive their name (Van et al., 2003). All species of *Meloidogyne* cause galls but they vary in shape and size (Karssen and Moens, 2006). The galls obstruct water and nutrient uptake (Budai et al., 2005), and also increase the susceptibility of the root system to invasion by disease causing fungi and bacteria (Rahman, 2003).

2.3.7 Management of root-knot nematodes

Plant protection against nematodes is difficult because nematodes cannot be eradicated completely from the field (Budai et al., 2005), a nematode management strategy therefore need to involve manipulation of nematode densities to non-injurious and sub-economical
threshold levels (Viaene et al., 2006). Various techniques have been used to reduce nematode levels in the soil, these measures include; prevention of introduction and spread of nematodes, cultural practices of crop protection, applications of organic matter and green manure, chemical control, organic amendments and green manure and biological control measures (Stirling, 1991; Rahman, 2003).

Prevention of nematodes, introduction and spread is done through disinfecting contaminated tools, hands, clothing or shoes of farm workers, disinfecting areas where transplants are grown, sanitation of greenhouse structures, crates, benches, tools, use of sterilized soil, locating seed beds away from infested fields, and procuring transplants from reputable sources and by irrigation water (Rahman, 2003; Cerkauskas, 2005; Mulrooney 2012; Van et al., 2012).

**2.3.7.1 Cultural practices for the management of root-knot nematodes**

Cultural practices of crop protection include crop rotation, fallow cropping, resistant and tolerant varieties. Crop rotation can be achieved by alternating poor hosts, non-hosts or resistant crops with susceptible or tolerant crops (Swamy et al., 1995). Poor host plants include maize, peas, onions, wheat, Rhodes grass and asparagus. Studies done on plants such as *Tagetes* spp., *Crotalaria* spp., *Asparagus* spp., sesame and neem found out that they are antagonistic to root-knot nematodes due to release of root exudates that are toxic to the nematodes (Vargs-ayala et al., 2000). Crop rotation have its own limitations because the suitability of the crop is determined by its efficiency in suppression of the nematode and the economic returns, most of the studied crops are of low commercial value and this becomes a major hindrance especially in commercial agriculture (Johnson et al., 1992). The challenge to research is therefore, to identify nematode suppressive crops that satisfy the economic considerations in crop production.
Otipa et al., (2003) demonstrated that there are beneficial crops such as cotton, desmodium, Rhodes grass, sorghum, sweet corn, alstroemeria, capsicum and peanuts which were suppressive to root-knot nematodes under greenhouse and field conditions, further testing of rotational crops before implementation of the rotation programme is necessary since some of the alternate crops may be unknown to the farmers, are expensive to grow and may enhance multiplication of other pathogenic nematodes. Nevertheless plant resistance can be cost effective and environmentally friendly and can be used extensively for crop protection against many pests and pathogens (Bridge, 1996). Other cultural control methods that can be practised include; desiccation and flooding. In intensive cultivation heating of the soil by solarisation or by electricity can be used as a management strategy but cultural practices do not have full control of the nematodes, there is therefore need to use other feasible methods (Mulrooney, 2012).

2.3.7.2 Chemical control of root- knot nematodes

Chemical control of root - knot nematodes have primarily been achieved through nematicides which can be non-fumigants or fumigants (Widmer and Abawi, 2000; Rahman, 2003). Non fumigants are applied during planting and are systemic affecting the nematodes behaviour. Examples are Nemacur®, Aldicarp®, Oxamyl®, and Carbofuran®. Fumigants are volatile liquids that are fast acting and dissolve in the soil killing the nematodes and their eggs. Examples of fumigants are 1, 3 dichloropropene methyl bromide, ethylene, Metham sodium® and Dazomet®. Although chemical nematicides have been widely used in commercial agriculture to control nematodes, they are both highly toxic and very expensive (Van et al., 2012), leading to banning of some fumigants like methyl bromide. They are also eco-friendly and may in the long run cause serious threat to the environment (Taouseef et al.,
2011). In this respect chemical nematicides are currently being appraised with respect to environmental hazards, human health, and development of nematicide resistance. These significant drawbacks of chemical use, has emphasized the need for researchers, to invent into alternative strategies for management of root-knot nematodes.

2.3.7.3 Organic amendments in management of root-knot nematodes

Organic amendments and green manure are potential alternatives to the harmful chemical control means currently used against plant-parasitic nematode and have been found to reduce plant feeding nematodes and increase tomato yields (Rahman 2003; Pakeerathan et al., 2009; Hassan et al., 2010; Mulrooy, 2012). They have also been found to release nutrients and improve the water holding capacity of the soil, and build-up of beneficial micro flora thereby improving plant growth and enhancing tolerance to nematode attack. Other advantages of antagonistic plants are their biodegradability, selective toxicity to target pests, safety to non-target organisms and environment (Bridge, 1996; Oka et al., 2007).

Use of these organic amendments is limited by the large quantities required for effective management and their availability (Bridge, 1996). Some of the soil amendments that can be used are manure from domestic animals, sewage sludge, refuse dump, rice husks, saw dust, crop residues, cotton seed hulls and oil cake (Hassan et al., 2010). Green manures of cauliflower leaves, chopped pineapple leaves, dry rice straw, oats or rye, cabbage, clove bud, cotton, Tagetes minuta, Leucaena Leucocephalata, Desmodium uncinatum, and Crotolalia juncea, have also been reported to reduce the incidence of root-knot nematodes in the field (Akhtar and Mashkoor, 1993; Widmer and Abawi, 2000; Kimenju et al., 2008).
2.3.7.4. Botanical control of root-knot nematodes

Various researchers have reported on the management of root-knot nematodes on tomato by utilizing plant products (Goswami and Vijayalakshmi, 1981; Zaki and Bhatti, 1989; Philippe et al., 2004). Several plants belonging to different botanical families have been found to contain nematicidal effects and investigations on various indigenous plants and neem (A. indica) products have revealed that they are effective against insects and nematodes (Sharma, 2000). Kumar and Khama (2006) tested the effect of five neem based nematicide products and found them to suppress nematode multiplication and improve plant growth. Neem tree; A. indica and chinaberry tree; M. azadirachta have been used as nematicides for their pesticidal, antifungal, and anti feedant properties, A. Indica also increased plant growth and yields (Akhtar et al., 2005). Studies done by Abid (1996) and by Jourand et al., (2004), showed that use of neem, mustard, sesame and castor oil cakes significantly reduced root galls in tomato, brinjal, mung bean, sponge gourd and okra and also increased plant growth.

Use of botanicals as nematicidal and nematostatic products are economical and eco-friendly and in order to make its application cost effective, an attempt was made with seed treatment and seedling dip treatment of plant products against root-knot nematode infestation in tomato crop (Anita and Rabeth, 2009). Other crops that can be used for management of nematodes include; Marigolds; Targetes sp., rattle box; Crotalaris spectabilis, Chrysanthemums spp, and castor bean; Riccinus communis (Iruthaya and Aruna, 2011).

2.3.7.5 Biological control of root-knot nematodes

Biological control has become a promising alternative to the use of chemicals (Stirling (1991), but not as a replacement because it is slow acting, their efficacy is controlled by the environment, time of application and their multiplication rate. It refers to the purposeful
utilization of introduced or resident living organisms, other than disease resistant host plants to suppress the activities and populations of one or more plant pathogens (Barker and Koenning, 1998).

According to Atkins et al., (2005), the egg- pathogenic fungus \textit{Paecilomyces lilacinous} is the most widely tested biological measure for plant parasitic nematodes, and has shown promising potential as an alternative to chemical control at both pre-planting and planting applications. \textit{P. lilacinus} significantly reduced \textit{M. incognita} soil and root population and increased tomato production (Kalele et al., 2010). Studies done by Baharullah et al., 2008, using various concentrations of \textit{Trichoderma harzianum} on root- knot nematode, \textit{Meloidogyne javanica} showed that the antagonistic activity of \textit{Trichoderma harzianum} make it an appealing alternative to chemical use. Nasrinasab et al., (2010) reported that \textit{T. harzianum} is an effective egg parasite of \textit{M.incognita} because \textit{T. harzianum} were able to grow on the egg surface and penetrated the egg cell reducing the incidence of root- knot nematodes and increasing plant growth through enhanced root growth.

\textbf{2.4 Fusarium wilt of Tomato}

\textbf{2.4.1 Causal agent of Fusarium wilt of tomato}

\textit{Fusarium} wilt of tomato is caused by \textit{Fusarium oxysporum} f. sp. \textit{lycopersici} (Sacc.) which is a soil borne plant pathogen in the class Hyphomycetes. The fungus consist of serious pathogenic strains, that infects plants roots at all stages of plant growth causing economic losses (Houssien et al.,2010). The pathogen penetrates through the roots mainly through wounds and proceeds into the vascular system leading to the collapse of the system. This destroys the root of the plants causing reduction in yields of up to 40\% (Suárez-Estrella et al., 2007) especially on susceptible varieties when the soil and air temperatures are high.
(Houssien et al., 2010). The disease is widely distributed in Kenya especially in Eastern province, Central Province, Nairobi and coast province. It is also prevalent in western province where it was introduced with bananas from Uganda and has caused a lot of damage in greenhouses and the open field where farmers have confused it with bacterial wilt and other abiotic factors (MOA, 2010).

2.4.2. Host range of Fusarium wilt disease

_Fusarium oxysporum_ affects a wide variety of hosts, Some of the most susceptible crops are potatoes, tomato, bananas, tobacco, peas, lentils, muskmelon, water melon, and sweet potatoes, legumes, peppers, egg plant (Netzwerk, 2010; Egel and Martyn, 2013). There are over 100 formae speciales each within the species which are host-specific. Example; _F. oxysporum_ f. sp. _batatas_ affects sweet potato; _F. oxysporum_ f. sp. _cubense_ causes Panama disease on banana. _F. oxysporum_ f. sp. _lycopersici_ causes vascular wilt in tomato. _F. melonis_ attacks muskmelon and cantaloupe. Cob rots in maize, cause mainly by _F. graminearum_ and _F. verticillioides, F.Cubense_ causes _Fusarium_ wilt of banana (Erwin and Ribeiro, 1996).

2.4.3. _Fusarium_ wilt disease development

The fungus penetrates the root of the plant and invades the xylem vessels, by fungal mycelium (Iannoti et al., 2012), with occurrence of unfavourable conditions and in the absence of a host, the fungus forms chlamydospores in the soil. Germination of chlamydospores is stimulated by nutrients from host root exudates or extraneous food base introduced into the soil (Varela and Seif, 2004). The mycelium invades the root tips or enters through root wounds and advances through the root cortex to the xylem and grows by production of micro conidia which germinate to cause new infection points. This invasion blocks the xylem vessels and the plant begins to wilt. The fungus spreads through the plant and invades the parenchymatous tissue and reaches the host surface and sporulates profusely.
and the spores are blown by wind or carried by water and continues to infect neighbouring plants (Narla, 2010).

2.4.4 Factors that favour *Fusarium* wilt disease development

The ideal conditions for *Fusarium* wilt development include warm, dry weather with temperatures between 25 and 32° C, and acidic soils of PH 5–5.6 (Cerkauskas, 2005; Iannoti 2012), the wilt is also severe in light sandy soils (Egel and Martyn, 2013). Since the fungus produces resting spores, it can survive in the soil indefinitely even when no host plants are grown. The fungus can also survive in fibrous roots of weeds such as *Amaranthus*, *Digitaria* and *Malva*. *Fusarium* wilt disease development is also promoted by presence of Ammonium nitrate and urea in the soil and by infestation of the plant by root-knot nematodes (Rahman, 2003).

2.4.5 Symptoms of *Fusarium* wilt disease of tomato

Symptoms of *Fusarium* wilt are dependent on the susceptibility of the plant, amount of inoculum in the soil, environmental factors and nutrients particularly nitrogen, (Egel and Martyn, 2013). The pathogen enters the plant through the roots mainly through wounds and is then spread throughout the plant by the vascular system leading to wilting and death of the infected plant (Steinkellner *et al.*, 2005). The tissues near the soil base show brownish colour starting about 1/8 inch below the bark. The stem of a wilted plant shows no soft decay, but if sliced, brown discoloration of the water-conducting tissue is evident between the pith and the outer green part of the stem. In the roots, root systems of the tomato plants appear partially or totally reddish-brown (Edmund and Potter, 2009). Yellowing begins on the lower tomato leaves, which then turn a dingy brown and begin to wilt and progress upwards (Gleason and Edmund, 2006; Edmund and Potter, 2009). The oldest leaves turn yellow and begin to droop. Often, only the leaves on one side of the stem turn yellow and wilting only occurs during the
hottest part of the day (Cerkauskas, 2005; Iannoti et al., 2012). As the disease progresses, yellowing and wilting continue up the stem until all of the foliage is killed and the stem dies. If the disease attacks the plant early in the season and the air temperatures are above 27°C for a long period, there may be little or no normal fruit formation. Plants attacked later in the growing season, may have the lower fruit clusters normal, but those on the upper part are small and inferior (Snyder and Hans, 2003).

2.4.6 Dissemination of *Fusarium* wilt disease

The fungus is disseminated by infected seed, plant transplants grown in infested soil, and can also be introduced into a field by contaminated equipment, training stakes, packing crates or shoes (Gleason and Edmund, 2006; Iannoti et al., 2012). Soil particles from infested fields may also be carried into disease-free fields by wind or water (Cerkauskas, 2005). The pathogen can be transported long distance through seed and plant transplants (Yun Wong, 2003). Plant parasitic nematodes may enhance infection through wounding of the plant favouring entrance of pathogens into the plant (Rahman, 2003).

2.4.7 Management of *Fusarium* wilt disease of tomato

*Fusarium* wilt of tomatoes is managed through various measures which include the use of cultural control, resistant varieties, chemical control and biological control. The best control method found for *F. oxysporum* is planting resistant varieties, although not all have been bred for every forma specialis (Lambart and white, 1997).

2.4.7.1 Chemical control of *Fusarium* wilt disease

Chemical control measures involve using soil and systemic fungicides to eradicate the disease from the soil, the most effective method in preventing tomato from *Fusarium* wilt was found to be mixing of tomato seeds with chemical fungicides (Widmer and Abawi, 2000). However,
chemical fungicides are harmful to human health and other living organisms and reduce soil microorganisms on repeated applications (Lewis et al., 1996; Akila, 2011). Some of the fungicides used are; benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin.

2.4.7.2 Cultural control of Fusarium wilt disease

Cultural control strategies may include improvement of soil conditions because *F. oxysporum* spreads faster through soils that have high moisture and poor drainage; this requires that tomatoes are grown in well-drained soils. Other measures include removal and destruction of infected plant materials from the field, seed beds and in green houses to prevent overwintering of the disease. Flood fallowing, use of clean seeds and seedlings and resistant varieties (Akila et al., 2011). A four year crop rotation with non-host plants can be utilised as a management measure but due to the small scale land sizes and limited economic enterprise diversity it may not be applicable under small scale farming in Kenya. Disinfesting soil by treating with steam can also be used (Gleason and Edmunds, 2006). According to Brayford, (1992) cultural control measures have been found to be difficult to apply and less effective hence other strategies aiming at replacement of hazardous chemical pesticides with friendly control methods are necessary.

2.4.7.3 Bio control of Fusarium wilt disease

Most of the available approaches for biocontrol of plant diseases are involved in the use of a single biocontrol agent to a single pathogen, this has led to inconsistent performance of biocontrol agents and poor activity in the soil environments (Raupach and Kloeppep, 1998). To overcome these problems, applications of mixtures of biocontrol agents having multiple mode of actions are advocated particularly under field conditions, where they are highly influenced by abiotic and biotic conditions (Duffy et al., 1996; Raupach and Kloeppep, 1998;
Guetsky et al., 2001). Integration of biocontrol with agronomic practices may also improve the efficacy of the biocontrol organisms and the health of the host plants, which may be sensitive to environmental changes. Several studies have indicated that botanicals with anti-fungal compounds can be used. They are advantageous due to their low mammalian toxicity, low concentrations of active ingredients and biodegradability (Harish et al., 2008).

Various studies have been done by scientists on use of bio control agents; Kagale et al., (2004) found that extract of Datura metel was able to reduce mycelia growth of Rhizoctonia solani and its leaf extract was found to be antifungal against leaf spot and rust pathogens. Shervin et al., (2011) in their study on effect of neem on Fusarium wilt of tomato found that neem was able to increase the biomass of tomato plants and reduce the symptoms of Fusarium wilt through reduction of mycelia growth while, Obongoya et al., (2010) on the study on use of crude plant extracts against soil borne diseases found that neem extract, tobacco, mexican marigold and peri – winkle controlled Fusarium oxysporum schl. f sp.phaseoli of common beans. Research findings on banana on a combination of botanical formulations with fungicidal effect and two bio control agents (Pseudomonas fluorescence and Bacillus subtillis) indicated that they were able to reduce Fusarium wilt incidence in bananas (Akila et al.,2011). Compost has also been reviewed extensively by various authors and can been used as an alternative method for disease control (suarez – Estrella et al., 2004).A combination of vegetable compost and Posidonia oceanic mixture was found to completely inhibit Fusarium wilt in tomatoes (Kouki et. al., 2012).
2.4.7.4 Biological control of *Fusarium* wilt disease

Biological control of *Fusarium* wilt involves inundative (massive) inoculation of propagules of an antagonist isolate (Fuchs *et al*., 1997), and can be used means to achieve sustainable crop protection which is less reliant on chemicals (Anitha *et al*., 2009). Their limitation is their availability, they are expensive and it’s hard to get the massive quantities required and the skills for application (Fuchs *et al*., 1997). Successful biological control systems commonly employ naturally occurring, antagonistic microorganisms that are able to reduce the activities of plant pathogens. They compete with pathogens for nutrients, inhibit pathogen growth by secreting antibiotics, or reduce pathogen populations through parasitism. In addition, some of these microorganisms induce resistance in host plants, which enhances the plant’s ability to defend itself from pathogen attack (Adams, 1990).

Numerous studies have demonstrated reduced incidence of diseases in different crops after supplementing the soils with fungal or bacterial antagonists (Singh *et al*., 2002; Akrami *et al*., 2011; Ahmed, 2011). This has resulted in accelerated commercialization of fungal biological control products (Fravel *et al*., 2003). Non-pathogenic strains of *Fusarium* spp. have been found to suppress soil borne diseases caused by pathogenic *Fusarium* spp. under greenhouse and field conditions (Miwa *et al*., 2005). Several isolates of non-pathogenic *Fusarium* spp. have also been identified to control *Fusarium* wilt of tomato and water melon (Larkin and Fravel, 2005). Inam-Ul-Haq *et al*., (2007); Srinivasan *et al*., (2009) on their research on bacterial isolates associated with entomopathogenic nematodes (*Xenorhabdus nematophila* and *Xenorhabdus* spp) and from used rock wood (*Pseudomonas genus*) respectively found that these bacteria had increased the tomato plant biomass and were antagonistic on *Fusarium Oxysporum f.sp., lycopersici*.  

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Several studies have investigated the ability of *P. fluorescens* to suppress *Fusarium* wilt disease; Fluorescent pseudomonad species such as *Pseudomonas fluorescens* (Sakthivel and Gnanamanickam 1987; Asha et al., 2011), *Pseudomonas putida* (de Freitas and Germida, 1991) and *Pseudomonas chlororaphis* (Chin-A-Woeng et al., 1998) have been used to suppress pathogens as well as to promote growth and yield in many crop plants. Sivamani and Gnanamanickam, (1988) reported that the seedlings of bananas treated with *P. fluorescens* showed less severe wilting and internal discoloration, better root growth and enhanced plant height after *Fusarium* infection. Several reports have also previously demonstrated the successful use of different species of *Trichoderma*, *Pseudomonas*, *Streptomyces*, and nonpathogenic *Fusarium* of both rhizospheric and endophytic in nature against *Fusarium* wilt disease under both glasshouse and field conditions (Lemanceau and Alabouvette, 1991; Alabouvette et al., 1993).

*Trichoderma* species are free living fungi that are common in the soil and root eco system and have been found to produce antibiotics and parasite other free living fungi and compete with deleterious plant micro-organism (Harman et al., 2004a). *Trichoderma* spp. was also found to stimulate plant growth (Harman et al., 2004a) and effectively suppress *Fusarium* wilt pathogens several reports indicate that *Trichoderma* (Sivan and Chet, 1986; Thangavelu et al., 2004). *T. harzianum* was also found to reduce wilt and nematode populations of *M. incognita* by reducing root lesions and galling index (Thangavelu, 2002). Raghuchander et al.,(1997) while *Trichoderma viride* was found to Induce defense related enzymes, and produce antibiotics and reduce the external and internal symptoms of *Fusarium* wilt disease (Thangavelu and Mustaffa, 2010).
Bacillus subtilis has been identified as a potential biological control agent. These strains produce a wide range of antifungal compounds, and some enzymes, which can degrade fungal cell wall (Berg et al., 2001). This plays a major role in plant disease suppression (Leelasuphakul et al., 2006). In addition, some antagonistic mechanisms of these Bacillus species are involved in the competition for nutrients, space and induction of plant resistance (Guerra-Cantera et al., 2005). Actinomycetes particularly Streptomyces spp. are important soil dwelling micro-organisms which are saprophytic, and spend majority of their life cycle as spores and are best known for their ability to produce antibiotics. They influence plant growth and protect plant roots against invasion by root pathogenic fungi (Crawford et al., 1993). Streptomyces species have been used extensively in the biological control of several formae speciales of F. oxysporum, which cause wilt disease in many plant species (Reddi and Rao, 1971; Lahdenpera and Oy, 1987; Smith et al., 1990). Dipping of roots in Streptomyces griseus was found to be effective against Fusarium wilt (Anitha and Rabeeth 2009). Fusarium wilt of tomatoes was also found to be controlled by bioformulation of Streptomyces griseus in green house conditions.
CHAPTER 3: MATERIALS AND METHODS

3.1 Raising and transplanting of tomato seedlings

Tomato seedlings of money maker variety were raised in a green house on seedling trays using coco peat as the planting media. The seedlings were fertigated with water containing a starter fertilizer (NPK, 19:19:0) at the rate of one gram per litre of water. Seedlings were transplanted at four weeks after sowing. The seedlings were carefully lifted from the tray and drenched with the treatments before transplanting into pots containing 2 kg of sterilized mixture of soil and sand at a ratio of 1: 2 each and two grams of di-ammonium phosphate (DAP, 18:18:0) fertilizer at a rate of 1g/kg growth media to enhance root development.

3.2 Extraction and preparation of root- knot nematode inoculum

Root- knot nematode inoculum was extracted from infested spinach roots by maceration method described by (Stetina et al., 1997). The roots were cleaned and blotted dry, cut into 2mm pieces and transferred to a one litter glass blender. They were then macerated in 100ml water for six seconds. The suspension was then sieved using three sieves of 0.0165inch, 0.0035inch and 0.0014 inch sieves respectively and the nematodes were held into a small beaker. One ml of aliquot was placed in a nematode counter and the nematodes were observed 40 mm magnified microscope and counted. The average nematode count per one ml was 1,000 eggs/juveniles each of the experiment pot was inoculated with 3 ml of inoculum which was equivalent to 3,000 eggs/juveniles per pot.

3.3 Isolation and culture of Fusarium wilt pathogen

Isolation of the fungus was done from diseased tomato plants obtained from farmer’s field in Embu, Kirinyaga and Nairobi Counties. The diseased plant stem base was cut into 5mm sections and then surface sterilized in 2.5% sodium hypochlorite for three minutes. They
were then rinsed in distilled water and dried with sterile tissue. Four sections were aseptically placed on each plate containing potato dextrose agar (PDA) and incubated at 25°C for 7 days. Pure cultures of the fungus were obtained by transferring mycelial tips on to fresh PDA and allowed to grow for 10 days. The fungal inoculum was then harvested by flooding the cultures with sterile distilled water and scraping the mycelia with a sterile glass rod followed by sieving through muslin cloth and standardized to $1.0 \times 10^6$ spores per millilitre using a haemocytometer.

3.4 Determination of the effect of Pre-treatment of tomato seedlings with biological and chemical agents for control of root-knot nematodes

3.4.1 Experimental design and layout

Green house experiment was conducted for two crop cycles at Faculty of Agriculture, University of Nairobi. The growth media consisted of potted sterilized soil and sand at a ratio of 2:1 inoculated with 3000 eggs/juveniles of root knot nematodes 7 days before administering the treatments. After transplanting the seedling were pre-treated with the following agents; (i) Marshal® 250 Ec whose active ingredients were 250g of carbosulfan a (chemical agent procured from Juanco SPS limited) at a rate of 2.5 ml/litre (ii) Neemraj® which is derived from neem kernel whose active ingredient is Azadiractin 0.30% Ec, (a botanical agent procured from Amiran Kenya limited) at the rate of 2.5 ml/litre of water (iii) Pl plus® a biological agent which contains spores of a fungus, Paecilomyces lilacinus (procured from Juanco SPS limited) at the rate of 1.9gm / litre of water. The controls were unpre treated seedlings in growth media containing (i) water alone, (ii) Root-knot nematode inoculum. Each pot was planted four seedlings. Each experiment was replicated four times, and experiment was laid out in a randomized complete design and watering was done daily throughout the duration of the experiment. Data on plant height was collected every week for six weeks. The experiment was terminated six weeks after transplanting. The plants were
carefully lifted and soil removed from the roots for the determination of galling index, dry weight, and nematode population in potting media at the end of experiment. Dry matter was determined by drying shoots and roots from each treatment in an oven at 60°C until constant dry weight was achieved.

3.4.2 Assessment of root-knot nematode infestation on roots and soil

After termination of the experiment, all the plants were carefully pulled out, separated into shoot and roots. The root system was washed and number of galls per plant were counted. The root gall severity was rated on a 0-5 scale described by Taylor & Sasser (1978), where; 0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; and 5 more than 100 galls. The Juveniles (J2) in the soil were extracted from the growth media using the following modified extraction tray method described by Whitehead and Hemming (1965). The potting media samples were collected from each treatment from around the plant roots and 100 g from each pot was placed on two layers of filter paper tissue and placed on top of a 38 µm sieve. Water was added to the pan so that the soil and the mesh are slightly covered with water. Nematodes were allowed to filter through the sieve by gravity for 24 hours. The aliquot was collected in 25ml bottles. 1 ml aliquot transferred to a nematode counting slide and observed under a microscope at 40µm magnification to count the number of nematodes. The number of Juveniles (J2) was expressed per 100 grams of soil.
3.5 Determination of the effect of Pre-treatment of tomato seedlings with biological and chemical agents for control of Fusarium wilt

3.5.1 Experimental design and layout

The experiment consisted of 2kg potted sterilized growth media composed of soil and sand at a ratio of 2:1. Each pot was inoculated with the Fusarium wilt pathogen spore suspension at a concentration of $1.0 \times 10^6$ ml at the rate of 5% (100ml) of the weight of the growth media 7 days before transplanting the seedlings. The seedlings were carefully uprooted from the seedling trays and their roots were dipped in the following treatments (i) Root guard® which contains a mixture of Trichoderma, Bacillus, Aspergillus, Chaetomium, Escherichia, Azotobacter spp. as biological control agent procured from Juanco SPS limited at the rate of 10gms/ litre of water, (ii) Neemraj® which is derived from neem kernel whose active ingredient is Azadiractin 0.3% Ec (a botanical agent procured from Amiran Kenya Ltd) at the rate of 2.5 ml/litre of water, (iii) Phosphite® a fungicide whose active ingredient is potassium phosphate 53% at the rate of 5ml/litre of water. Controls consisted of seedlings grown in growth media (i) innoculated with Fusarium wilt pathogen and (ii) water only. The seedlings were then transplanted into the growth media. Each treatment was replicated four times; each replicate consisting of one treated pot containing 4 plants each. The experiment was laid out in a randomized complete design.

3.5.2 Assessment of Fusarium wilt infection incidence and severity

Data on yellow and dead leaves was collected on 2nd, 4th and 6th week after transplanting. At the 6th week, the plants were carefully lifted and soil washed off and Fusarium infection in the stem determined by longitudinal sectioning of each stem and measuring the total stem length and length showing brown discoloration. One centimetre portions was cut at stem base of each plant and cut into 3 mm$^3$ pieces plated on Potato Dextrose Agar (PDA). (4 Petri-dish
plates per treatment) and incubated for 7 days. The number of stem pieces showing growth of *Fusarium* was counted. The percentage disease reduction was calculated using the formula:

\[
\text{Percentage reduction (\%)} = \frac{\text{innoculated control} - \text{pre treated}}{\text{Inoculated control}} \times 100
\]

Dry matter was determined by drying shoots and roots from each treatment in the oven at 60°C until constant dry weight was achieved.

### 3.6 Determination of the synergistic effect of botanical and biological agents in the management of root- knot nematodes and *Fusarium* wilt

Two crop cycle experiments were conducted at the University of Nairobi, Kabete Campus in a green house. The experiment consisted of potted sterilized growth media composed of soil and sand at a ratio of 2:1. The growing media was inoculated with $1.0 \times 10^6$ ml spore suspension of *Fusarium* pathogen at the rate of 5% (100ml) of the weight of the growth media and 3,000 eggs/juveniles of root- knot nematodes, at the same time the seedlings were uprooted and pre treated with the following treatments before transplanting into the growth media. 

(i) Root guard® which contains *Trichoderma*, *Bacillus*, *Aspergillus*, *Chatomium*, *Escherichia*, *Azobacter* spp. as biological control agent against *Fusarium* wilt procured from Juanco SPS limited at the rate of 10gms/litre of water  

(ii) Neemraj® which is derived from neem kernel whose active ingredient is Azadiractin 0.30% Ec, a botanical agent procured from Amiran Kenya limited at a rate of 2.5 ml/litre of water  

(iii) Pl plus® a biological agent which contains spores of the fungi, *Paecilomyces lilacinus* procured from Juanco SPS limited at the rate of 1.9g/litre of water  

(iv) Pl plus® and Root guard®  

(v) Pl plus® and Neemraj®  

(vi) Root guard® and Neemraj® in the same proportions respectively  

and two controls consisting of (i) growth media with water only and (ii) media inoculated with both nematode and *Fusarium* inoculums. The experiment was laid out in a randomized
complete design and replicated four times with each treatment consisting of a pot with four plants. Watering was done daily throughout the duration of the experiment. The experiment was terminated six weeks after transplanting. The plants were carefully lifted and the stem and roots were separated.

3.7 Data collection
Data on plant height, the number of plants showing *Fusarium* wilt symptoms and mortality was collected every week for six weeks. The soil was washed off the roots he number of galls in the roots and nematode juvenile population in potting media at end of experiment counted. The lengths of stem showing brown discoloration, number of stems per treatment showing presence of *Fusarium* after re-isolation on culture media was determined and shoots and root dried at 60 °C until a constant weight is achieved.

3.8 Data analysis
Data on plant height shoot and root dry weight; root galling, root- knot nematode second stage Juveniles (J2) in the soil and *Fusarium* stem discoloration and re isolation was statistically analyzed to determine the differences among the treatments using analysis of variance (ANOVA) with Genstat 14th edition software. Comparison of means was done using Fischer’s protected least significance difference (LSD) test.
CHAPTER 4: RESULTS

4.1 Effect of pre-treatment of tomato seedlings with chemical and biological agents in control of root-knot nematodes

4.1.1 Plant biomass and plant height

Pretreatment of tomato seedlings with Marshal®, Neemraj® and Pl plus® significantly increased shoot dry weight in both experiments (Table 2). Pre-treatment with Pl plus® was the most effective, resulting in the highest shoot weight of 78% compared to the inoculated control. However, the treatments had very little effect on root dry weight. Only biological pretreatment with Neemraj® significantly increased plant height of the tomato seedlings at the 6th week after transplanting for experiment 1 and 2 (Table 3).

Table 2: Effect on plant height (cm), Shoot and root dry weight (g) of tomato plants pre-treated chemical and biological control agents grown media inoculated with Meloidogyne spp.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
<th>Plant height (cm)</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marshal®</td>
<td>43.1b</td>
<td>4.4b</td>
<td>1.4b</td>
<td>38.0b</td>
<td>4.0b</td>
<td>1.3c</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>50.8a</td>
<td>4.1b</td>
<td>1.8a</td>
<td>43.1a</td>
<td>4.0b</td>
<td>1.5b</td>
</tr>
<tr>
<td>Pl plus®</td>
<td>41.9b</td>
<td>5.1a</td>
<td>1.9a</td>
<td>39.0b</td>
<td>5.0a</td>
<td>1.7a</td>
</tr>
<tr>
<td>Inoculated</td>
<td>38.5b</td>
<td>2.9c</td>
<td>1.8a</td>
<td>37.6b</td>
<td>2.6c</td>
<td>1.6b</td>
</tr>
<tr>
<td>control</td>
<td>58.5a</td>
<td>5.1a</td>
<td>1.5b</td>
<td>44.8a</td>
<td>5.0a</td>
<td>1.4bc</td>
</tr>
<tr>
<td>Water alone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.S.D</td>
<td>4.9</td>
<td>0.4</td>
<td>0.2</td>
<td>3.7</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>17.0</td>
<td>7.2</td>
<td>7.5</td>
<td>16.1</td>
<td>12.0</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Means followed by the same letter (s) along the column are not significantly different. Marshal® 250 Ec-chemical agent, whose active ingredients is carbosulfan, Neemraj® botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30% Ec, Pl plus®-biological agent containing spores of the fungus Paecilomyces lilacinus
Table 3: Plant height (cm) of tomato seedlings pre-treated with chemical and biological control agents grown in media inoculated with *Meloidogyne* spp

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks after transplanting</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Marshall®</td>
<td>13.8c</td>
<td>32.9bc</td>
<td>43.1b</td>
<td></td>
</tr>
<tr>
<td>Neemraj®</td>
<td>21.9a</td>
<td>34.8ab</td>
<td>50.8a</td>
<td></td>
</tr>
<tr>
<td>Pl plus®</td>
<td>20.1ab</td>
<td>36.5a</td>
<td>41.9b</td>
<td></td>
</tr>
<tr>
<td>Inoculated control</td>
<td>19.8b</td>
<td>30.7c</td>
<td>38.5b</td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>18.3b</td>
<td>37.5a</td>
<td>52.9a</td>
<td></td>
</tr>
<tr>
<td><strong>L.S.D.</strong></td>
<td>1.8</td>
<td>3.2</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td><strong>C.V.%</strong></td>
<td>17.0</td>
<td>14.6</td>
<td>17.0</td>
<td></td>
</tr>
</tbody>
</table>

Experiment 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks after transplanting</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Marshall®</td>
<td>18.9c</td>
<td>30.2bc</td>
<td>38.0b</td>
<td></td>
</tr>
<tr>
<td>Neemraj®</td>
<td>19.0c</td>
<td>27.9c</td>
<td>39.0b</td>
<td></td>
</tr>
<tr>
<td>Pl plus®</td>
<td>22.7a</td>
<td>32.0ab</td>
<td>43.1a</td>
<td></td>
</tr>
<tr>
<td>Inoculated control</td>
<td>20.4bc</td>
<td>30.5bc</td>
<td>37.6b</td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>21.0b</td>
<td>34.9a</td>
<td>44.8a</td>
<td></td>
</tr>
<tr>
<td><strong>L.S.D.</strong></td>
<td>1.6</td>
<td>3.5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td><strong>C.V.%</strong></td>
<td>14.1</td>
<td>20.1</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter (s) along the column are not significantly different.

Marshal® 250 Ec-chemical agent, whose active ingredients is Carbosulfan, Neemraj®-botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30% Ec, Pl plus®-biological agent containing spores of the fungus *Paecilomyces lilacinus*
4.1.2 Number of root galls and nematode population

The chemical, botanical and biological formulations significantly reduced the number of root galls, galling index and the population of second stage juveniles (J2) in the soil (Table 4). Marshal® 250 Ec was the most effective in reducing both the number of galls and juvenile (J2) population in both experiments while Pl plus® was the least effective (Figure 1). High levels of nematode population were extracted in soils where seedlings were not pre-treated.

Table 4: Effect on the number of galls, galling index and juveniles (J2) /100gms soil in tomato pre-treated with chemical and biological control agents grown in soil inoculated with Meloidogyne spp.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
<th></th>
<th>J2 count/100g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Galling</td>
<td>Mean</td>
<td>Galling</td>
<td>Mean</td>
<td>Galling</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galls(No.)</td>
<td>index</td>
<td>J2/100g.</td>
<td>index</td>
<td>J2/100g.</td>
<td>index</td>
<td>J2/100g.</td>
<td></td>
</tr>
<tr>
<td>Marshall®</td>
<td>4.6d</td>
<td>1.6d</td>
<td>2.8c</td>
<td>3.2d</td>
<td>1.6d</td>
<td>2.0c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neemraj®</td>
<td>21.3c</td>
<td>3.0c</td>
<td>13.8b</td>
<td>23.3c</td>
<td>3.0c</td>
<td>12.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl plus®</td>
<td>44.5b</td>
<td>4.0b</td>
<td>21.8b</td>
<td>44.5b</td>
<td>4.0b</td>
<td>18.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated control</td>
<td>92.1a</td>
<td>4.5a</td>
<td>255.0a</td>
<td>95.2a</td>
<td>4.6a</td>
<td>283.0a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>0.0e</td>
<td>0.0e</td>
<td>0.0c</td>
<td>0.0e</td>
<td>0.0e</td>
<td>0.0c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.S.D</td>
<td>3.5</td>
<td>0.1</td>
<td>9.0</td>
<td>3.0</td>
<td>0.1</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>28.4</td>
<td>14.0</td>
<td>14.0</td>
<td>24.1</td>
<td>13</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter (s) along the column are not significantly different. Galling index: 0= no galls; 1= 1-2 galls; 2= 3-10 galls; 3 = 11-30 galls; 4= 31-100 galls; and 5 more than 100 galls
Figure 1: Percentage reduction in the number of galls, galling index and the number of juveniles (J2)/100g soil in tomato seedling pre-treated with chemical and biological control agents and grown in media inoculated with Meloidogyne spp.
4.2 Effect of pre-treating tomato seedlings with biological and chemical agents against *Fusarium* wilt

4.2.1 Biomass and plant height

All the treatments significantly increased the root weight and plant height when compared with the inoculated control (Table 5). Pre-treatment with Root guard® was most effective in increasing the dry shoot and root dry weight. The plant height of the tomato seedlings started increasing from the 2nd week after transplanting in experiment 1 and week 4 in experiment 2 respectively. The biological agent, Root guard® and chemical agent Phosphite® were the most effective in enhancing tomato plant height in both experiments (Table 6).

Table 5: Effect on plant height (cm) and shoot and root dry weight of tomato plants pre-treated with chemical and biological control agents and grown in media inoculated with *Fusarium oxysporum* spores.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant height(cm)</td>
<td>Shoot dry weight(g)</td>
<td>Root dry weight(g)</td>
<td>Plant height(cm)</td>
<td>Shoot dry weight(g)</td>
<td>Root dry weight(g)</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>43.7b</td>
<td>3.7b</td>
<td>0.6a</td>
<td>54.6b</td>
<td>7.2b</td>
<td>0.5b</td>
</tr>
<tr>
<td>Phosphite®</td>
<td>51.9a</td>
<td>4.1b</td>
<td>0.5b</td>
<td>56.6b</td>
<td>7.5b</td>
<td>0.6b</td>
</tr>
<tr>
<td>Root-guard®</td>
<td>54.2a</td>
<td>4.6a</td>
<td>0.7a</td>
<td>63.3a</td>
<td>8.3a</td>
<td>0.9a</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>31.2c</td>
<td>3.0c</td>
<td>0.4b</td>
<td>39.7c</td>
<td>5.3c</td>
<td>0.2c</td>
</tr>
<tr>
<td>Water only</td>
<td>51.0a</td>
<td>4.3a</td>
<td>0.7a</td>
<td>59.2a</td>
<td>7.5b</td>
<td>0.6b</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>5.7</td>
<td>0.4</td>
<td>0.1</td>
<td>4.6</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>17.1</td>
<td>0.3</td>
<td>6.6</td>
<td>12.2</td>
<td>4.6</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Means followed by the same letter (s) along the same column are not significantly different; Neemraj® - Botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30% ec, Phosphite®- Fungicide whose active ingredient is potassium Phosphite® 53%, Root guard® - Biological agents contains *Trichoderma, Bacillus, Aspergillus, Chatomium, Escherichia, Azotobacter* spp
Table 6: Effect on Plant height (cm) of tomato plants pre-treated with chemical and biological control agents grown in media inoculated with *Fusarium oxysporum* spores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weeks after transplanting</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>Weeks after transplanting</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>24.4c</td>
<td>34.8d</td>
<td>43.7b</td>
<td></td>
<td>25.5bc</td>
<td>41.4b</td>
<td>54.6b</td>
<td></td>
</tr>
<tr>
<td>Phosphite®</td>
<td>33.6a</td>
<td>47.4a</td>
<td>51.9a</td>
<td></td>
<td>28.4a</td>
<td>42.1b</td>
<td>56.6b</td>
<td></td>
</tr>
<tr>
<td>Root-guard®</td>
<td>25.3c</td>
<td>42.8b</td>
<td>54.2a</td>
<td></td>
<td>27.9ab</td>
<td>44.3b</td>
<td>63.3a</td>
<td></td>
</tr>
<tr>
<td>Inoculated control</td>
<td>18.2d</td>
<td>29.0e</td>
<td>31.2c</td>
<td></td>
<td>23.8c</td>
<td>36.0c</td>
<td>39.7c</td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>27.6b</td>
<td>38.6c</td>
<td>51.0a</td>
<td></td>
<td>29.8a</td>
<td>49.1a</td>
<td>59.2ab</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D. 1.4 3.3 5.7 2.64 3.6 4.6
C.V (%) 7.6 12.1 17.1 13.8 12.2 12.2

Means followed by the same letter(s) along the same column are not significantly different; Neemraj® - Botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30% ec, Phosphite®- Fungicide whose active ingredient is potassium Phosphate 53%, Root guard® - Biological agents contains *Trichoderma, Bacillus, Aspergillus, Chatomium, Escherichia, Azotobacter spp*

### 4.2.2: Effect on *Fusarium* wilt severity

The chemical and biological agents were effective in reducing the number of yellow leaves, dead leaves, stem discoloration and the *Fusarium* re isolated in both experiments 1 and 2 (Table 7). The length of stem discoloured on the tomato seedlings was reduced by over 70% by all the treatments. The biological treatment Root guard® was very effective in reducing the stem discolouration (100%) in both experiments (Figure 2).

### 4.3 Effect of combining botanical and biological formulations on root- knot nematode and *Fusarium* wilt infection

#### 4.3.1 Plant biomass and plant height

Pre-treatment with the individual treatment, Root guard® was most effective resulting in the highest shoot dry weight (Table 8). Among the combined formulations pre-treatment with a combination of Pl plus® and Neemraj® was the most effective in increasing the shoot dry weight while a combination of Root guard® and Neemraj® was the least effective. All the
treatments significantly increased the tomato plant dry root weight with the individual treatments being more effective as compared to the combined treatments. Among the individual treatments Root guard® was the most effective in increasing the root dry weight while a combination of Root guard® and PI plus® had very little effect in the root dry weight in experiment 1 and no effect in experiment 2. All the treatments had a significant effect on the plant height. There was significant increase in plant height from week 4 in experiment 1 and in week 6 in experiment 2 (Table 9).

Table 7: Number of yellow and dead leaves, % stem discoloration, % Fusarium re isolation of tomato plants pre-treated with chemical and biological control agents and grown in media inoculated with Fusarium oxysporum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yellow leaves (no.)</td>
<td>Dead leaves (no.)</td>
<td>Stem discoloration (%)</td>
<td>% Fusarium re isolation (%)</td>
<td>Yellow leaves (no.)</td>
<td>Dead leaves (no.)</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>11.4c</td>
<td>5.3b</td>
<td>8.0b</td>
<td>55.0c</td>
<td>12.5bc</td>
<td>7.1b</td>
</tr>
<tr>
<td>Phosphite®</td>
<td>15.3b</td>
<td>5.8b</td>
<td>5.5b</td>
<td>75.0b</td>
<td>14.7b</td>
<td>7.8b</td>
</tr>
<tr>
<td>Rootguard®</td>
<td>13.3c</td>
<td>4.3b</td>
<td>0.0c</td>
<td>15.0d</td>
<td>12.0bc</td>
<td>5.2b</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>28.4a</td>
<td>29.3a</td>
<td>27.2a</td>
<td>100.a</td>
<td>23.1a</td>
<td>20.6a</td>
</tr>
<tr>
<td>Water only</td>
<td>11.5c</td>
<td>3.2b</td>
<td>0.0c</td>
<td>0.0e</td>
<td>10.3bc</td>
<td>4.4b</td>
</tr>
</tbody>
</table>

L.S.D. 2.7 2.6 4.4 11.7 5.2 6.2 3.9 6.7
C.V. (%) 14.1 18.1 35.6 15.8 23.7 45.0 40.7 8.8

Means followed by the same letter(s) along the column are not significantly different.

Neemraj® - botanical agent derived from Neem kernel active ingredient - Azadiractin 0.30% ec, Phosphite® - fungicide whose active ingredient is potassium phosphate 53%, Root guard® - biological agents, contains Trichorderma, Bacillus, Aspergillus, Chatomium, Escherichia, and Azotobacter spp.
Figure 2: Percentage reduction in the stem discoloration and pathogen re-isolation in tomato seedling pre-treated with chemical and biological control agents and grown in media inoculated with *Fusarium oxysporum.*
Table 8: Effect on plant height (cm), Shoot and root dry weight (g) of tomato plants pretreated with individual and combination of biological control agents grown in media inoculated with *Meloidogyne* spp. and *Fusarium oxysporum* spores.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>Shoot dry weight (g)</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>57.0bc</td>
<td>9.7b</td>
</tr>
<tr>
<td>Pl plus®</td>
<td>52.9c</td>
<td>5.8e</td>
</tr>
<tr>
<td>Root guard®</td>
<td>64.3a</td>
<td>12.0a</td>
</tr>
<tr>
<td>Neemraj® + Pl plus®</td>
<td>54.0c</td>
<td>8.3c</td>
</tr>
<tr>
<td>Root guard® + Neemraj®</td>
<td>58.4abc</td>
<td>5.4f</td>
</tr>
<tr>
<td>Root guard® + Pl plus®</td>
<td>61.2ab</td>
<td>7.7d</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>46.2d</td>
<td>5.0g</td>
</tr>
<tr>
<td>Water only</td>
<td>61.7ab</td>
<td>11.7a</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>6.6</td>
<td>0.3</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>6.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) along the same column are not significantly different; Neemraj® - botanical agent derived from Neem kernel active ingredient - Azadiractin 0.30%; Pl plus®- biological agent Containing spores of the fungi *Paeclomyces lilacinus*; Root guard® - biological agents contains *Trichoderma, Bacillus, Aspergillus, Chatomium, Escherichia, Azotobacter* sp.

Table 9: Effect on Plant height (cm) of tomato plants pretreated with individual and combination of biological control agents and grown in media inoculated with *Meloidogyne* spp. and *Fusarium oxysporum* spores.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks after transplanting</th>
<th>Experiment 1</th>
<th>Weeks after transplanting</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Neemraj®</td>
<td>16.3c</td>
<td>37.5a</td>
<td>48.8c</td>
<td>39.5a</td>
</tr>
<tr>
<td>Pl plus®</td>
<td>24.6a</td>
<td>33.2b</td>
<td>42.3d</td>
<td>34.6a</td>
</tr>
<tr>
<td>Root guard®</td>
<td>17.4b</td>
<td>37.1a</td>
<td>60.9a</td>
<td>34.5a</td>
</tr>
<tr>
<td>Pl plus® + Neemraj®</td>
<td>22.4ab</td>
<td>31.4b</td>
<td>48.3c</td>
<td>35.1a</td>
</tr>
<tr>
<td>Root guard® + Neemraj®</td>
<td>16.5c</td>
<td>37.1a</td>
<td>50.3c</td>
<td>35.0a</td>
</tr>
<tr>
<td>Root guard® + Pl plus®</td>
<td>19.0b</td>
<td>36.9a</td>
<td>56.0b</td>
<td>37.0a</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>16.2c</td>
<td>29.3b</td>
<td>33.3e</td>
<td>24.6b</td>
</tr>
<tr>
<td>Water only</td>
<td>18.8b</td>
<td>34.7a</td>
<td>47.3c</td>
<td>39.1a</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>5.0</td>
<td>3.4</td>
<td>4.2</td>
<td>7.7</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>15.4</td>
<td>5.7</td>
<td>5.0</td>
<td>22.1</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) along the same column are not significantly different; Neemraj® - Botanical agent derived from Neem kernel active ingredient - Azadiractin 0.30%; Pl plus®- Biological agent Containing spores of the fungi *Paeclomyces lilacinus*; Root guard® - Biological agents contains *Trichoderma, Bacillus, Aspergillus, Chatomium, Escherichia, Azotobacter* spp.
4.3.2 Effect on number of root galls and Juveniles (J2)

Both the individual and combined treatments were very effective in reducing the number of galls, galling index and juveniles in the soil significantly (Table 10). Among the individual treatments Root guard® was the most effective (93%) in reducing the number of galls in the roots of the tomato plants. A combination of Root guard® and Neemraj® was the most effective combination among the combined treatments reducing the number of galls by about 80%. All the treatments were very effective in reducing the number of juveniles in the soil (90%) except for Root guard® which reduced the number of juveniles in the soil by about 60% (Figure 3).

4.3.3 Effect on Fusarium wilt symptoms and stem infection

The individual and combined biological pretreatment of tomato seedlings significantly reduced the number of yellow leaves, dead leaves and stem discoloration and the Fusarium re-isolated from the tomato seedlings (Table 11). Root guard® was the most effective individual treatment reducing stem discolouration by 100% and the Fusarium re-isolated by 80%. All the combined treatments; Pl plus® and Neemraj®, Root guard® and Neemraj® and Pl plus® and Root guard® were very effective in reducing the stem discoloration (100%) and the amount Fusarium re isolated to over (80%) respectively (Figure 4).
Figure 3: Percentage reduction in the number of galls, galling index and the number of juveniles in tomato plants pre-treated with individual and combined biological control agents and grown in media inoculated with *Meloidogyne* spp. and *Fusarium* spores.
Table 10: Effect of different treatments on the number of root galls, galling index and juvenile (J2) in the soil in tomato plants grown in media inoculated with both *Meloidogyne* *spp* and *Fusarium oxysporum* spores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galls</td>
<td>Gall index</td>
<td>J2/100g soil</td>
<td>Galls</td>
<td>Gall index</td>
<td>J2/100g soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neemraj®</td>
<td>23.5d</td>
<td>3.0b</td>
<td>14.5c</td>
<td></td>
<td>24.0c</td>
<td>3.0c</td>
<td>13.7c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl plus®</td>
<td>39.2b</td>
<td>4.0a</td>
<td>35.2c</td>
<td></td>
<td>37.7b</td>
<td>4.0b</td>
<td>21.8c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root guard®</td>
<td>21.6d</td>
<td>3.0b</td>
<td>88.1b</td>
<td></td>
<td>24.0c</td>
<td>3.0c</td>
<td>69.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl plus + Neemraj</td>
<td>28.3c</td>
<td>3.3b</td>
<td>19.6c</td>
<td></td>
<td>29.4c</td>
<td>3.3c</td>
<td>17.6c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rootguard+ Neemraj</td>
<td>19.5d</td>
<td>3.0b</td>
<td>33.0c</td>
<td></td>
<td>19.1d</td>
<td>3.0c</td>
<td>22.5c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rootguard + Pl plus</td>
<td>26.2cd</td>
<td>3.0b</td>
<td>19.6c</td>
<td></td>
<td>29.4c</td>
<td>3.3c</td>
<td>17.6c</td>
<td></td>
<td></td>
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<tr>
<td>Inoculated control</td>
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<td>0.0d</td>
<td>229.2a</td>
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<td>106.8a</td>
<td>5.0a</td>
<td>224.3ac</td>
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<tr>
<td>Water only</td>
<td>0.0e</td>
<td>0.0d</td>
<td>0.0c</td>
<td></td>
<td>0.0d</td>
<td>0.0e</td>
<td>0.0d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.S.D.</td>
<td></td>
<td>4.0</td>
<td>0.4</td>
<td>26.8</td>
<td>6.3</td>
<td>0.4</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td></td>
<td>7.4</td>
<td>7.0</td>
<td>31.2</td>
<td>11.0</td>
<td>6.7</td>
<td>12.6</td>
<td></td>
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</tbody>
</table>

Means followed by the same letter(s) along the same column are not significantly different; Neemraj® - Botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30%; Pl plus® - Biological agent Containing spores of the fungi *Paecilomyces lilacinus*; Rootguard® - Biological agents contain *Trichoderma*, *Bacillus*, *Aspergillus*, *Chatomium*, *Escherichia*, *Azotobacter* spp

Table 11: Effect on Number of yellow, dead leaves, % stem discoloration and % re isolation of *Fusarium* from tomato plants pre-treated with a combination of biological control agents and grown in media inoculated with *Meloidogyne* *spp* and *Fusarium oxysporum* spores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of yellow leaves</td>
<td>No. of dead leaves (%)</td>
<td>Stem discoloration (%)</td>
<td><em>Fusarium</em> re-isolation (%)</td>
<td>No. of yellow leaves</td>
<td>No. of dead leaves (%)</td>
<td>Stem discoloration (%)</td>
<td><em>Fusarium</em> re-isolation (%)</td>
<td></td>
</tr>
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<td>Neemraj®</td>
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<td>15.1b</td>
<td>60.0b</td>
<td>8.8c</td>
<td>6.0b</td>
<td>18.8b</td>
<td>60.0b</td>
<td></td>
</tr>
<tr>
<td>Pl plus®</td>
<td>10.5b</td>
<td>6.6b</td>
<td>11.9b</td>
<td>53.3b</td>
<td>13.0b</td>
<td>5.7b</td>
<td>7.4c</td>
<td>53.3c</td>
<td></td>
</tr>
<tr>
<td>Root guard®</td>
<td>4.4c</td>
<td>4.5b</td>
<td>0.0c</td>
<td>20.0c</td>
<td>5.0cd</td>
<td>2.6c</td>
<td>0.0d</td>
<td>20.0c</td>
<td></td>
</tr>
<tr>
<td>Plplus+Neemraj</td>
<td>4.7c</td>
<td>5.8b</td>
<td>0.0c</td>
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<td>8.3c</td>
<td>5.0b</td>
<td>0.0d</td>
<td>20.0c</td>
<td></td>
</tr>
<tr>
<td>Neemraj®+RG®</td>
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<td>4.5b</td>
<td>0.0c</td>
<td>6.7d</td>
<td>5.4cd</td>
<td>4.4b</td>
<td>0.0d</td>
<td>13.3d</td>
<td></td>
</tr>
<tr>
<td>Rootguard+PL®</td>
<td>4.0cd</td>
<td>5.8b</td>
<td>0.0c</td>
<td>20.0c</td>
<td>7.0c</td>
<td>3.9b</td>
<td>0.0d</td>
<td>26.7c</td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>17.1a</td>
<td>15.0a</td>
<td>46.1a</td>
<td>100.0a</td>
<td>16.5c</td>
<td>12.7a</td>
<td>24.0b</td>
<td>100.0a</td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>5.1c</td>
<td>6.9b</td>
<td>0.0d</td>
<td>0.0e</td>
<td>0.0e</td>
<td>2.7bc</td>
<td>0.0d</td>
<td>0.0e</td>
<td></td>
</tr>
<tr>
<td>L.S.D.</td>
<td></td>
<td>2.0</td>
<td>3.5</td>
<td>12.8</td>
<td>15.8</td>
<td>2.8</td>
<td>2.9</td>
<td>2.4</td>
<td>21.2</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td></td>
<td>14.4</td>
<td>13.0</td>
<td>80.7</td>
<td>26.1</td>
<td>19.0</td>
<td>31.4</td>
<td>22.2</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) along the same column are not significantly different; Neemraj® - botanical agent derived from Neem kernel active ingredient- Azadiractin 0.30%; Pl plus®- biological agent containing spores of the fungi *Paecilomyces lilacinus*; Rootguard® - biological agents contain *Trichoderma*, *Bacillus*, *Aspergillus*, *Chatomium*, *Escherichia*, *Azotobacter* spp
Figure 4: Percentage reduction in the stem discolouration and pathogen re-isolation in tomato seedling pre-treated with individual and combined biological control agents and grown in media inoculated with *Meloidogyne* spp and *Fusarium oxysporum* spores.
CHAPTER 5: DISCUSSION

5.1 Effect of chemical and biological pre-treatment of tomato seedlings on root-knot nematode infestation

Pre-treatment of tomato seedlings with Marshal®, Pl plus® and Neemraj® was effective in increasing the tomato plant biomass and in suppressing the root-knot nematodes. The results support those by Kienwnicks and Sikora, (2004) that soil treated with *P. lilacinus* before planting increased the tomato fruit yield. Studies conducted by Davide and Zorilla, (1986) demonstrated that root galling in okra treated with *P. lilacinus* remained at trace and slight levels, and those by Davide and Batino, (1985) on undelinted and delinted cotton seeds treated with *P. lilacinus* the number of galls, nematode and egg masses were significantly reduced. Studies done by Kienwnicks and Sikora, (2004) found that *P. lilacinus* applied 24 hours before transplanting reduced the number of galls and nematodes in the soil.

The fact that the inoculated control registered a higher dry root weight can be attributed to the presence of large galls which concurs with findings of Oclarit and Cumagin, (2009) where the root weight of tomato plants treated with *M. incognita* alone was higher than those treated with *P. lilacinus* and those pre-treated with the chemical agent. *P. lilacinus* reduced the number of nematodes and the number of galls by 95%. *P. lilacinus* is parasitic to nematodes (Oclarit and Cumagin, 2009), destroying their egg shells, larva cuticles and interferes with their morphology (Morgan Jones *et al.*, 1984), this leads to the death of the larvae (Morgan Jones and Rodriguez Kabana, 1985) and also causes deformation and killing of eggs and occasionally killing of juveniles (Jatala *et al.*, 1985).

Neemraj® was found to increase the plant height, root and shoot biomass and reduce the number of galls in the roots and also the nematodes in the soil. This is in line with the
findings of (Shervin et al., 2011), where Neem was able to reduce the galling index from 5.0 in the inoculated treatment to 2.0 in chilli and brinjals using Neem oil formulations. Kumar and Khama, (2006) found that Neem products were able to improve the plant growth and suppress nematode multiplication, root galling index, number of egg masses per gram of root and final nematode population in soil. The results of the experiment also agrees with Agbenin et al., (2004) where Neem seed powder increased the root, shoot weights and plant heights and decreased root galling index. The study by Motha et al., (2010) indicated that dry neem seed treatment was most effective in suppressing root- knots and parasitic nematodes, Saravanapriya and Sivakumar, (2005) documented that root dip with neem extract reduced the nematode population by 83% after 45 days after transplanting, it also increased the fruit yield and seed germination and establishment of uniform and vigorous seedlings. Agbenin et al., (2005), found that neem was able to inhibit hatching of egg masses and was lethal to the larvae.

5.2 Effect of chemical and biological pretreatment of tomato seedlings on Fusarium wilt Infection
Pre - treatment by use of Phosphtite®, Neemraj® and Root guard® respectively on tomato seedlings significantly increased the tomato plant biomass and suppressed Fusarium disease. This is in line with studies done by Obongonya et al., (2010) who found that neem extract was effective against F. oxysporum schl.f. Sp phaseoli and also promoted the shoot and root of beans. Hanaa et al., (2011) demonstrated the same results on their study on effect of neem and willow aqueous extracts on Fusarium wilt disease in tomato seedlings. The results also concur with those of Shervin et al., (2011) where neem powder was found to significantly suppress the F.oxysporum reducing the wilt severity and vascular discoloration. Kimaru et al., (2004) found that neem powder used as soil amendment suppressed the wilt symptoms
and improved the growth of the plant. The same results were documented by Ogechi et al., (2006) where crude extracts of neem leaf and neem seed inhibited mycelia growth.

Rootguard® contains *Trichoderma, Bacillus, Aspergillus, Chatomium, Escherichia, and Azobacter*. *Trichoderma* spp. produces mycoparasites through production of chitinase, glyconases and solubilizes inorganic plant nutrients and therefore increasing the plant health, it also inactivates enzymes involved in the infection process suppressing the pathogens (Aktar et al., 2005). The study is in line with the findings of various authors on the effect of *Trichoderma* on *F. oxysporum*. Ramezani, (2010) found that soil treated with *T. harzianum* reduced the disease by 92% while. Mwangi et al., (2011) documented that *T. harzianum* when applied to tomatoes increased the height and dry root weight. Houssien et al., (2010) working on activation of tomato plant defense response against *Fusarium* wilt disease using *T. harzianum* and Salicylic acid under greenhouse conditions found that tomato plants treated with *T. harzianum* by seedling root dipping or soil treatment one week before inoculation with *F. oxysporum* f. sp. *lycopersici* were healthy and had no wilt symptoms and the disease incidence was reduced up to 0%. Studies by Larkin et al., (1998) found that isolates of *Trichoderma* spp. and commercial biocontrol agents containing *T. harzianum* effectively controlled *Fusarium* disease.

Research on bacterial isolates associated with entomopathogenic nematodes (*Xenorhabdus nematophila and Xenorhabdus spp*) and from used rock wood (*Pseudomonas* genus) found that these bacteria had increased the tomato plant biomass and were antagonistic on *F. Oxysporum* f.sp. *Lycopersici* (Inam-Ul-Haq et al., (2007); Srinivasan et al., (2009) respectively. Research findings on banana using *P. Fluorescence* and *Bacillus Subtitillis* indicated that they were able to reduce *Fusarium* wilt incidence (Akila et al., 2011) the same
result were demonstrated by Asha et al., (2011) on biological control of *F. oxysporum* f. sp. *lycopersici* by *P. fluorescens* which resulted in increased emergence of tomatoes and reduced *Fusarium* wilt disease incidence.

### 5.3 Effect of combining chemical and biological formulations against root-knot nematode and *Fusarium* wilt infection

From the study the combined biological formulations were more effective in suppressing the root-knot nematodes and *Fusarium* wilt disease of tomatoes and improving the plant growth more than the individual formulations. This study is in line with that of Goswami et al., (2006) where *P. lilacinous* and *Trichoderma* spp. or in combination with mustard seed reduced the number of galls per plant and promoted plant growth. Sharon et al., (2001) on the control of root-knot nematodes, *M. javanica* by *T. harzianum* found that it resulted into increase in shoot weight. Dadabat and Sikora (2007) got the same results in their study on biological control of *M. incognita* using *T. Harzianum* and *T. viride*.

The results also concurs with the findings of Aktar et al., (2005) that *T. harzianum* and Neem (*A. indica*) seed powder increased plant growth and yield significantly. The study is also in line with that conducted by Yigt F and Dikilitas, (2007) which showed that a combination of biocontrol agents applied against *Fusarium* wilt gave better results than individual bio control agents. A study done by Mugo, (2012) on integrated management of *Fusarium* wilt of tomatoes using fungicides, organic matter and neem extract showed that neem extract was effective in suppressing the disease and that a combination of control measures was more effective than use of single methods. Mwangi et al., (2011) in the study on inoculation of tomato seedlings with *T. harzianum* and Arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings also documented that disease incidence was
generally low in tomatoes grown with *T. Harzianum* and AMF either individually or combined together.

According to Akila *et al.*, (2011), in their study on combined application of botanical formulations and biocontrol agent for the management of *F. Oxysporum F. sp. Cubense(Foc)* causing *Fusarium* wilt in banana application of mixtures of botanical and biological formulations were found to be effective in control of *Fusarium* wilt incidence both under greenhouse and field conditions. The study is also in line with that of Shervin *et al.*, (2011) on comparing Neem extract with chemical control on *F. oxysporum* and *M. incognita* complex of tomato, where Neem powder was found to significantly suppress the *F.oxysporum* reducing the wilt severity and vascular discoloration and also by Aktar *et al.*, (2005) on studies on the management of root-knot nematode, *M. incognita* wilt fungus, *F. oxysporum* disease complex of green gram, *Vigna radiata* cv ML-1108 which found that *T. harzianum* significantly suppressed both *M. incognita* and *F. oxysporum* and *A. indica* seed powder and was also moderately effective against both the pathogens, Nagesh *et al.*, (2006) on the study of management of *M. incognita* and *F. oxysporum F.Sp. lycopersici* wilt complex using antagonistic fungi in tomato found that tomatoes treated with *P. lilacinus* along with *T. harzianum and Neem cake* were free from nematode and did not wilt and their roots had no *Fusarium*.
CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study indicated that pre-treatment of seedlings with biological and botanical fungicides is effective in managing root-knot nematode and *Fusarium* wilt in tomato. Combined application of both biological and botanical products improves their efficacy against the root–knot nematode - *Fusarium* disease complex than when the products are applied alone. Pre-treatment of seedlings with biological or chemical products may also offer a more cost effective or cheaper method of crop protection where less quantities of product is used and also is degraded before the plant matures therefore reducing the hazardous effect to the environment, animals and human beings.

Use of biological and botanical control methods by pre-treating of seedlings can therefore go a long way in management of plant soil borne diseases and pests. This study concurs with the hypothesis that biological and chemical pre-treatment of tomato seedlings in management of root-knot nematodes will suppress infestation of tomato plants by root-knot nematodes; Biological and pre-treatment of tomato seedlings in management of *Fusarium* wilt will suppress infestation of tomato plants by *Fusarium* wilt disease of tomatoes and the combined effect of botanical and biological agents in management of root-knot nematodes and *Fusarium* wilt of tomato will suppress infestation of tomatoes by root-knot nematodes and infection by *Fusarium* wilt in a nematode – *Fusarium* infected soil. Combined treatments are also more efficient than individual treatments in control of *Fusarium* wilt.

6.2 Recommendations

1. Advice farmers on the use Pl plus® and Neemraj® as effective biological and botanical control agents in suppression of root-knot nematodes and improvement of plant growth.
2. Root guard® and Neemraj®, used for pre-treatment of tomato seedlings are effective biological formulations for the suppression of *Fusarium* wilt of tomatoes and also increases the plant growth.

3. Harness synergistic effect of combining individual formulations for effective control of root- knot nematodes and *Fusarium* wilt disease of tomatoes in soils infested with root-knot nematodes and *Fusarium* wilt.
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