CAUSAL AGENT OF PYRETHRUM WILT AND FACTORS INFLUENCING THE DISEASE DEVELOPMENT

BY

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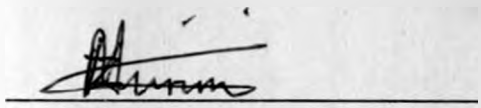
DECLARATION

This thesis is my original work and has never been presented for award of a degree in any university.



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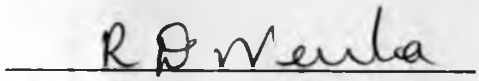

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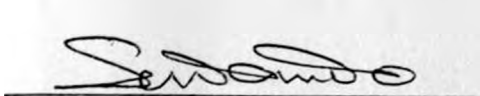

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DEDICATION

To my beloved parents,

Mary and Peter.

ACKNOWLEDGEMENTS

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ABSTRACT

Pyrethrum production has recently been threatened by a disease characterized by wilting and subsequent death of pyrethrum plants. Information on etiology and epidemiology of the disease is scanty and generally not reliable. This study was carried out to identify the pathogen(s) that cause the disease and to investigate the conducive environmental conditions for the disease development. In addition to the above objectives, some commercially recommended clones and varieties were also screened for resistance to the wilt disease.

Diseased plant and rhizosphere soil samples were collected from five different pyrethrum-growing areas, namely Kisii, Molo, Mwongoris, Sotik and Limuru for isolation and identification of pathogens.

Identification of fungal isolates from diseased plant materials revealed the presence of *Fusarium oxysporum* at all the sampling sites. All the isolates were found to be pathogenic to clone L/75/487 plants, colonizing the vascular (xylem) system of inoculated plants.

Nematodes isolated from all the five sites were identified as *Meloidogyne hapla* and *Pratylenchus penetrans*. However, samples from Sotik had *Tylenchus* species at very low population levels.

Use of high levels of either fungal inoculum alone or nematode-fungus combination led to increased severity as well as early onset of the wilt disease during pathogenicity tests. These results indicate that inoculum build up in the soil, where no control measures are practised, can have disastrous effects on the pyrethrum field stands as well as crop productivity.

Plants inoculated through root dipping method developed symptoms much earlier than those inoculated through soil drenching, though the symptoms of the disease were basically similar at comparable inoculum levels. However, unilateral symptom development was observed in plants inoculated by soil drenching in addition to other symptoms.

Symptoms of the wilt disease consisted of initial chlorosis of lower leaves, beginning with the leaf margins and progressing towards the leaf petioles. This was followed by necrosis of the chlorotic regions and eventual drying up of the leaves. At high inoculum levels, symptoms were more severe, with withering and epinasty of leaves even without initial chlorosis.

Influence of temperature on wilt development was clearly demonstrated by exposing plants of clone L/75/487 to ambient temperatures of 17.7°, 21.5°, 25.0° and 30.0°C. Plants inoculated and incubated at high temperatures of 25° and 30°C developed wilt symptoms much earlier and more severely than plants incubated at lower temperatures of 17.7° and 21.5°C.

Different soil moisture levels of 25-30, 45-50, 65-70 and 90-100% displayed significant differences in their influence on wilt development in pyrethrum. In general, wilt symptoms developed earlier at low moisture levels than at high moisture levels.

A field experiment on the effect of prevailing temperatures and rainfall (a pre-requisite for soil moisture levels) was carried out at Kisii, Molo, Ol Joro-Orok and Limuru. These sites experienced ambient temperatures of 19.9°, 13.1°, 13.7° and 13.0°C respectively and corresponding rainfall amounted to 164.9, 215.5, 98.4 and 16.9mm, during the first four months of the experimental period (June to September 1994). Wilt symptoms developed earliest on plants at the Kisii trial site followed by plants grown at Limuru, Ol Joro-Orok and Molo in that order.

A test carried out to investigate the effect of pH of agar medium on growth of fungal isolates indicated that high pH levels favour colony growth. Colony diameters on the fourth day of incubation increased from 2.3cm to 5.3cm as pH increased from 4 to 8.

Screening of different clones and varieties for resistance to wilt disease gave no promising result since all the eight clones and three varieties screened succumbed to the disease. The longest time taken for wilt to develop under greenhouse conditions was 5½ months (for clone L/75/487) and 6 months under field conditions at Mwongoris (for clone Kr/74/122).

CHAPTER ONE

1. INTRODUCTION

Pyrethrum (*Chrysanthemum cinerariaefolium* Vis.), a small daisy-like perennial plant and a source of insecticidal pyrethrins (Kumar, 1984), is a major foreign exchange earner and provides income to over 200,000 families in Kenya.

Pyrethrum is a high-priced cash crop that can be grown satisfactorily in areas with an average annual rainfall of more than 875mm. Rainfall of about 100mm a month is ideal for optimum growth. The crop also grows satisfactorily in the Kisii Highlands of South-West Kenya which receive an annual rainfall of 1800 to 2000mm/year (Brown and Cocheme, 1969).

High altitude areas with low temperatures favour flowering of pyrethrum almost throughout the year (Brown and Cocheme, 1969). There is a strong positive correlation between low temperatures and high pyrethrins content in pyrethrum flowers (Kroll, 1964).

With respect to soil requirements, pyrethrum does well on fertile, deep, well-drained, loamy soils. The crop can also grow satisfactorily on shallow soils provided that rainfall or other forms of moisture are adequate (Brown and Cocheme, 1969).

Pyrethrum is mainly grown in high-altitude areas such as Nakuru, Nyandarua, Kisii, Uasin Gishu, Kiambu, Keiyo Marakwet, Baringo, Laikipia, Nyeri, Kericho, Meru, Trans Nzoia, West Pokot and Bungoma with altitudes between 1900 and 2500 metres above sea level, except for some areas in Kisii district which have lower altitudes of 1700m above sea level (Wanjala, 1991).

Small-scale farmers with plots ranging from 0.2 to 2 hectares produce about 95% of some 12,000 to 15,000 tonnes of dried pyrethrum flowers produced in Kenya annually. Kenya produces about 80% of the annual world production of dried flowers (Kimani, 1992). Ninety-eight percent

(98%) of Kenya's annual produce is exported, leaving only 2% for local consumption (Gichuru, S.P.- Personal communication).

The natural pyrethrins are commonly used in household insect sprays and aerosols, as livestock sprays and upon edible produce where treatment is needed just prior to harvest. Their wide acceptance as insecticides is due to an inherent lack of toxicity and negligible adverse residual effect (Metcalf, 1955).

Because of the high efficacy of pyrethrins against insects and their safe use in the environment, adequate amounts of pyrethrum flowers should be produced to ensure an adequate and sustainable supply of the insecticides. Pyrethrum production, however, is limited by many constraints including diseases, pests and poor husbandry practices (Anonymous, 1992b).

Robinson (1963) gave a list of pyrethrum diseases in Kenya such as true bud disease, false bud disease, Ascochyta disease and root rots which are caused by various pathogens. Some fungi whose pathogenicity is not known are also included in the list.

Among the diseases, pyrethrum wilt is a major drawback in production of pyrethrum. The wilt problem in pyrethrum has recently become increasingly important, not only in farmers' fields, but also in the pyrethrum research stations (Kimani, 1992).

Various fungal and nematode pathogens have been associated with wilted pyrethrum plants (Nattrass, 1945; Nattrass, 1950; Whitehead, 1958; Parlevliet and Brewer, 1971; Ondieki and Anyango, 1980).

Because success in disease management requires a clear understanding of the disease etiology, there was need to study the etiology of pyrethrum wilt as a pre-requisite to formulation of a viable control strategy. The etiology of pyrethrum wilt is poorly understood.

Fungal wilt pathogens, especially species of the genera *Fusarium* and *Verticillium*, are often involved in interactions with plant-parasitic nematodes, where they are synergistic in action

and lead to increased disease incidence and severity (Mace *et al*, 1981). The role of fungal pathogens and plant-parasitic nematodes in pyrethrum wilt development should be established.

Various environmental factors such as soil temperature, moisture, pH and nutrients are known to govern the degree of vascular colonization by vascular wilt pathogens and, therefore, influence symptom expression (Wheeler, 1969; Garrett, 1970). The role of such environmental factors in wilt development in pyrethrum has not been studied.

Attempts to control pyrethrum wilt in Kenya using fungicides have not been successful (Kimani, 1992). Use of resistant varieties and clones has not been tried in Kenya. Therefore, screening the available germplasm for resistance to pyrethrum wilt could be a step forward in alleviating the disease problem. Varieties/clones found to exhibit resistance to the disease could be used in breeding for the wilt resistance.

In view of the importance of pyrethrum and the problem of the wilt disease as a constraint to higher production of the crop, this study was carried out with the following objectives:

1: To identify the pathogen(s) associated with the wilt disease in pyrethrum.

2: To study the effect of temperature, moisture and pH on wilt development in pyrethrum.

3: To screen various commercially recommended pyrethrum clones/varieties for their resistance to the wilt disease.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Wilt Disease of Pyrethrum in Kenya

Wilt disease is a major problem in pyrethrum production in Kenya (Kimani, 1992). Plants wilt and die off at various stages of growth, sometimes even before any economic yields are realized. Economic losses due to the disease can reach about 50% (Anon, 1980). The losses due to the disease are in terms of both labour expenses of planting and re-planting and the subsequent reduction in yields due to low field stands after some of the plants wilt and die.

Wilt diseases can be induced by various plant pathogens and/or adverse environmental conditions (Wheeler, 1969). Nematodes and fungal pathogens have been known to be associated with wilt in pyrethrum (Nattrass, 1945; Nattrass, 1950; Whitehead, 1958; Parlevliet and Brewer, 1971; Ondieki and Anyango, 1980). Root-rotting and browning (discolouration) of the vascular system associated with pyrethrum wilt are indicators of nematode and fungal involvement in wilt development (Parlevliet and Brewer, 1971; Ondieki and Anyango, 1980).

Fusarium graminearum Schwabe, *Rhizoctonia solani* Kühn, *Sclerotinia minor* Jagger, and *Sclerotinia sclerotiorum* (Lib.) de Bary are known to cause root rots that result in wilting and death of pyrethrum plants (Nattrass, 1950; Whitehead, 1958).

Sclerotinia minor has been known to be the most common fungus associated with pyrethrum wilt in Kenya. The fungus spreads from plant to plant by means of mycelium and sclerotia. Adverse weather conditions and/or poor husbandry practices predispose pyrethrum plants to *S. minor* infection. Lettuce and sunflower are also good hosts of *S. minor* (Nattrass, 1945; Nattrass, 1950). *Sclerotinia sclerotiorum*, with much larger sclerotia, has less frequently been found attacking pyrethrum in Kenya (Nattrass, 1950).

Both *Sclerotinia minor* and lesion nematodes, *Pratylenchus* spp., have been found associated with pyrethrum wilts at Molo Research Station (Nattrass, 1950). Lesion nematodes were first implicated as causal agents of wilt and root rot of pyrethrum in 1958 (Whitehead, 1958). Later, *Fusarium solani* was found associated with wilt of pyrethrum on clones 4331, Ks/71/6 and Ks/70/64 in Kisii district (Ondieki and Anyango, 1980).

2.2. Symptomatology of Pyrethrum Wilt

Wilt caused by *Sclerotinia minor* in pyrethrum is characterized by a sudden wilting, drying of leaves and eventual death of infected plants. Fungal mycelia and numerous sclerotia around the root system of the plant were usually associated with *Sclerotinia* wilt (Nattrass 1950).

Lesion nematodes cause the formation of dark lesions and pruning of a root system, yellowing, stuntedness and eventual wilting of the infected plants (Whitehead,1958). Root-knot nematodes, especially *Meloidogyne hapla* Chitwood, cause galling of the root system (Robinson, 1963). Nematode invasion leads to wounding of roots and this can provide entry points for the fungal wilt pathogens (Conroy and Green, 1974).

Fusarium wilt of pyrethrum is characterized by yellowing, vascular discolouration and wilting of infected plants without any visible external, above-ground decay symptoms. The plants rarely collapse as roots are not usually affected, except during wet conditions (Ondieki and Anyango, 1980).

2.3. Control of Pyrethrum Wilt

Recommended control measures against pyrethrum wilt include planting of clean (disease-free) seeding materials on nematode-free land. This kind of sanitary practice can offer control against nematodes for 1-3 years. Breeding for resistance/tolerance to nematodes may also offer a solution to the wilt disease problem (Parlevliet and Brewer, 1971).

Nemacur (5%) can minimize losses due to root-knot nematodes, *Meloidogyne* spp., and lesion nematodes, like *Pratylenchus penetrans* (Ondieki and Anyango, 1980).

Use of fungicides to control pyrethrum wilt has generally proved unsuccessful (Kimani, 1992). Perhaps more understanding of the wilt complex is required first to be able to identify effective fungicides (Nattrass, 1945; Nattrass, 1950).

2.4. Disease Predisposition

There is a paucity of information regarding pyrethrum wilt in relation to interaction of wilt pathogens with different environmental factors. A brief account is hereby given for other crops with respect to such aspects as nematode-fungus interaction and the influence of temperature and soil moisture on wilt development and expression.

2.4.1. Nematode-fungus Interactions in Wilt Enhancement

Plant-parasitic nematodes predispose plants to infection by other pathogens such as fungi and bacteria (Norton, 1978; Dropkin, 1980; Mace *et al*, 1981). Interaction between the tomato wilt fungus, *Fusarium oxysporum* fsp. *lycopersici*, and *Heterodera marioni* (a cyst nematode) increased wilt severity even in resistant cultivars of tomato. The disease was less severe when

the fungus was used alone. There were no wilt symptoms on plants inoculated with nematodes alone (Young, 1939).

A combination of *Heterodera marioni* and *Fusarium oxysporum* fsp. *vasinfectum*, the causal agent of cotton wilt, also increased disease severity. A relatively lower disease incidence was recorded when the wilt fungus was used alone. No wilt developed on plants inoculated with nematodes alone (Miles, 1939).

Significant infection of tobacco with *Fusarium oxysporum* fsp. *nicotianae* occurs in the presence of the root-knot nematodes, *Meloidogyne incognita, M. arenaria* and *M. javanica*, as compared to the presence of the fungus alone (Porter and Powell, 1967). Galled tissues were equally predisposed to *Fusarium oxysporum* fsp. *nicotianae* in wilt-susceptible as well as wilt-resistant tobacco plants in the presence of root-knot nematodes (Melendez and Powell, 1967).

By increasing the nematode population of *Pratylenchus penetrans*, Conroy *et al* (1972) were able to increase the incidence of infection of roots of tomatoes by *Verticillium albo-atrum* Reink & Berth. Nematodes alone did not induce any wilt symptoms. Increase in *Verticillium* wilt incidence in the presence of *P. penetrans* has also been recorded on eggplant (Mountain and McKeen, 1960).

Bergeson *et al* (1970) reported numerically more propagules of *Fusarium oxysporum* fsp. *lycopersici*, the causal agent of *Fusarium* wilt of tomato, in the rhizosphere of tomato plants infected with *Meloidogyne javanica* than in rhizospheres of non-infested plants.

Nematodes can act as wounding agents, alter the physiology or genetic resistance of host plants, or serve as vectors to some groups of pathogens such as viruses and bacteria (Mace *et al*, 1981). Wounding and alteration of the host's physiology or genetic resistance by nematodes can result in increased severity of diseases caused by an interaction of different pathogens (Conroy and Green, 1974).

2.4.2. Enhancement of Wilt Development by High Temperatures

Temperature influences vascular wilt development (Roberts and Boothroyd, 1984). Hood and Stewart (1957) demonstrated that increases in temperature between 15°C and 32°C reduced the time taken for symptoms to develop and also increased the severity of *Dianthus* wilt, caused by *Fusarium oxysporum* fsp. *dianthi*.

The frequency of occurrence of *Fusarium oxysporum* propagules on the root surface of dwarf bean seedlings has been shown to increase with increase in temperature (Taylor and Parkinson, 1964).

Emberger and Nelson (1981) reported that terminal leaves of *Chrysanthemum* plants grown at soil temperatures of 29°C to 32°C after inoculation with *Fusarium oxysporum* fsp. *chrysanthemi* became chlorotic and twisted after two weeks. Eventually, the plants wilted and died. However, lower temperatures of 18° to 24°C delayed or prevented both the development of foliage symptoms and anatomical changes in inoculated plants.

The severity of *Chrysanthemum* wilt caused by *Fusarium oxysporum* fsp. *chrysanthemi* increased with increase in air temperature (Gardiner *et al*, 1987; Gardiner *et al*, 1989).

Enhanced vascular wilt development with increased temperature could be due to a breakdown of tolerance or resistance mechanisms in the infected plants (Gardiner *et al*, 1987). High temperature has been related to direct effects on the growth and/or sporulation of *Fusarium oxysporum* fsp. *chrysanthemi* in Chrysanthemum plants (Gardiner *et al*, 1989).

Vessel element plugs of pectinaceous materials, wound gums, hydrolyzed gels, phenolic compounds and polysaccharides have all been quoted as contributory factors in the resistance to water flow in the xylem vessels of plants infected with vascular wilt pathogens (McClure, 1950; Dimond and Waggoner, 1953; Pierson *et al*, 1955; Deese and Stahman, 1962; Pennypacker and Nelson, 1972). An increase in temperature would be expected to increase the activity of the

vascular pathogens inside their hosts, leading to increased production of pectic and cellulolytic enzymes which lead to production of the above mentioned materials (Beckman *et al*, 1953). These materials clog the xylem vessels, thus affecting the lifting force by which water moves through the xylem from roots and soil (Beckman, 1964). Tyloses in vessels may also reduce water flow (Beckman *et al*, 1953). All these aspects lead to rapid wilt development and increased disease severity.

2.4.3. Effect of Soil Moisture on Wilt Development

Soil moisture content and the duration for a given moisture regime are important in the development of wilts and other diseases (Wheeler, 1969; Garrett, 1970). *Thielaviopsis basicola* causes appreciable root-rot damage to poinsettia at 36% of the soil moisture holding capacity (MHC), and the disease severity increases with increase in soil moisture content up to 70% MHC (Bateman 1961).

Rhizoctonia root- and stem-rots are most severe on poinsettia at moisture levels below 40% MHC and the disease severity decreases with increase in soil moisture. Above 80% MHC, *Rhizoctonia* causes little or no damage to poisenttia. Soil moisture levels above 70% MHC are associated with serious problems of *Pythium* wilt of poinsettia (Bateman, 1961).

Significant reduction in severity of common scab of potato, caused by *Streptomyces scabies*, was achieved by holding soil moisture above 90% of available soil moisture (ASM) for either 6 or 9 weeks. However, maintaining the high moisture level for 3 weeks did not reduce scab severity (Davis *et al,* 1974).

High levels of soil moisture have further been shown to be effective in controlling common scab of potatoes. Irrigation throughout the growing season at either -0.45 or -0.65 bars

of soil moisture tensions resulted in significantly less potato scab than irrigation at -0.96 or -1.60 bars (Davis *et al*, 1976).

Increased soil moisture reduces the amount of soil aeration which is essential for survival and multiplication of aerobic fungi. This, therefore, reduces the inoculum potential of a pathogen in the soil, with the result that high soil moisture levels lead to slow wilt development and reduced disease severity in comparison to rapid wilt development at low soil moisture levels where soil aeration is adequate (Stover, 1953a; Stover, 1955; Stover *et al*, 1953).

CHAPTER THREE

3. MATERIALS AND METHODS

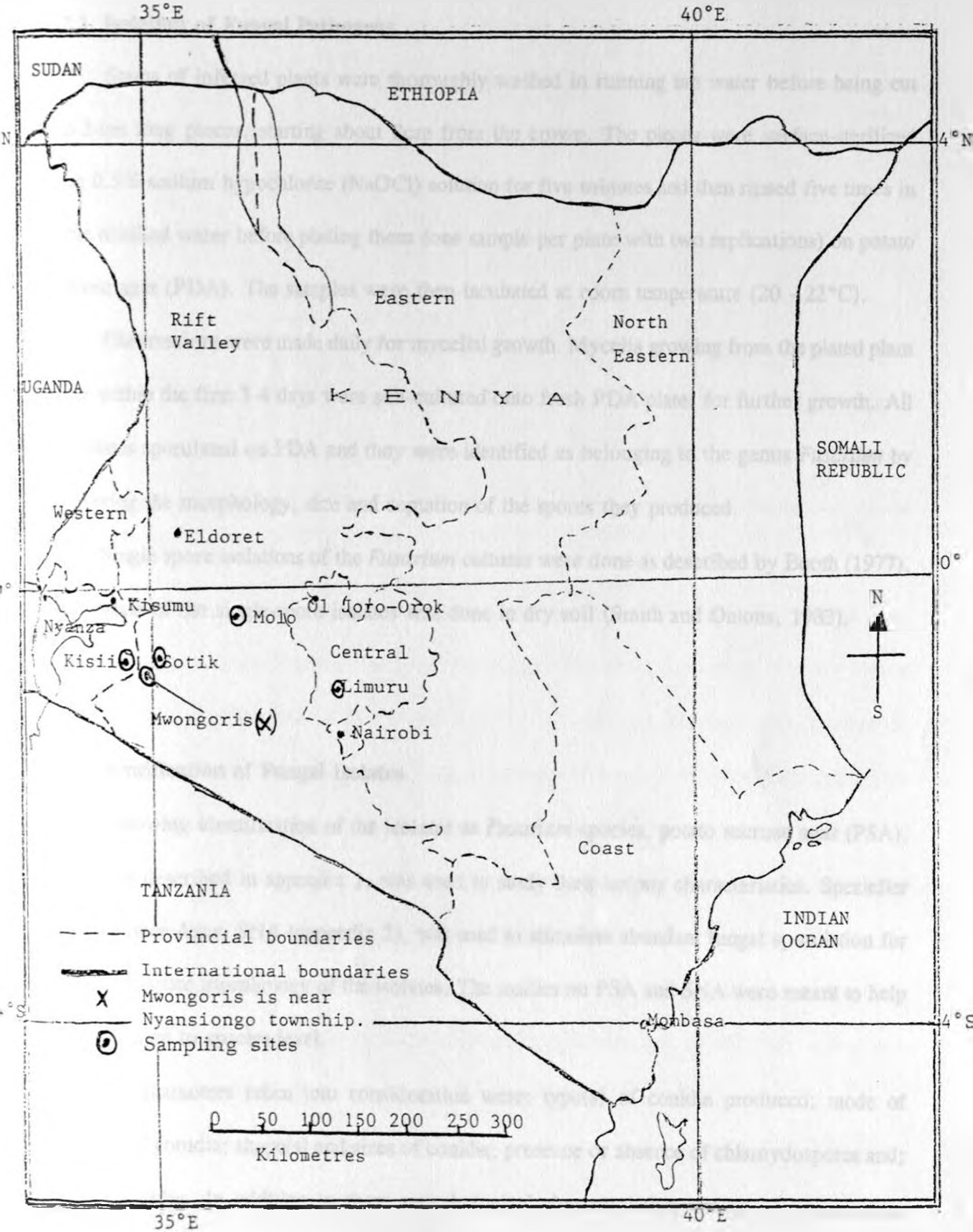
3.1. Sampling of Diseased Plant Materials and Soils

Rhizosphere soil and plant samples were collected from five different pyrethrum-growing areas, namely Sotik in Bomet district, Mwongoris in Nyamira district, Molo in Nakuru district, Kisii Research Station in Kisii district and Limuru in Kiambu district (Figure 1). Ten sampling points were selected at random in each area for sample collection.

Sampling was done from completely wilted plants by uprooting and examining them for infection in root and crown areas. Those plants showing vascular discolouration were collected. Rhizosphere soil samples were taken from an 8-10cm depth by hand-scooping of the soil.

The soil and infected plant samples thus collected were separately put in polythene bags and transported to the Plant Pathology Laboratory (Kabete Campus of the University of Nairobi) for fungal isolation and nematode extraction. Symptoms of the wilted plants in the field were recorded.

Fig 1. Sampling sites for wilt-affected pyrethrum plants and soils.



3.2. Isolation and Identification of Pathogens

3.2.1. Isolation of Fungal Pathogens

Stems of infected plants were thoroughly washed in running tap water before being cut into 2-cm long pieces, starting about 2cm from the crown. The pieces were surface-sterilized using 0.5% sodium hypochlorite (NaOCl) solution for five minutes and then rinsed five times in sterile distilled water before plating them (one sample per plate with two replications) on potato dextrose agar (PDA). The samples were then incubated at room temperature (20 - 22°C).

Observations were made daily for mycelial growth. Mycelia growing from the plated plant pieces within the first 3-4 days were sub-cultured onto fresh PDA plates for further growth. All the isolates sporulated on PDA and they were identified as belonging to the genus *Fusarium* by considering the morphology, size and septation of the spores they produced.

Single spore isolations of the *Fusarium* cultures were done as described by Booth (1977). Preservation of the single-spore isolates was done in dry soil (Smith and Onions, 1983).

3.2.2. Identification of Fungal Isolates

Following identification of the isolates as *Fusarium* species, potato sucrose agar (PSA), prepared as described in appendix 1, was used to study their colony characteristics. Spezieller Nahrstoffarmer Agar, SNA (appendix 2), was used to stimulate abundant fungal sporulation for studying the spore morphology of the isolates. The studies on PSA and SNA were meant to help in identification to species level.

The characters taken into consideration were: type(s) of conidia produced; mode of production of conidia; shape(s) and sizes of conidia; presence or absence of chlamydospores and; type of phialides. In addition to these morphological characteristics, colour of sporodochia,

growth on PSA (pH 6.7 ± 0.1) at 37°C, pigmentation on PSA at 25°C, and growth rate on PSA at 25°C (colony diameters on the 4th day of incubation) were also used in the identification process. A synoptic key was then used to identify the isolates to species level (Anonymous, 1992a). Fungal descriptions given by Booth (1971) were also used in identification.

3.2.3. Extraction of Nematodes from Roots

Nematodes were extracted from roots by using a modified maceration-seiving technique (Fallis, 1943; Hooper, 1970; Southey, 1970). Roots of wilt-affected pyrethrum plants were washed in running tap water before being cut into 1-cm long pieces. Thirty grammes of the root pieces of each sample were macerated in a blender, after adding sufficient tap water to cover the material, for five seconds at low speed and then ten seconds at high speed.

The macerate was emptied into 500-ml beakers and allowed to stand for 24 hours in order for nematodes to wriggle from the plant material into the water. Each macerate was then sieved through a 30-mesh sieve nested over a 200-mesh sieve with two 375-mesh sieves below it. Backwashing was done on the fine-pore sieves to transfer nematodes to separate beakers by use of a jet of tap water. The water:nematode suspension thus obtained was allowed to stand for 2-3 hours to let nematodes settle at the bottom of the beaker (Flegg and Hooper, 1970; Hunt and Bridge, 1993). The top layer suspension was then decanted and fresh clean water added to the remaining nematode suspension. This process continued until the suspension became clear. The volume of the suspension was subsequently reduced by siphoning out the top layer supernatant in order to concentrate nematodes into a 20-ml suspension.

Phytoparasitic nematodes belonging to various genera contained in 1-ml aliquots of the resulting suspensions were counted, six counts per sample, under a dissecting microscope.

3.2.4. Extraction of Nematodes from Soil

Extraction of nematodes from the rhizosphere soil samples was done by using the sieving technique developed by Cobb in 1918 (Flegg and Hooper, 1970). Soil from each collection site was thoroughly mixed and a 200-ml sample was taken from the mixture. This was then put in a 20-litre bucket to which about half a litre of tap water was added. The muddy mixture was stirred for about five minutes and the resulting suspension was sieved through a series of sieves as described in section 3.2.3. Clearing and concentration were done as for the root sample suspensions. Nematode counts were made as described in section 3.2.3.

3.2.5. Maintenance and Multiplication of Nematodes

Nematode suspensions from roots and soil samples obtained from each of the five sample-collection sites were mixed after the determination of nematode populations to get a mixed nematode suspension for each site. Sterile soil in wooden flats, with tissue culture-produced plants of clone Mo/74/223 planted onto them, were inoculated with the mixed suspensions, one suspension per wooden flat, for multiplication of the nematodes to be used in later inoculation experiments.

3.2.6. Nematode Preservation, Mounting and Identification

Preservation of nematode specimens was carried out by first killing the nematodes through partial immersion of nematode suspensions, contained in universal bottles (vials), in a large volume of water at a temperature of 80-90°C for 10 minutes. The killed nematodes were then fixed by adding an equal volume of 'double strength' TAF fixative (prepared as in appendix 4) to the cooled nematode suspension. Nematode slide mounts were prepared as explained by

Hooper (1990) and Hunt and Bridge (1993) for examination under a compound light microscope.

Identification involved the use of observed morphological characteristics and morphometric measurements and subsequent application of the Commonwealth Institute of Helminthology (C.I.H) descriptions of Plant-Parasitic Nematodes and appropriate synoptic keys.

The morphological characteristics and morphometric measurements employed included the following: body description (shape); head skeleton offsetting; tail terminus appearance; lip region description; body annulation; median bulb description; lip region annules; body length; greatest body width; spear (stylet) knob appearance; spear length; spear base width; spear knob size; median bulb size; length of esophagus; tail length; V-value; length of spicules and length of gubernaculum. For *Meloidogyne* species specimens, slides of perineal patterns of mature females were prepared and examined for identification to species level.

3.3. Pathogenicity Tests

3.3.1. Planting Medium and Materials

A steam-sterilized homogenized mixture (v/v) of forest soil, peat moss and gravel in the ratio of 20 : 8 : 5 respectively, which is recommended for growing of Chrysanthemums, was used for pot experiments in the greenhouse (Dr. Okioga, Personal Communication, 1993).

A wilt-susceptible clone (L/75/487) was used as the planting material. Tissue culture-produced plants were obtained from Molo Tissue Culture laboratory.

Black polythene paper sleeves (pots), about 15cm in diameter and 20cm in depth, were used for pot experiments.

3.3.2. Fungal Inoculum Preparation

Fungal inoculum was prepared from 10-day-old single-spore cultures of *Fusarium oxysporum* on SNA. One representative isolate was chosen from each of the five sample-collection sites for inoculation purposes.

Conidial suspensions were obtained by flooding the culture plates of each isolate with 10ml of sterile, distilled water and then dislodging the conidia gently from the mycelium using a sterile wire loop. The resulting suspensions were separately sieved through two layers of cheesecloth to remove hyphal strands. The conidia were subsequently counted using a haemocytometer and the final concentration of conidia adjusted appropriately to the levels stated in section 3.3.4.

3.3.3. Nematode Inoculum Preparation

Excised nematode-infested roots of the pyrethrum plants used for maintenance and multiplication of nematodes (Section 3.2.5) were cleared of debris and soil particles using running tap water and macerated using a blender after chopping them into pieces of about 1cm length. The macerate was sieved through a series of sieves to get separate nematode suspensions as in section 3.2.3. The five separate nematode suspensions representing the five sample-collection areas were used in inoculation experiments after adjusting the inoculum to the levels required.

Only plant-parasitic nematodes (juveniles and adults) were taken into consideration when making the appropriate counts.

The various levels of nematode inoculum used, either singly or in combination with fungal inoculum, consisted of mixed populations of three nematode genera, *Meloidogyne, Pratylenchus* and *Tylenchus* in proportions shown in table 1.

Table 1. Relative proportions (%) of nematodes in the genera *Meloidogyne*, *Pratylenchus* and *Tylechus* in samples used in pathogenicity tests.

Collection site (Isolate)	Proportions (%) of nematodes in the genera		
	Meloidogyne	*Pratylenchus*	*Tylenchus*
Kisii (I)	68.0	32.0	—
Molo (II)	54.5	45.5	—
Mwongoris (III)	58.6	41.4	—
Sotik (IV)	39.0	35.0	26.0
Limuru (V)	72.4	27.6	—

3.3.4. Inoculation Techniques

The five *Fusarium oxysporum* isolates and plant-parasitic nematodes (mixtures of *Meloidogyne hapla, Pratylenchus penetrans* and *Tylenchus* spp.) obtained from the five sample-collection sites were used for inoculation purposes.

Treatments were fungal isolates alone (each representative isolate used separately), nematodes alone (mixed suspensions of nematodes from different sites taken separately) and nematode plus fungal inoculum (prepared by combining nematodes from a particular site with the respective representative fungal isolate).

Four different levels of fungal inoculum, $1.25x10^6$, $2.50x10^6$, $5.0x10^6$, and $1.0x10^7$ conidia, were used for inoculation with fungus alone; three levels of 500, 1000 and 1500 nematodes of each nematode mixture for inoculation with nematodes alone and; four different levels of $2.5x10^6$: 500, $2.5x10^6$: 1500, $1.0x10^7$: 500 and $1.0x10^7$: 1500 conidia and nematode ratios were used in the nematode plus fungus treatments. Control plants were included in each treatment. The treatments were replicated three times.

Two methods of inoculation, root-dipping and soil drenching, were used to inoculate plants with the different inoculum levels prepared as described above. The root-dipping method involved washing plants in running tap water to remove adherent soil from their root systems and then dipping them in an appropriate inoculum for 30 minutes before re-planting onto sterile planting medium (Section 3.3.1). Soil drenching involved making a furrow in the planting medium around each potted plant and pouring 10ml of the appropriate inoculum into the furrow which was then covered with a small amount of soil.

The test plants were incubated under greenhouse conditions (where watering was done evenly at regular intervals on all pots) in a completely randomized design (CRD) with three replications.

The pH of the planting medium was determined using the Calcium Chloride method (Ahn, 1973; Page *et al*, 1982) and found to be at pH value of 6.6. Air temperatures in the greenhouse were recorded two times (at 9am and 3pm) on a daily basis. The average of the two values was taken as the daily mean temperature.

Plants were observed on a daily basis for symptom development and wilt disease assessment.

3.3.5. Disease and Plant Performance Assessment

Disease assessment was based on wilting and vascular discolouration. A plant which showed wilt symptoms for two consecutive days was scored as diseased and the time taken for wilting to occur since inoculation (incubation period) under each treatment was recorded. The symptoms used to signify wilting included gradual chlorosis of the lower leaves of plants starting from the leaf margins, epinasty of leaves during hot weather periods even in the presence of adequate soil moisture, and necrosis of chlorotic areas.

Positive infection of the test plants inoculated with fungal inoculum alone and nematode-fungus inoculum was confirmed by isolating the fungal pathogens from the infected plants as described in section 3.2.1. The re-isolated fungal isolates from the stem pieces were grown on SNA for re-identification on the basis of their spore morphology.

Longitudinal and transverse sections of stems were disinfected for five minutes in 0.5% sodium hypochlorite (NaOCl), stained for 30 seconds in 1% cottonblue in lactophenol and finally cleared in concentrated lactophenol. This was done on both wilted and symptomless plants to check for vascular colonization by the fungus.

For those plants inoculated using nematode inocula alone, data were taken on the height of the plants, plant bush diameter and number of tillers as no wilt symptoms were observed on the plants even at the end of the experimental period.

Infection of plant roots by nematodes was determined by first washing roots from inoculated plants and immersing them for 3 minutes in a boiling solution of equal parts lactic acid, glycerol, distilled water and 0.05% cotton-blue. The roots were then cleared in a 50:50 mixture of glycerol and distilled water. Nematodes were seen embedded within the root tissues and they were stained blue.

The data collected on time taken (days) for symptom expression were subjected to analysis of variance. Further analysis (mean separation) was done using the Duncan's Multiple Range Test (Steel and Torrie, 1980).

3.4. Effect of Environmental Conditions on Wilt Development in Pyrethrum

3.4.1. Planting Materials, Experimental Sites and Inoculation Methods

Effect of environmental conditions (temperature and soil moisture) on wilt development in pyrethrum was studied under both greenhouse and field conditions. Field experiments were conducted at Kisii, Molo, Ol Joro Orok and Limuru while greenhouse experiments were conducted at the University of Nairobi, Kabete Field Station. Plants of the same susceptible clone, L/75/487, were used as test plants in these experiments .

The inoculum used for both greenhouse and field inoculations was prepared by mixing (in equal proportions by volume) all the five representative isolates of *Fusarium oxysporum* used in the pathogenicity tests (Section 3.3) after adjusting their concentrations separately to $1.5x10^6$ conidia/ml. This gave a bulked inoculum composed of the five *Fusarium oxysporum* isolates. Bulking of the isolates was done because all the isolates were shown to be pathogenic from the results of the pathogenicity tests and therefore all the isolates were used.

In the greenhouse tests, inoculation was done by dipping the roots of the test plants in the bulked inoculum for 30 minutes before planting in sterile soil mixture. Field inoculations were done by drenching 10ml of the bulked inoculum standardized to $1.5x10^6$ conidia/ml around each plant in a furrow, 10cm deep and 2cm from the plant.

Fusarium fungal spore counts were determined using the soil plate method as described by Warcup (1950) before setting up field experiments in order to establish the natural soil infestation levels with *Fusarium* at all the field experimental sites. A soil sample was prepared from each of the sites by combining five sub-samples taken randomly from the top 8-10cm soil layer. Four such samples were prepared from each site. Each soil sample was thoroughly mixed and sieved after air-drying for two days on a laboratory bench. From each sample, 0.01g of soil

was transferred into a sterile petri dish using a micro-spatula. A drop of sterile distilled water was mixed with soil particles to help in dispersion before 10-15ml of cooled molten Kerr's medium (Appendix 3) was added to the plate. The soil particles were further dispersed by shaking and rotating the plate before the medium solidified. Three plates per soil sample were incubated at room temperature (20-22°C).

Observations were made daily for the growth of fungal colonies. The resulting colonies identified as belonging to the genus *Fusarium* were subcultured onto SNA and subsequently identified to species level. Those colonies showing *Fusarium oxysporum* were counted and recorded.

3.4.2. Effect of Temperature on Wilt Development in Pyrethrum under Greenhouse Conditions

Plant inoculations were done by dipping the root systems of four-month-old tissue culture-produced plants of clone L/75/487 in the bulked inoculum (Section 3.4.1) for 30 minutes. The plants were then planted on a sterile soil mixture (Section 3.3.1) contained in 15-cm wide and 20-cm deep polythene paper sleeves.

The plants were incubated under four different air temperatures of 17.7°, 21.5°, 25.0° and 30.0°C. Temperature conditions of 17.7° and 21.5°C were attained by incubating the plants outside and inside a greenhouse respectively while growth chambers were used for the 25° and 30°C temperature treatments. Ambient temperatures were measured daily for the four incubation conditions so that the means at the end of the experimental period were obtained. Six plants in a completely randomized design (CRD) were used for each temperature treatment, four of which were inoculated (providing four replicates) while two were left as uninoculated controls.

Data were taken on only the four inoculated plants; the control plants merely served as comparison plants during assessments for vascular colonization by the fungus (carried out as in section 3.3.5).

Soil temperatures were measured daily (at 9am and 3pm) for a period of four months (June to September, 1994) for each air temperature regime. The average of the two daily temperature measurements was taken as the mean soil temperature for the day. The average daily soil temperatures were calculated for the four-month period.

Data taken for this experiment were the time taken (days) from the day of inoculation to initial expression of wilt symptoms for each temperature treatment. Time taken (days) for complete plant death after the initial wilt symptom expression was also recorded. All plants showing wilt symptoms were taken to be infected. In addition, all symptomless plants were sampled and cultured at the end of the experimental period to establish if there was infection by the fungus. This was done in a similar manner to the infection assessment done under section 3.3.5.

Data analysis (F-test) was performed to determine whether there were any significant differences among the treatments. Further analysis (mean separation) was done using the Duncan's Multiple Range Test.

3.4.3. Effect of Soil Moisture on Wilt Development in Pyrethrum under Greenhouse Conditions

Four moisture levels of 25-30%, 45-50%, 65-70% and 90-100% soil saturation were used in this experiment.

The moisture content at saturation level for the planting soil mixture (Section 3.3.1) was first determined by using the oven-dry weight method. Four samples of the soil mixture were put in separate polythene paper sleeves (pots) and watered to saturation, until water started dripping from the holes at the base of each pot. The soil was then allowed to drain freely under gravity for 24 hours (one day). The moisture content of the saturated soil mixture was determined by taking 40g of the saturated mixture from each sample and drying for 24 hours at 105°C. This moisture content was regarded as the pot capacity (PC) which was then used as a reference (100% moisture content) for calculation and establishment of the other moisture levels above. For instance, the amount of water added to a completely dry soil mixture in pots to get the 45-50% moisture level was half of that added to the 90-100% moisture content pots.

Air-dried samples of the sterilized soil mixture (Section 3.3.1), each weighing 2400g, were put in pots. Water was then added to the pots in accordance with the level of moisture required. Pyrethrum plants (clone L/75/487) were planted on the potted soil mixture with different moisture levels, one plant per pot, after inoculating them by root-dipping in the bulked *Fusarium oxysporum* inoculum (Section 3.4.1). Control (uninoculated) plants were only washed in running tap water prior to planting.

For each soil moisture level, four inoculated and two uninoculated plants were used. The soil from uninoculated pots was used for determining the moisture levels which subsequently helped in regulation and maintenance of the established soil moisture levels. Plants from such plants also provided comparison samples during the assessment of plant colonization by the

fungus (Section 3.3.5). Each treatment representing one moisture level was replicated four times and the experiment was laid out in a completely randomized design (CRD).

In order to maintain the moisture levels within the designed ranges, moisture contents were regularly determined on the dry-weight basis using soil mixture samples from the two uninoculated control pots. The samples were taken from the 8-10cm deep layer from each of the two pots per moisture level. In case the moisture content for a particular moisture level was found to be one unit or less above the minimum value for that level, the appropriate amount of water was calculated and added accordingly to bring the value for that particular moisture level to the maximum level.

Data collected for this experiment were the time taken (days) for initial wilt symptoms to appear from the start of the treatments. The time taken for complete death of the wilted plants was also recorded. Symptomless plants were sampled at the end of the experimental period to establish if there was any infection as in section 3.3.5. All those plants showing wilt symptoms were considered to be infected.

The data collected were subjected to analysis of variance, with further analysis being done using the Duncan's Multiple Range Test.

3.4.4. Effect of Combined Field Conditions on Wilt Development in Pyrethrum

The field experiment was set up in June, 1994 at Kisii, Molo, Ol Joro-Orok and Limuru, these being areas with different ecological conditions. Recorded average air temperatures for the period 1986 to 1993 were 20.4°, 14.3°, 14.4° and 14.1°C for Kisii, Molo, Ol Joro-Orok and Limuru respectively, with respective altitudes of 1723, 2523, 2400 and 2115 metres above sea level. The average annual rainfall figures for the period 1986 to 1993 were 2025.9, 1085.6,

789.9 and 962.2mm for the same areas respectively.

Eight plots, each measuring 180cm by 90cm, were prepared at each experimental site. The experiment was set up by first planting each plot with 16 plants of clone L/75/487 at the recommended spacing of 60cm between rows and 30cm between plants within a row (Anon, 1976). The plots were laid out in a randomized complete block design (RCBD) of four blocks (replicates), each block consisting of two plots. In each block, one plot was taken at random and its plants were inoculated with the bulked fungal inoculum through the soil-drench method (Section 3.4.1). Inoculation was done four weeks after planting. Plants in the second plot in each block were left uninoculated as controls.

Meteorological data on temperature and rainfall for the period June to September, 1994 were taken for each of the four experimental sites. Field capacity moisture determinations were also carried out for the four sites (Doneen and Westcott, 1984).

Only the central four plants per plot were considered for data collection while the rest of the plants were considered as guard row plants. Observations were made daily to record the time taken for the first wilt symptoms to appear at each experimental site. At the end of the experimental period, all the symptomless plants were sampled and examined for the presence or absence of pathogen infection (Section 3.3.5).

The data collected were subjected to analysis of variance and mean separation was conducted using the Duncan's Multiple Range Test.

3.4.5. Influence of pH on Wilt Expression and Fungal Growth

3.4.5.1. Soil Sampling

Soil samples were collected from experimental sites at Kisii, Molo, Mwongoris, Ol Joro Orok and Limuru. The samples, each about 200cm^3, were taken from eight points that were randomly selected at each site. Samples from each site were paired at random, mixed thoroughly and then spread out on the laboratory bench to dry for two days. This resulted in four samples per site.

After the air-drying process, each sample was sieved through a 2-mm sieve to get the fine earth fraction, which was subsequently reduced by quartering until a homogenous sub-sample of about 50g was obtained. Thus, there were four 50-g samples from each collection site.

In addition, four 50-g samples were prepared in the same way from the planting soil mixture used for greenhouse experiments.

3.4.5.2. Determination of pH

Measurement of pH was carried out on each of the 50-g samples using the Calcium Chloride ($CaCl_2$) method as described by Ahn (1973) and Page *et al* (1982). To each sample a 100ml-solution of 0.01M $CaCl_2$ was added and the mixture allowed to come into equilibrium for 30 minutes with stirring at regular intervals of 5 minutes. After the 30-minute period, the resulting suspension was allowed to settle to give a layer of fairly clear supernatant water. A pH-meter electrode was then lowered into the clear supernatant water and the pH value read on the meter.

The data obtained were analyzed by analysis of variance and means separated using the Duncan's Multiple Range Test.

3.4.5.3. Effect of pH of Medium on Growth of Fungal Isolates

A laboratory experiment was conducted to determine the influence of various pH levels of the culturing medium on the growth of five *Fusarium oxysporum* isolates obtained from Kisii, Molo, Mwongoris, Sotik and Limuru.

Potato sucrose agar (PSA) was prepared with five different pH levels of 4.0, 5.0, 6.0, 7.0 and 8.0. and poured into sterile Petri dishes. Culturing was done by dipping a sterile inoculating needle in a conidial suspension of each representative isolate and transferring the adhering conidia to the centre of a PSA plate.

For each isolate, three PSA plates were used at each pH level; hence there were three replicates in the experiment which was laid out in a completely randomized design (CRD).

The plates were incubated at room temperature (20-22°C) and colony diameter measurements were made daily for seven days for all the fungal isolates. The data collected on the 4th day for colony diameters were subjected to analysis of variance and mean separation was done using the Duncan's Multiple Range Test.

3.5. Screening of Pyrethrum Clones and Varieties for Resistance to Wilt Disease

3.5.1. Planting Materials and Inoculation Methods

Pyrethrum clones (Ks/70/64, Ks/75/313, Kr/74/122, Mo/74/223, Ma/70/1013, Ma/71/443, L/75/487 and 4331) and varieties (K218, K235, P_4) that are adapted to different ecological conditions were screened for resistance to wilt disease under both greenhouse and field conditions. Clone 4331 was used as a control because of its known susceptibility to the wilt disease (Previous field observations).

Planting materials (splits) were obtained by splitting healthy plants from the National Pyrethrum and Horticultural Research Station at Molo.

Root-dipping method of inoculation was used in the greenhouse experiments while soil drenching method was employed in field experiments. The inoculum used was prepared as in section 3.4.1.

3.5.2. Greenhouse Experiment

Four plants per clone/variety mentioned above were inoculated by root-dipping in a $1.5x10^6$ conidia/ml suspension for 30 minutes and potted in a sterile planting soil mixture (Section 3.3.1). Two uninoculated control plants of each clone/variety were only washed in tap water prior to potting. The four inoculated and two uninoculated plants constituted an experimental unit.

The experimental units were laid out in a completely randomized design (CRD) with three replicates, such that there were 18 plants of each clone/variety in the experiment.

The plants were allowed to establish and grow under greenhouse conditions and observations were made weekly for the expression of wilt symptoms. Data were taken on the time taken (in days) for wilt symptoms to appear from the time of inoculation for each

clone/variety per replicate.

At the onset of wilt symptoms on plants of each clone/variety, shoot samples were taken and cultured on PSA plates to establish the length of vascular colonization by the fungus. The same was done for all symptomless plants of each clone/variety at the end of the experimental period. Stems of the sampled plants were cut into 8-cm length portions, starting from the crown level, and sterilized in 0.5% NaOCl solution for five minutes before being rinsed in five changes of sterile distilled water. The portions were then aseptically split longitudinally, placed on PSA plates and incubated at room temperature (20-22°C). Stem portions from the same shoot were plated separately on one PSA plate. The cutting into 8-cm lengths was done in order to accommodate the shoots inside the PSA plates which measured 9cm in diameter.

The distance along the portions showing mycelial growth was measured and recorded. The total length of vascular colonization by the fungus along any shoot was obtained by adding up the measured lengths from the individual 8-cm length portions of the same shoot.

The data collected on days to symptom expression and vascular colonization were subjected to analysis of variance and mean separation was carried out using the Duncan's Multiple Range Test.

3.5.3. Field Experiment

Experiments to screen the eleven clones/varieties mentioned above (Section 3.5.1) for resistance to wilt were set up at Molo and Mwongoris in June 1994. At each site, four blocks were used, each consisting of twelve plots. Each plot measured 180cm by 90cm.

The eleven clones/varieties were planted in various plots at random in each block, with clone 4331 (a control clone) being allocated to two plots at random and the other ten clones/varieties being allowed one plot each. On each plot, the plants were planted at a spacing

of 60cm by 30cm, such that there were 16 plants per plot. In each plot, only the central four plants, making an experimental unit, were considered for data collection; the rest were regarded as guard-row plants. The experiment was laid out in a randomized complete block design (RCBD) with four replicates.

The plants were allowed to establish for four weeks before inoculation by drenching the soil with 10ml of the bulked inoculum (prepared as in section 3.4.1) around each plant. One plot planted with clone 4331 in each block was left as a uninoculated control.

Observations were made on a weekly basis and the time taken (days) for wilt symptoms to appear, from the date of inoculation, was recorded for each clone or variety at each site. Other data taken included the number of flowers picked and their weights from the central four plants at fortnightly intervals. At each experimental site, the clones/varieties were sampled for all the symptomless and wilted plants and cultured on PSA to determine vascular colonization at the end of the experimental period. This was done as in the case of the greenhouse experiment (Section 3.5.2). The data taken were subjected to analysis of variance.

CHAPTER FOUR

4. RESULTS

4.1. Wilt Symptoms on Pyrethrum in the Field

Field observations on occurrence of pyrethrum wilt were made at the time of collection of infected plant materials and corresponding rhizosphere soil samples in September 1993 from the areas mentioned in section 3.1.

At Kisii, breeding and soil fertility trials conducted by the Pyrethrum Board of Kenya had poor plant stands with large gaps remaining behind after plants wilted and dried up (Plate 1). Pyrethrum stands of clone Ks/70/64 were frequently affected by wilt at Mwongoris, a plant multiplication centre (Plate 2). Molo and Sotik areas did not have serious wilt problems except for some isolated cases of wilted plants in the fields. In Limuru area, the wilt problem was particularly critical where clone Ma/70/1013 (Plate 3) was planted but other clones were affected to a lesser extent.

In all the areas mentioned above, plants displayed varied symptoms of the disease, perhaps depending on the length of time that each plant had been infected. Some plants showed initial chlorosis of leaves on one side of the plant while others showed chlorosis of all the leaves. Chlorotic leaves on some plants were withered, with the mid-veins slowly becoming yellowed and chlorosis extending towards the leaf petioles. Plants at a more advanced stage of the disease displayed drooping of leaves (Plate 4 background) and some had no indication of chlorosis, either on one side of the plant or the whole plant. In all the cases, the symptoms started with the lower leaves.

Plate 1. Poor plant stands due to wilt disease at Kisii Research Station.

Plate 2. Poor performance of multiplication plants at Mwongoris due to the wilt disease.

Plate 3. Pyrethrum field of clone Ma/70/1013 affected by the wilt disease, leaving large gaps, at Limuru.

Plate 4. Typical wilt expression in the field. Note the upright stems even after the drying up of leaves. At the background is a plant in its initial wilting stages.

Occasionally, well-established healthy pyrethrum bushes were found with totally dried up leaves but with erect stems (Plate 4). On up-rooting these plants, the root systems were found to be intact in most cases, although some roots had started rotting. The rotting was mainly observed at the root tips. Most of the plants exhibited brown to dark grey, parallel streaks on the split roots and stems (especially near the crown region). These parallel streaks were manifestations of infection on the vascular tissue, as evidenced by the growth of fungal mycelia from such wilted plants upon culturing their sections on PDA. However, some plants with external symptoms did not show any vascular discolouration, as in the case of symptomless plants.

4.2. Identification of Pathogens

4.2.1. Identification of Fungal Isolates

Fungal isolation as described in section 3.2.1 resulted in five different isolates from the diseased materials obtained from Kisii, Molo, Mwongoris, Sotik and Limuru and designated as I, II, III, IV and V respectively.

4.2.1.1. Colony descriptions

All isolates I, II, III, IV and V had white mycelia with a cottony appearance, a flattened top and uniform (regular) margins on PSA, seven days after incubation. Colony diameters were 5.6, 4.3, 5.1, 4.4 and 4.8cm for isolates I, II, III, IV and V respectively, four days after inoculation on PSA (pH 6.7 $\pm$ 0.1) and incubation at 25°C. Colony diameters on the seventh day of incubation were 0.6, 0.8, 0.8, 1.1 and 0.6cm for isolates I, II, III, IV and V respectively incubated on PSA at 37°C.

Each isolate had a shade of pinkish pigmentation on the reverse side of the culture plates incubated at 25°C. The pinkish pigmentation could be seen slowly penetrating the upper surface so that the cottony mycelium became faintly pinkish.

4.2.1.2. Spore morphology

Three types of spores, macroconidia, microconidia and chlamydospores, were produced by all the five isolates.

The macroconidia had only transverse septa, ranging from 3 to 7 in number, and majority of them were 4-celled (Table 2b). All the macroconidia were tapered at both ends (Plates 5 and 6) and had their maximum diameter, ranging from 3.6 to 5.4μm, around the curved (sickle-shaped) spore middle. They also had a foot-shaped basal cell (Plate 6). Isolates I, III and V had clearly visible macroconidial septation unlike isolates II and IV which had faint septa. The general shape of isolates I, II and V spores was the same (falcate) whereas isolates III and IV had sausage-shaped to falcate macroconidia.

Macroconidia of isolate I were the largest, ranging in length from 36.0μm to 46.8μm (average being 40.7μm), whereas macroconidia of isolate III were the smallest with an average length of 31.0μm, ranging from 28.8 to 32.4μm (Table 2a).

Table 2a. Spore characteristics of five different isolates of *Fusarium oxysporum*

	Isolates*				
	I	II	III	IV	V
Macroconidia					
Length (μm)	36.0-46.8 (40.7)	32.4-36.0 (34.2)	28.8-32.4 (31.0)	28.8-36.0 (34.2)	32.4-43.2 (39.2)
Maximum width (μm)	3.6-5.4 (4.3)	3.6	3.6	3.6	3.6
General shape	Falcate	Falcate	Sausage-shaped to falcate	Sausage-shaped to falcate	Falcate
Septa (range)	3-7	3-5	3-4	3-5	3-7
Microconidia					
Length (μm)	7.2-10.8 (9.4)	7.2-10.8 (9.4)	7.2-10.8 (9.7)	7.2-10.8 (8.6)	7.2-10.8 (8.6)
Maximum width (μm)	3.6	3.6	3.6	3.6	3.6
General shape	Clavate	Clavate	Fusoid	Fusoid	Clavate
Septa (range)	0-1	0-1	0-1	0-1	0-1

Table 2b. Conidia septation of five different isolates of *Fusarium oxysporum*

	Isolates*				
	I	II	III	IV	V
Number of macro-conidia considered	20	20	20	20	20
Number with:					
a) 3 septa	11 (55%)	12 (60%)	11 (55%)	10 (50%)	12 (60%)
b) 4 septa	- (0%)	5 (25%)	6 (30%)	7 (35%)	3 (15%)
c) 5 septa	5 (25%)	3 (15%)	3 (15%)	3 (15%)	3 (15%)
d) 6 septa	2 (10%)	- (0%)	- (0%)	- (0%)	- (0%)
e) 7 septa	2 (10%)	- (0%)	- (0%)	- (0%)	2 (10%)
Number of micro-conidia considered	20	20	20	20	20
Number with:					
a) 0 septa	15 (75%)	15 (75%)	17 (85%)	16 (80%)	15 (75%)
b) 1 septum	5 (25%)	5 (25%)	3 (15%)	4 (20%)	5 (25%)

*Isolates I, II, III IV and V were collected from Kisii, Molo, Mwongoris, Sotik and Limuru respectively. The figures in brackets are the means for the respective ranges, taken for 20 spores.

Macroconidia were hoisted on greatly branched conidiophores in the early stages of colony sporulation. During later stages (when cultures were ten days old and beyond), numerous, clear to light-coloured sporodochia were evident with many macroconidia clustered together (Plate 5) and having no apparently visible conidiophores (the sporodochia appeared to be formed on the hyphae without visible conidiogenous cells).

Microconidia of all the isolates were mostly single-celled (with occassional bicelled conidia), fusoid to clavate in shape, with smoothly-rounded ends. They were almost as thick as the macroconidia (Table 2a). Microconidia of all the isolates formed false heads on monophialides and when detached from the "cluster", they aligned themselves alongside hyphae in a sticky manner (Plate 7).

All the isolates produced smooth-walled chlamydospores which were both terminal and intercalary. The terminal chlamydospores were mostly borne singly whilst intercalary ones were in chains.

By use of the above colony characteristics (Section 4.2.1) and spore morphology descriptions in addition to application of a *Fusarium* identification key (Anonymous, 1992a) and descriptions of *Fusarium* species by Booth (1971), all the five isolates were identified as *Fusarium oxysporum* Schlecht. emend. Sny. & Hans.

Plate 5. Macroconidia from Mwongoris isolate produced in sporodochia (x500).

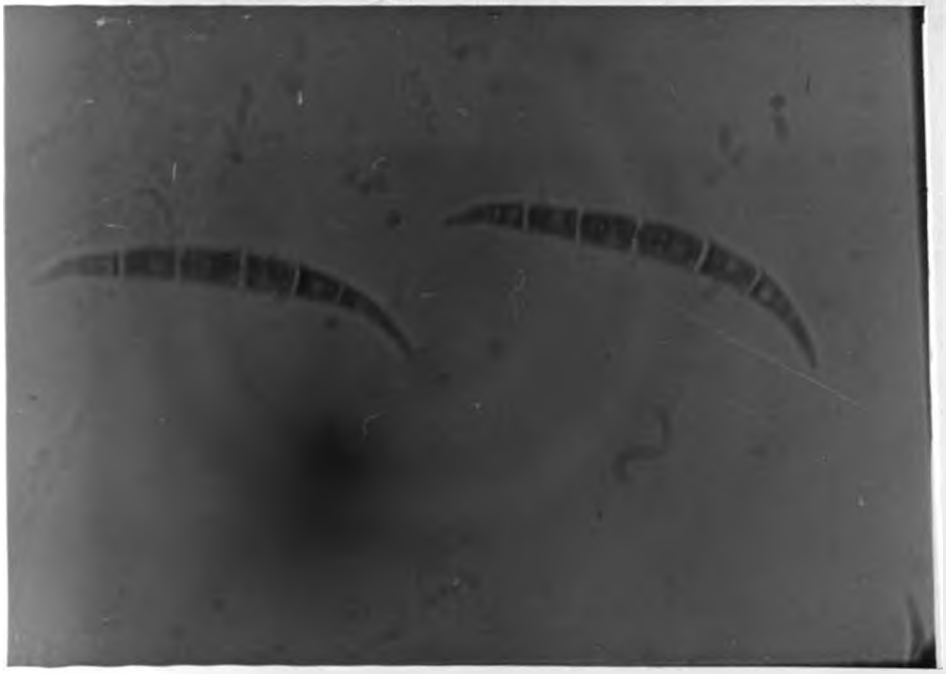


Plate 6. Macroconidia from Limuru isolate showing pedicellate basal cells. Note the typical tapering at both ends (x1250).

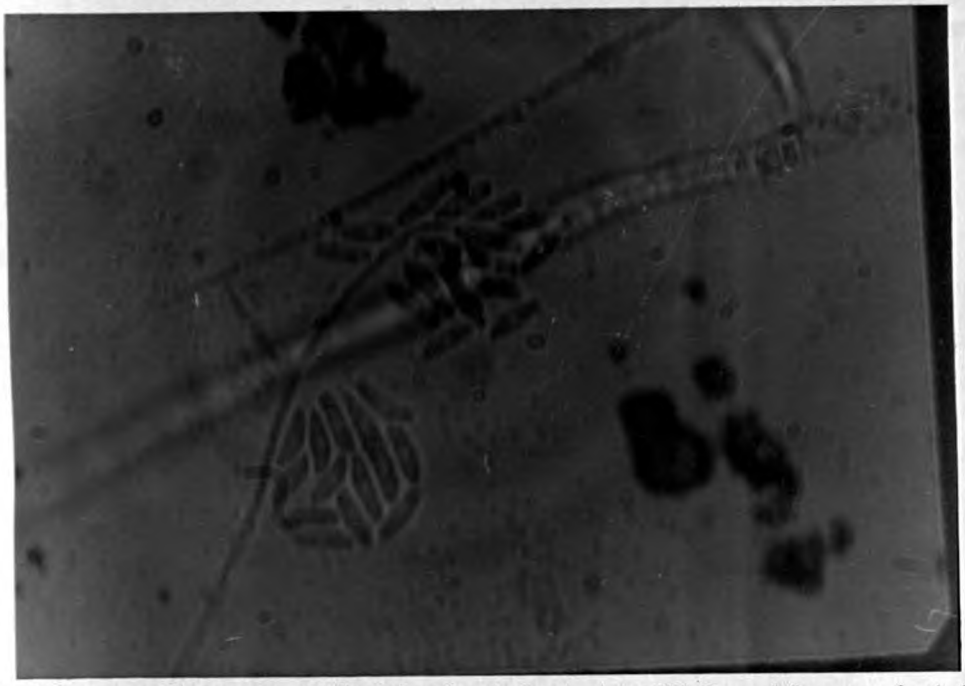


Plate 7. Microconidia produced in false heads by isolate III from Mwongoris (x1250).

4.2.2. Identification of Nematodes

Examination of nematode samples from each nematode-collection site was done and various morphological measurements were taken. From both root and soil samples, nematodes belonging to the genera *Meloidogyne*, *Pratylenchus* and *Tylenchus* were identified as being parasitic on pyrethrum plant roots. These genera represented stylet-bearing nematodes. Table 3 summarizes the relative numbers of nematodes counted for each genus from the samples examined.

Table 3. Nematode counts* from five sample-collection sites

Collection site	Sample type	Nematode genera			Totals
		Meloidogyne	*Pratylenchus*	*Tylenchus*	
Kisii (I)	Roots[a]	2940	1340	-	4280
	Soil[b]	720	900	-	1620
Molo (II)	Roots[a]	766	588	-	1354
	Soil[b]	284	440	-	724
Mwongoris (III)	Roots[a]	960	544	-	1504
	Soil[b]	372	562	-	934
Sotik (IV)	Roots[a]	460	260	40	760
	Soil[b]	220	380	580	1180
Limuru (V)	Roots[a]	880	376	-	1260
	Soil[b]	570	124	-	694

*Only juveniles and vermiform adults were considered in making the counts. The figures represent 1ml aliquot counts adjusted to whole volume counts for the nematode suspensions.

[a]Nematodes in 30g of root samples from wilted plants.

[b]Nematodes in 200ml of soil from rhizosphere of wilted plants.

4.2.2.1. General descriptions of nematodes

a) *Meloidogyne* species specimens

i) Females

The whitish females had a swollen, pyroid body with a short neck and a bluntly rounded anterior end (Plates 8 & 9). The swelling was more pronounced posteriorly. The body annules were only faintly visible around the body middle. However, annulations around the neck and in the region of the anus and vulva were clearly visible. The median bulb was conspicuously rounded, with the median bulb valves clearly visible (Plate 10).

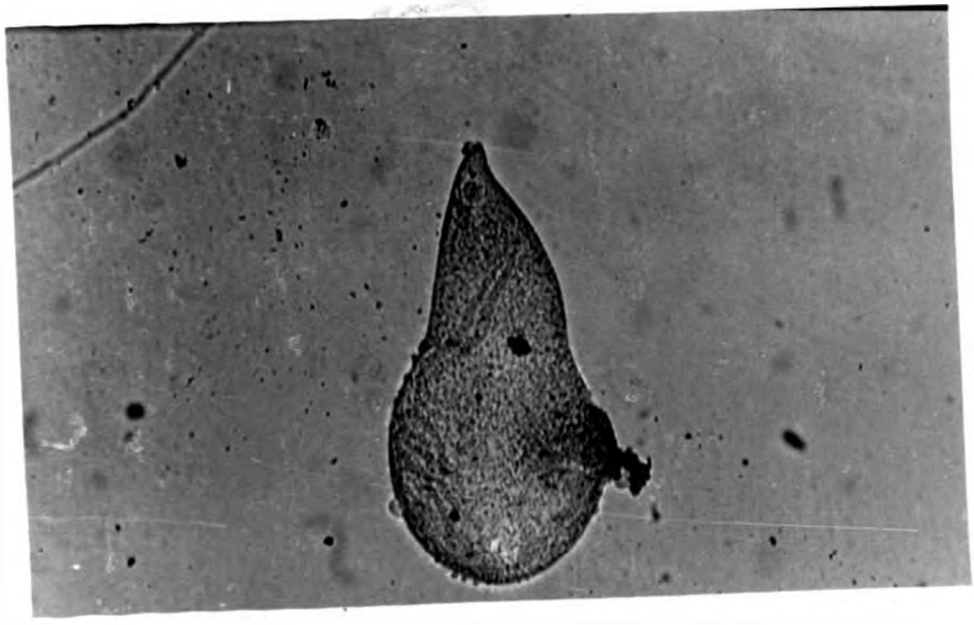


Plate 8. A mature *Meloidogyne hapla* female. Note the swollen posterior end and short neck (x79).

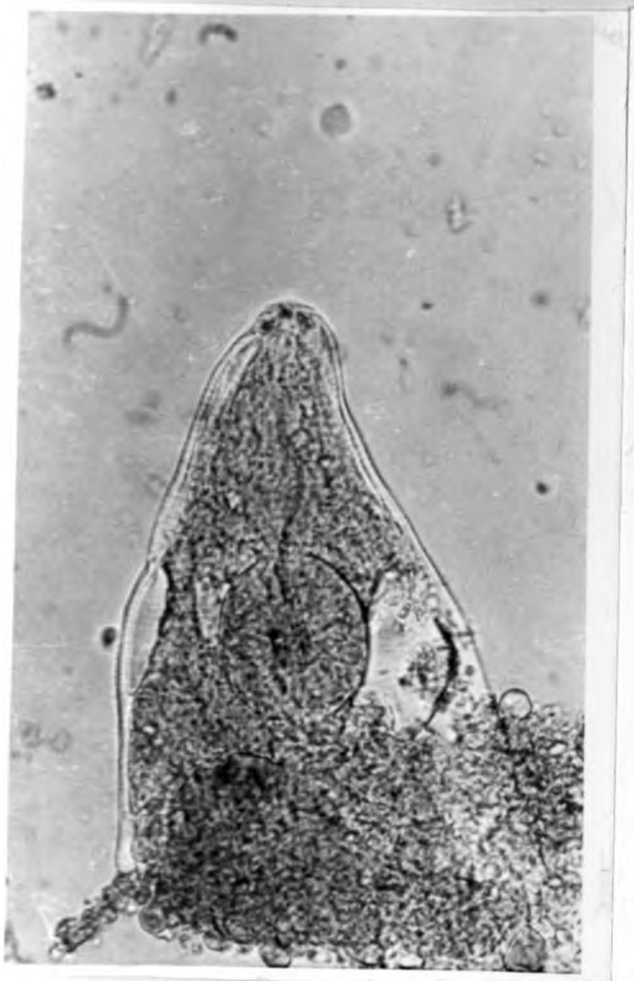


Plate 9. Head and neck regions of a mature *Meloidogyne hapla* female (x500)

Plate 10. Perineal pattern of a *Meloidogyne hapla* female (x1250)

The adult females possessed a distinctive pattern of striations surrounding the vulva and the anus (perineal pattern). The perineal patterns, formed by slightly wavy and closely spaced striae (Plate 10), were generally circular. The lateral fields of the perineal patterns were inconspicuous and largely invisible. The perivulval region was clear of striae and around the tail terminus, the striae were widely spaced to some extent. An important feature noticed was the presence of distinct stippling of the area between the anus and the tail terminus by punctations

(Plate 10). The synoptic key of perineal patterns, photomicrographs and drawings provided by Franklin (1965) were used and these confirmed the perineal patterns to belong to *Meloidogyne hapla*.

ii) Males

Males had a general vermiform body with fine annulations. The head had a semi-circular outline with a well developed cephalic framework (Plate 11). The stylet was strongly developed with large backwardly sloping stylet knobs. The median bulb was rounded and clearly defined but not as strongly developed as in females.

The slightly curved paired spicules were located almost at the tip of a smooth, bluntly-rounded tail (Plate 12). The gubernaculum was simple and weakly developed compared to the spicules just anterior to it.

The length/width ratio ranged from 39.0 to 45.5. Dropkin (1989) gives this ratio as close to 45 for *Meloidogyne* species males.

iii) Juveniles

The larval stages examined were vermiform in shape, anteriorly and posteriorly tapering and had no clearly defined head skeleton.

The head was not offset and followed the same contour as the rest of the body. The weakly developed stylet had rounded basal knobs behind which was an ellipsoidal median bulb (Plate 13).

The tail tip was crooked in appearance for some specimens and bifid in others (Plate 14). Mobile juvenile specimens had a serpentine movement, showing some banding of the body.

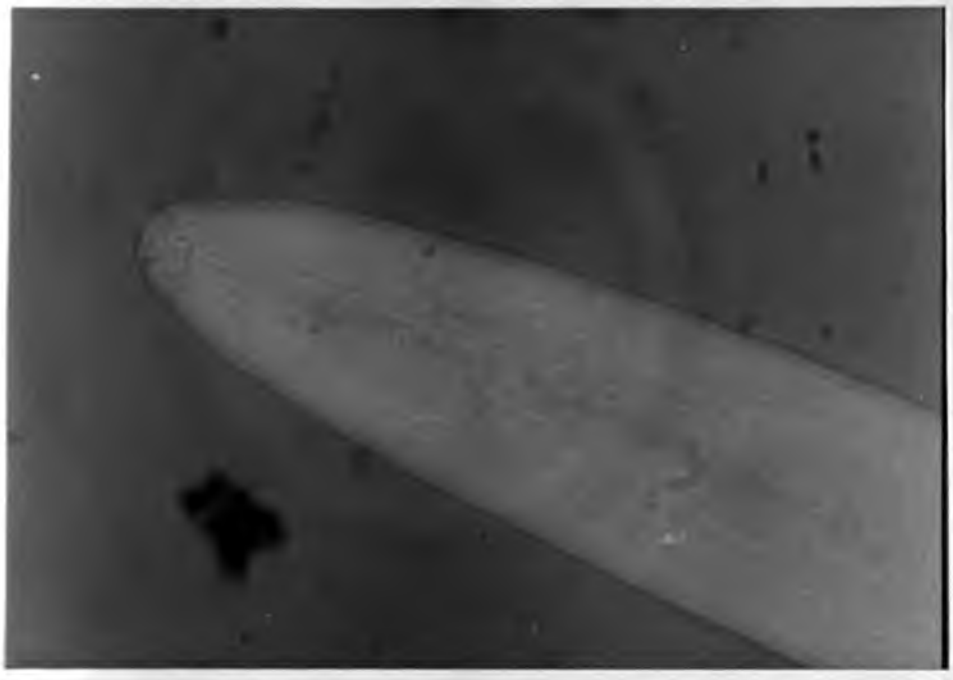


Plate 11. Anterior region (ventral view) of a *Meloidogyne* male specimen (x750).

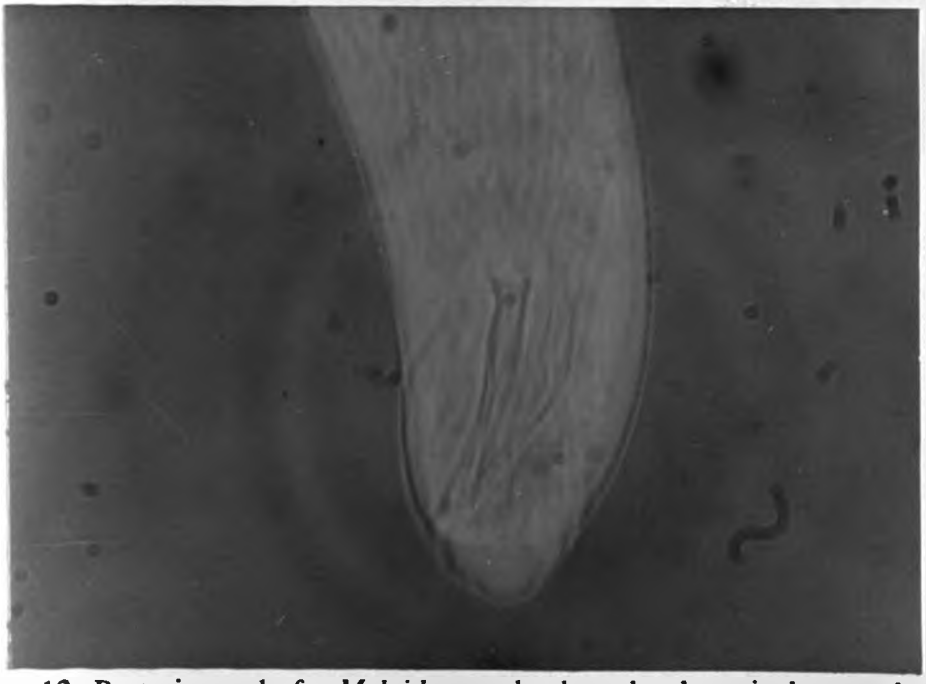


Plate 12. Posterior end of a *Meloidogyne hapla* male; the spicules are clearly visible near the bluntly rounded tail terminus (x750).

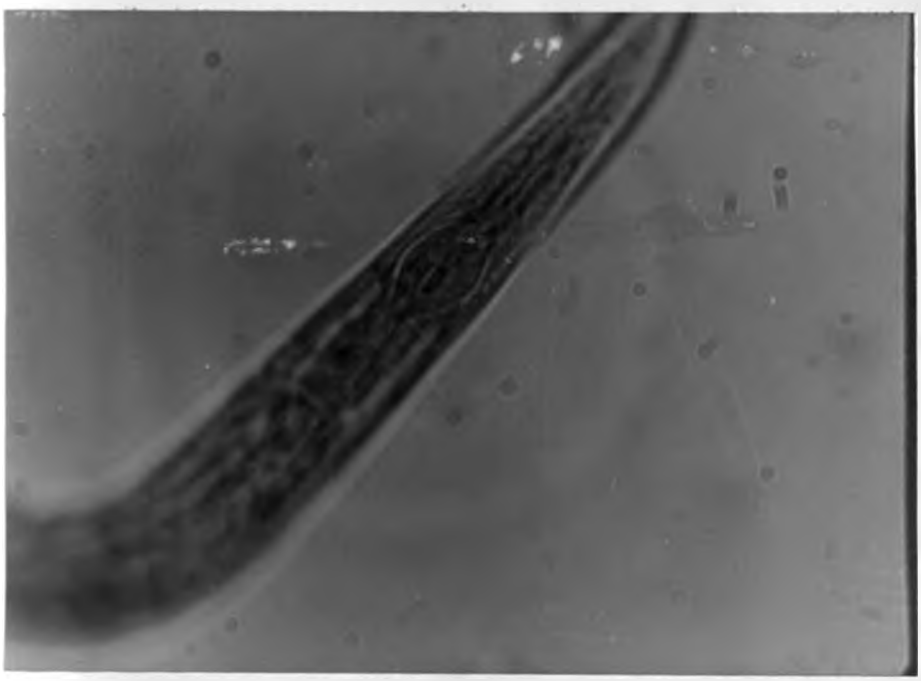


Plate 13. Ellipsoid median bulb of a second stage *Meloidogyne* juvenile. The bulb becomes more spherical with maturity (x1250).

Plate 14. Typically crooked tail terminus of a *Meloidogyne hapla* juvenile (x1250).

b) *Pratylenchus* species specimens

i) Females

Females had a cylindrical body with an untapered neck (no head offsetting) and clear but fine annulations. The body was generally opaque, appearing to be granulated (Plate 15) especially in the intestinal region.

The lip region was flat on the anterior end, having a well-sclerotized cephalic framework. The stylet was well-developed and had prominently rounded basal knobs (Plate 16). There were three head annules just behind the flat anterior end.

The median bulb was more ovate than spherical in shape, the basal bulb being only slightly visible due to a dorsal overlap of the intestine over the esophageal glands.

The ovary was single, prodelphic and outstretched with a possible second ovary having been reduced to only a small undeveloped post-vulval sac. The vulva was posteriorly positioned at about 83% of the body length from the anterior end (Appendix 5b). The tail was smooth and broadly rounded (obtuse) at the tip.

ii) Males

Males resembled the females in gross morphology. They were cylindrical and had an untapered neck just like the females. They were, however, smaller than the females in size as illustrated by the respective measurements (Appendix 5b).

The lip region was a truncate cone with a well-developed head frame and three head annules. The stylet was short and strong and backed by large broadly-rounded basal knobs.

The paired spicules were posteriorly located near the smooth, conoid tail tip which was enveloped by a bursa.

iii) Juveniles

The larval stages had the same basic body shape (vermiform appearance) as the adults but they were slightly smaller than the males. The tail was relatively longer than that of the males.

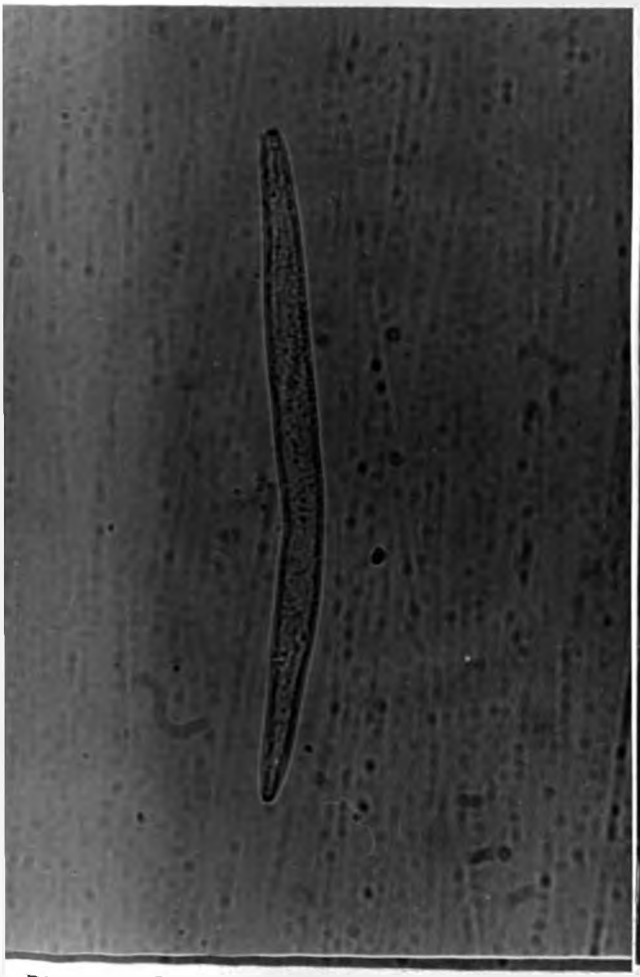


Plate 15. Opaque appearance of a Pratylenchus *penetrans* female (x200)

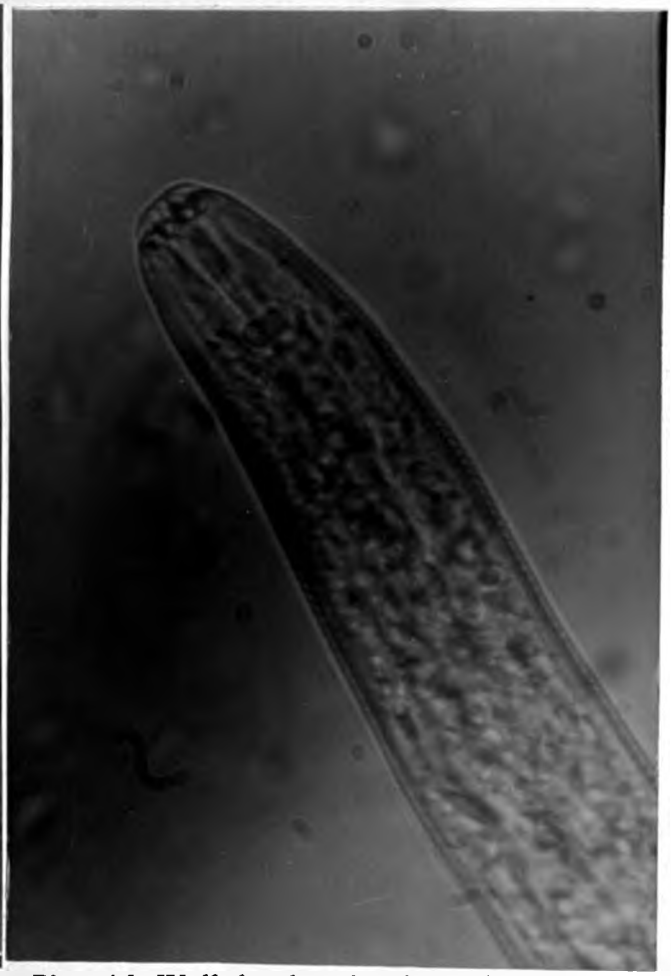


Plate 16. Well-developed stylet and knobs in *Pratylenchus penetrans* (x1250).

c) *Tylenchus* **species specimens**

i) Females

Generally, the females had a long vermiform body ending with a long filiform tail posteriorly and a narrowed down anterior part without any cephalic framework (Plate 17). The body was smooth and had no visible annulations although Heyns (1971) records the presence of transverse striae. The lip region was flat and not offset at all.

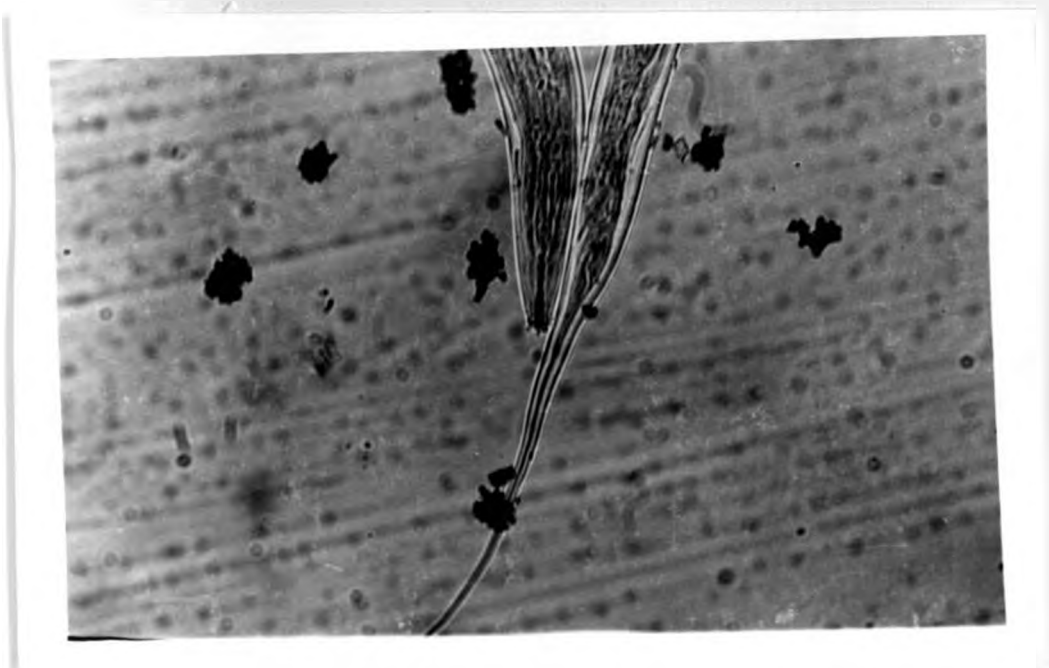


Plate 17. Head and tail regions of a *Tylenchus* species female (x200).

The ovary was single and anteriorly outstretched (prodelphic). The vulva was posteriorly placed but not far from the middle of the body (average V-value for the specimens was 52%; Appendix 5c).

There was no overlap between the intestine and the esophagus which ended with a pyriform basal bulb. The oval-shaped median bulb, posterior to a weakly-developed, knobless stylet, was only faintly visible. The stylet on most of the specimens examined was found protruding out of the head.

ii) Males

No male specimens were observed in the various samples that were examined.

iii) Juveniles

The juveniles were basically similar to the adult females although they had relatively shorter, filiform tails. Among those examined, no genital primordium was present except for one juvenile specimen which had a vulva at about 47% of the body length from the anterior end.

4.2.2.2. Morphometric measurements for nematode specimens

The following is a summary of measurements and ratios for the three nematode genera identified. Complete tables of the measurements and ratios for a few representative specimens are provided in appendix 5.

a) ***Meloidogyne*** **specimens**

Males (8♂♂)

L = 1131.0-1174.5 (1145.5μm); a = 39.0-45.5 (41.1); b = 14.2-15.0 (14.5); b' = 18.5-19.2 (18.7); c = 157.1-209.4 (172.2); Spicules length = 21.6-25.2 (24.3μm);

Gubernaculum length = 10.8μm.

Juveniles (27 larvae)

L = 349.2-377.0 (356.3μm); a = 20.0-24.8 (23.3); b' = 6.6-8.1 (7.2); c = 8.1-9.9 (8.9).

b) ***Pratylenchus*** **specimens**

Females (22♀♀)

L = 522.0-638.0 (600.3μm); a = 21.5-24.2 (22.6); b = 5.4-6.1 (5.8); b' = 9.7-11.8 (11.1); c = 15.7-17.7 (16.5); V = 81.4-83.7 (82.6%).

Males (6♂♂)

L = 536.5-551.0 (543.8μm); a = 21.3-21.9 (21.6); b = 5.9-6.0 (5.9); b' = 10.6-10.9 (10.8); c = 15.3-16.6 (15.9).

Juveniles (14 larvae)

L = 478.5-493.0 (488.2μm); a = 22.2-22.8 (22.6); b' = 10.5-11.4 (11.0); c = 12.1-12.5 (12.3).

c) *Tylenchus* specimens

Females (26♀♀)

L = 1725.5-1870.5 (1777.3μm); a = 24.2-30.0 (26.9); b = 3.9-4.1 (4.0); c = 3.8-6.5 (4.6); V = 51.2-52.9 (52.0%).

Juveniles (15 larvae)

L = 855.5-957.0 (893.2μm); a = 14.8-26.4 (18.8); b = 3.8-4.1 (4.0); c = 11.0-13.6 (12.0).

4.3. Pathogenicity Tests

4.3.1. Symptomatology of pyrethrum wilt

Inoculated plants of the susceptible clone L/75/487 exhibited symptoms of the wilt disease (Plate 18) but the responses varied depending on the inoculation method and level of inoculum used. Plants inoculated with high inoculum levels took shorter time periods to express wilt symptoms and the disease severity was also higher than in plants inoculated with lower inoculum levels (Plates 19 and 20). Wilt symptoms were only observed in cases where fungal inoculum was used, either alone or in combination with nematodes. Plants inoculated with nematodes alone showed no wilt symptoms, although differences in plant heights, plant bush diameters and number of tillers per plant were observed (Table 4) when compared with the healthy ones.

Plate 18. Typical wilt symptoms on an artificially inoculated clone L/75/487 plant; on the left is an uninoculated plant.

Plate 19. Clone L/75/487 plants inoculated with fungal inoculum alone. I_1, I_2, I_3 and I_4 are inoculum levels of $1.25x10^6$, $2.5x10^6$, $5.0x10^6$ and $1.0x10^7$ conidia respectively for Kisii isolate (I).

Plate 20. Clone L/75/487 plants inoculated with nematode-fungus inoculum. I_a, I_b, I_c and I_d are inoculum levels of $2.5x10^6$: 500, $2.5x10^6$: 1500, $1.0x10^7$: 500 and $1.0x10^7$: 1500 conidia and nematode combination ratios respectively for Kisii isolate (I).

Plate 21. Clone L/75/487 plants inoculated with *Fusarium oxysporum* isolates from Kisii (I), Molo (II), Mwongoris (III), Sotik (IV) and Limuru (V) at the same inoculum level of $1.0x10^7$ conidia.

Plate 22. Clone L/75/487 plants inoculated with *Fusarium oxysporum* isolates from Kisii (I), Molo (II), Mwongoris (III), Sotik (IV) and Limuru (V) combined with respective site nematodes in the ratio $1.0x10^7$: 1500 conidia : nematodes.

Plants inoculated by soil drenching took relatively longer periods to express wilt symptoms than the root-dipped plants. However, the symptoms expressed were basically similar for correspondingly similar inoculum levels in both methods. Equal inoculum levels of the different isolates of *Fusarium oxysporum* showed more or less similar levels of symptoms on inoculated plants (Plates 21 and 22).

Plants inoculated with $1.25x10^6$ conidia, $2.5x10^6$ conidia, and $2.5x10^6$: 500 conidia and nematode combination ratio expressed mild symptoms of the wilt disease (Plate 19, plants I_1 and I_2; Plate 20, plant I_a). The symptoms started with chlorosis of the lower leaves from the leaf margins and progressed towards the leaf petioles. As the disease progressed, necrosis followed chlorosis of the leaves which eventually dried up. At this stage, the plants had lost their green colour on the leaves, leaving behind only pale green stems with dried up leaves.

For inoculum levels of $5x10^6$ and $1.0x10^7$ conidia, $2.5x10^6$: 1500 and $1.0x10^7$: 500 conidia-nematode combination ratio, plants became diseased much earlier and more severely than those inoculated at the above inoculum levels (Plate 19, plants I_3 and I_4; Plate 20, plants I_b and I_c). In plants inoculated with these four inoculum levels, drooping of leaves (epinasty) was more pronounced, such that withering was clearly evident; necrosis occurred almost concurrently with yellowing of the leaves and the upward progression of the symptoms on the leaves was much faster. Differences in symptom expression in relation to inoculum levels were clearly displayed.

Plants inoculated using $1.0x10^7$ conidia combined with 1500 nematodes were the most severely affected. These plants exhibited wilting (withering of leaves and tender shoot tips) without any visible initial chlorosis of leaves. The plants had turgid leaves and looked healthy in the morning hours of the day but the withering was clearly visible towards the middle hours of the day, especially during hot weather. After about 4 to 5 days, the plants were unable to recover from the wilted condition and started drying up (Plate 20, plant I_d).

All the above symptoms developed in the plants inoculated through root-dipping. Similar observations were also made on plants inoculated by soil drenching, but with an additional characteristic namely, unilateral wilting, in some of the plants. Nevertheless, all the affected plants eventually became wholly wilted and dried out.

Even though affected plants wilted and dried up completely, the stems were always left upright without collapsing irrespective of the level of inoculum used (Plates 19-22).

Roots had no externally observable symptoms except for some slight blackening of the crown. On splitting open the roots and stems of wilted plants, brownish vascular discolouration was observed. This symptom was mainly observed with high inoculum level treatments. Plants inoculated with high inoculum levels of the fungus ($5.0x10^6$ and $1.0x10^7$ conidia), either singly or in combination with nematodes, exhibited more intensive vascular browning (visual assessment) than those inoculated with lower fungal inocula levels ($1.25x10^6$ and $2.5x10^6$ conidia).

Fungal infection was noted on all the inoculated plants when isolations from such plants were done on PSA. The two media used, PSA and SNA, supported good growth and profound sporulation of all the fungi. All the re-isolated fungi were identified as *Fusarium oxysporum*, the same fungus that had initially been inoculated onto the test plants. Staining of longitudinal and transverse sections of stems from wilted plants with 1% cottonblue-in-lactophenol displayed presence of the fungus in vascular bundles, which became stained bluish-green (Plates 23 and 24).

Uninoculated (control) plants showed none of the symptoms described above in all the cases.

Table 4. Heights, bush diameters and number of tillers for clone L/75/487 plants 120 days after inoculation with mixed nematode species at various levels*

	Heights (cm)		Bush diameters (cm)		Number of tillers	
Inoculum levels (Nematodes)	Root-dip	Soil-drench	Root-dip	Soil-drench	Root-dip	Soil-drench
0	45.0a	45.3a	18.5a	18.2a	16.9a	14.8a
500	34.8b	28.5b	17.5ab	17.0b	15.6ab	13.7ab
1000	30.7c	25.7c	16.8bc	16.6b	15.0b	13.1bc
1500	27.1d	21.4d	15.8c	15.4c	12.9c	11.7c
CV%	8.52	8.18	8.25	7.16	16.4	14.9

Means followed by the same letter in a column are not significantly different at 1% level of significance by DMRT.
*The mixed nematode suspensions consisted of *Meloidogyne hapla*, *Pratylenchus penetrans* and *Tylenchus*.

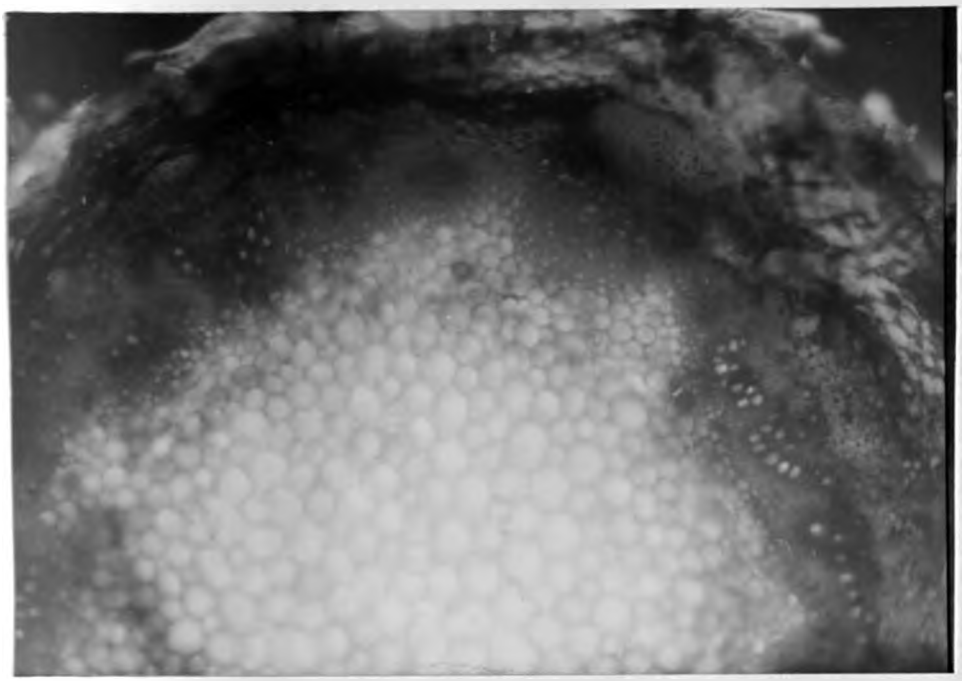


Plate 23. Transverse section of a stem of a clone L/75/487 plant infected by *Fusarium oxysporum*. The colonized vascular bundles are stained bluish-green, indicating presence of the fungus (x50).

Plate 24. Longitudinal section of a stem of a clone L/75/487 plant infected by *Fusarium oxysporum*. The colonized vascular bundles are stained bluish-green, indicating presence of the fungus (x50).

4.3.2. Effect of Inoculation Methods, Different Isolates and their Inoculum Levels on Wilt Expression

The two methods of inoculation used, root dipping and soil drenching, had a highly significant effect ($P \leq 0.01$) on the time required for expression of wilt symptoms (Appendix 6a). Root-dip-inoculated plants expressed wilt symptoms much earlier than soil drench-inoculated plants at comparable levels of inoculum (Tables 5a and 5b).

Data analysis for the ability of the isolates to incite the wilt disease indicated a significant difference ($P \leq 0.01$) among the isolates with regard to both root-dip and soil drench inoculation methods (Figures 2a and 2b; Table 5a)

For fungal inoculum alone using root-dip inoculation, isolates I, II, III and V, from Kisii, Molo, Mwongoris and Limuru respectively, showed no significant difference in the time required for them to cause wilting in test plants of clone L/75/487 (Table 5a; Figure 2a). However, isolate IV (from Sotik) was significantly different ($P \leq 0.01$) from all the other isolates, by taking a much longer time (an average of 104.4 days) as compared to the other isolates I, II, III and V which caused wilt development after 100.4, 99.3, 101.4 and 99.8 days respectively (Table 5a).

In case of soil drench inoculation using fungal inoculum alone, all the isolates were had no significant statistical differences among them. The isolates, however, displayed increased aggressiveness in the order of IV, V, III, II and I, with the respective incubation periods of 113.8, 111.8, 110.4, 109.9 and 109.8 days for wilt development (Table 5a; Figure 2b).

Isolate combinations involving pathogens from Kisii, Molo, Mwongoris and Limuru were not significantly different from each other in their ability to incite wilt symptoms in experiments in which combined nematode-fungus inocula were used. Pathogen combination from Sotik was significantly different ($P \leq 0.01$) from the others and was also the weakest wilt incitant with both inoculation methods (Figures 2c and 2d; Table 5b).

Results of the pathogenicity tests indicated that an increase in the level of inoculum decreased the time taken for disease development, irrespective of the isolate and the method of inoculation used (Tables 5a and 5b; Appendix 6b). The computed F-values were higher than the tabular F-value, which shows that the coeffients of determination (R^2 values) were significantly different from zero. The R^2 values were also quite high; the lowest value was 0.773 (Table 5c). This means that a high proportion of the total variation in the disease incubation periods could be explained by linear functions of the inoculum levels used. The linear regression coefficients for inoculum levels were negative, indicating that increases in inoculum levels led to reduced wilt incubation periods and vice versa (Table 5c).

For treatments involving the use of fungal inoculum alone, the incubation periods were shortened from an average of 117.1 days for an inoculum level of $1.25x10^6$ conidia through to 87.2 days for an inoculum level of $1.0x10^7$ conidia for the root-dip-inoculated plants (Table 5a; Fig. 2a) and from 123.2 to 97.0 days for soil drench-inoculated plants using correspondingly similar inoculum levels (Table 5a; Fig. 2b).

Use of increased nematode inocula with constant fungal inoculum levels also resulted in shorter incubation periods of the disease (Figures 2c and 2d; Table 5b). Even though fungal inoculum was held constant at $2.5x10^6$ conidia for treatments A and B in both inoculation methods, the differences in the numbers of nematodes combined with this quantity of fungal propagules resulted in significant differences ($p \leq 0.01$) between the two treatments. Plants inoculated with $2.5x10^6$: 500 and $2.5x10^6$: 1500 conidia and nematode ratios wilted after 107.7 and 99.5 days respectively for the root-dip inoculation method. With similar inoculum level combinations, wilting was noticed after 116.7 and 111.5 days respectively when plants were inoculated using the soil-drench method.

Similar results were obtained by varying the nematode inoculum combined with $1.0x10^7$

conidia from 500 to 1500 nematodes; again the two levels of inoculum combination gave significantly different ($p \leq 0.01$) results (Table 5b).

In all the treatments there was no significant interaction between the inoculum levels and isolates used.

Table 5a. Effect of different fungal isolates and their inoculum levels on time taken for wilt development.

(i) Root-dip inoculation method

Inoculum levels	Incubation period (days) Isolates I	II	III	IV	V	Inoculum level means*
1	117.3	115.3	118.3	121.0	113.3	117.1a
2	105.0	104.3	107.0	111.3	107.0	106.9b
3	94.0	92.3	92.7	96.0	92.3	93.5c
4	87.0	85.3	87.7	89.3	86.7	87.2d
Isolate Means*	100.4b	99.3b	101.4b	104.4a	99.8b	

(ii) Soil-drench inoculation method

Inoculum levels	Incubation period (days) Isolates I	II	III	IV	V	Inoculum level means*
1	122.3	122.7	125.0	124.7	121.3	123.2a
2	118.3	115.3	117.3	120.3	119.0	118.1b
3	103.3	107.0	104.0	109.7	107.7	106.3c
4	95.3	94.7	95.3	100.7	99.0	97.0d
Isolate Means*	109.8a	109.9a	110.4a	113.8a	111.8a	

*Means followed by the same letter are not significantly different at 1% level of significance.

Key to the inoculum levels used

1 → 1.25×10^6 conidia 2 → 2.5×10^6 conidia

3 → 5.0×10^6 conidia 4 → 1.0×10^7 conidia

Table 5b. Effect of different nematode-fungus combinations and their inoculum levels on time taken for wilt development.

(i) Root-dip inoculation method

Inoculum levels	Incubation period (days) Isolates I	II	III	IV	V	Inoculum level means*
A	108.3	106.3	104.3	113.7	105.7	107.7a
B	97.0	95.7	101.7	104.3	99.0	99.5b
C	88.3	86.3	86.3	91.0	87.7	87.9c
D	79.7	78.3	81.3	85.0	80.0	80.9d
Isolate Means*	93.3b	91.7b	93.4b	98.5a	93.1b	

(ii) Soil-drench inoculation method

Inoculum levels	Incubation period (days) Isolates I	II	III	IV	V	Inoculum level means*
A	116.3	114.7	117.0	121.0	114.7	116.7a
B	108.0	109.7	109.0	119.3	111.3	111.5b
C	94.3	97.3	97.0	104.3	96.3	97.9c
D	92.7	91.7	91.0	102.7	96.7	94.9d
Isolate Means*	102.8b	103.3b	103.5b	111.8a	104.8b	

*Means followed by the same letter are not significantly different at 1% level of significance.

Key to the inoculum levels used

A → 2.5×10^6 conidia and 500 nematodes

B → 2.5×10^6 conidia and 1500 nematodes

C → 1.0×10^7 conidia and 500 nematodes

D → 1.0×10^7 conidia and 1500 nematodes.

Table 5c. Regression coefficients and coefficients of determination for effect of inoculum levels on time taken for wilt development

Inoculum and inoculation method	R^2 values	ß values	Tabular F values 5%	Tabular F values 1%	F-value for inoculum levels
Fungus alone by root-dipping	0.904	-10.31	3.59	6.11	536.87***
Fungus alone by soil-drenching	0.863	-9.03	3.59	6.11	353.98***
Nematode-fungus by root-dipping	0.905	-9.20	3.59	6.11	540.16***
Nematode-fungus by soil-drenching	0.773	-7.90	3.59	6.11	186.58***

R^2 is the coefficient of determination.

ß is the regression coefficient for inoculum levels.

Fig 2a. Effect of fungal isolates and their inoculum levels on wilt expression (Root-dip inoculation).

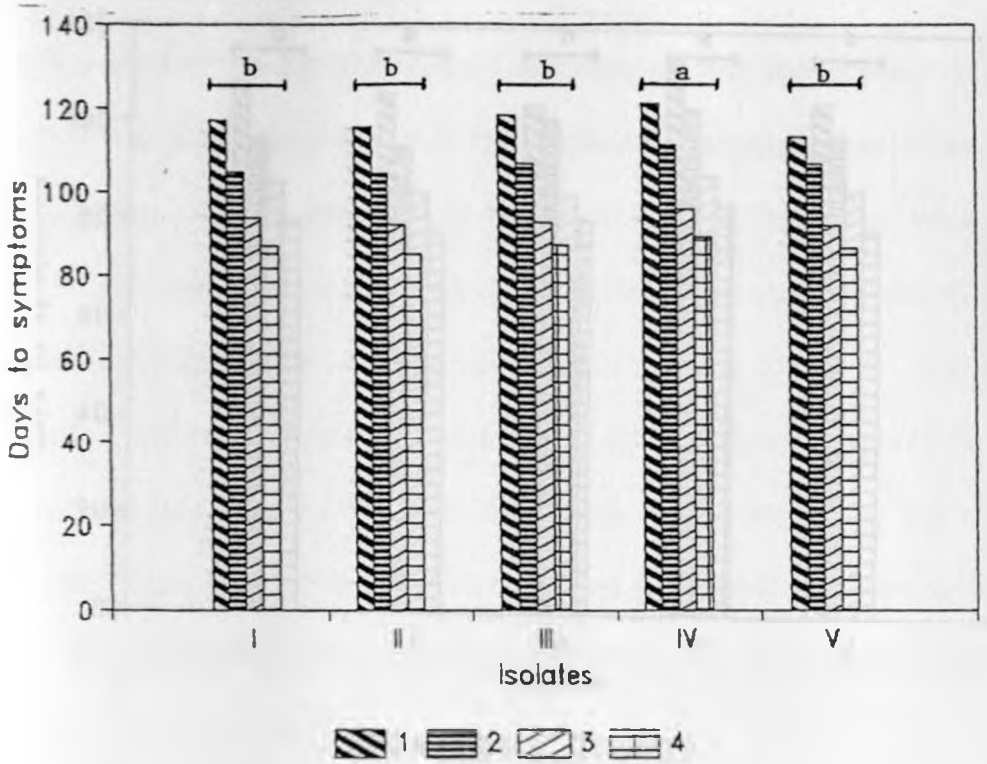
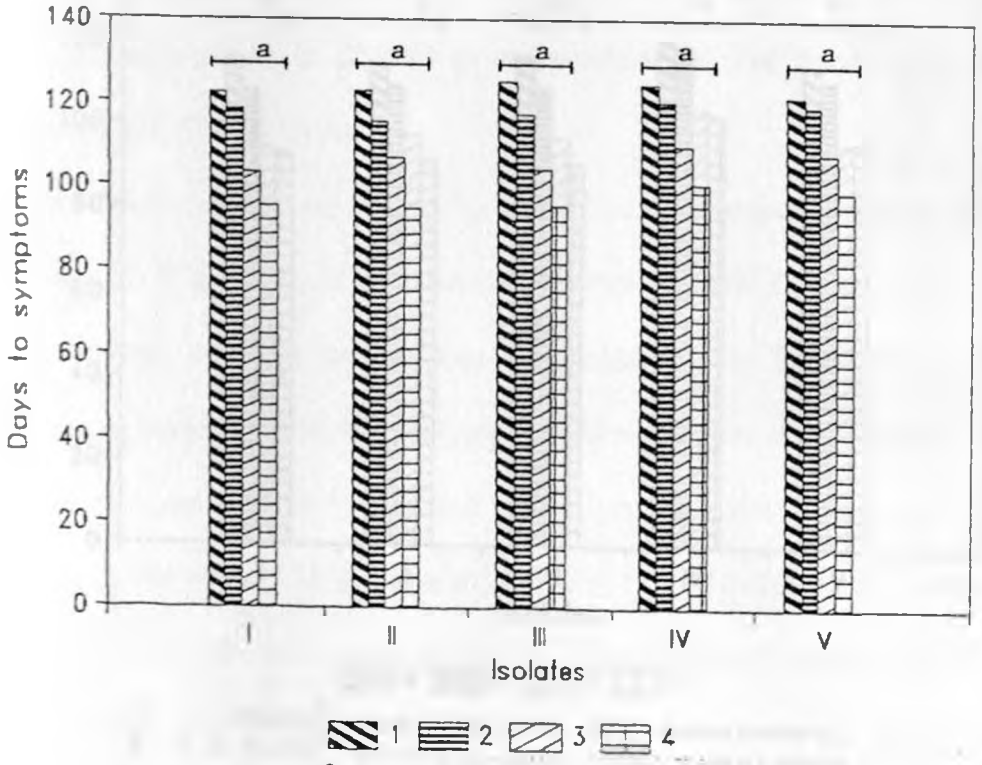


Fig 2b. Effect of fungal isolates and their inoculum levels on wilt expression (Soil-drench inoculation).



1 → 1.25×10^6 conidia 2 → 2.5×10^6 conidia
3 → 5.0×10^6 conidia 4 → 1.0×10^7 conidia

Bar stacks followed by different letters are significantly different at 1% level by DMRT.

Fig 2c. Effect of fungal isolates and nematode-fungus levels on wilt expression (Root-dip inoculation).

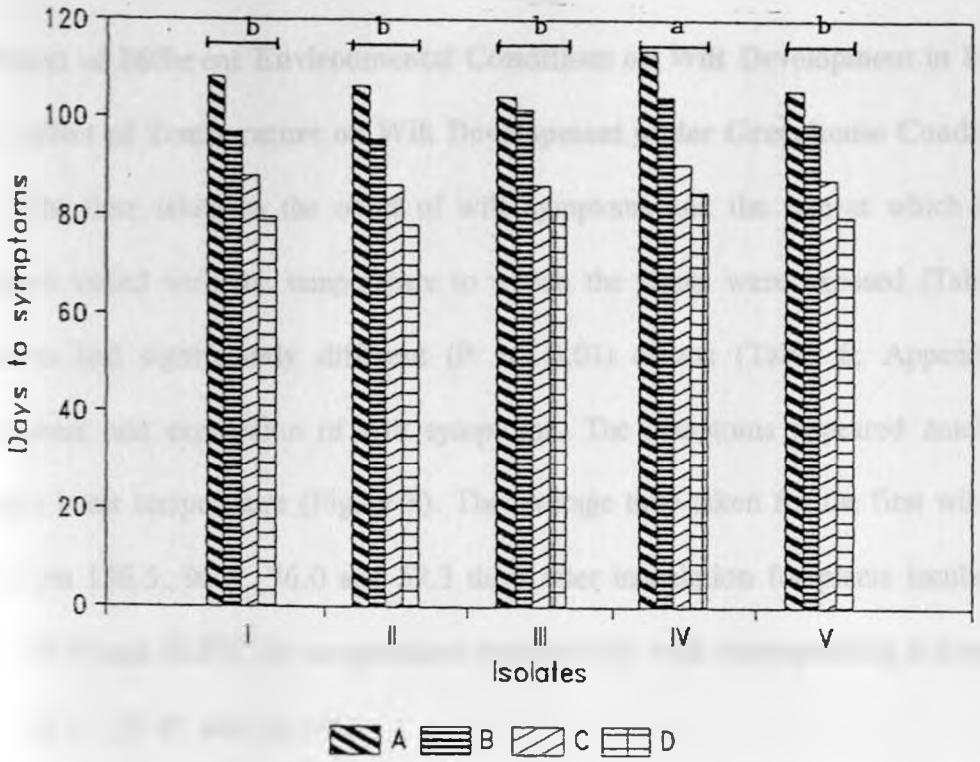
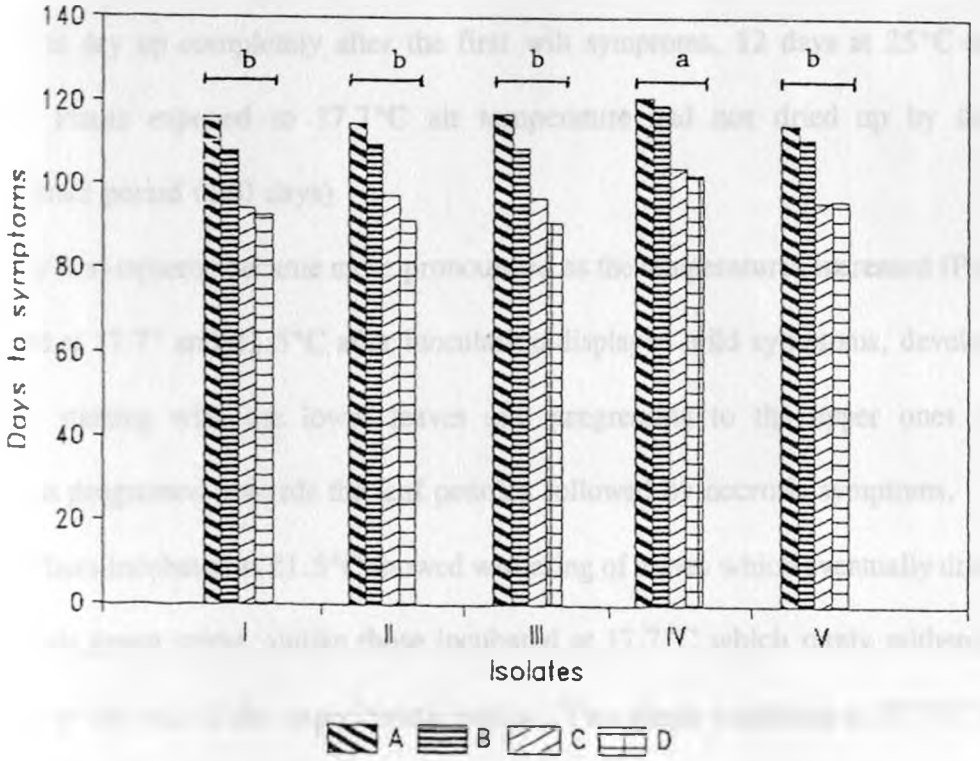


Fig 2d. Effect of fungal isolates and nematode-fungus levels on wilt expression (Soil-drench inoculation)



A → 2.5×10^6 conidia and 500 nematodes
B → 2.5×10^6 conidia and 1500 nematodes
C → 1.0×10^7 conidia and 500 nematodes
D → 1.0×10^7 conidia and 1500 nematodes.

Bar stacks followed by different letters are significantly different at 1% level by DMRT.

4.4. Effect of Different Environmental Conditions on Wilt Development in Pyrethrum

4.4.1. Effect of Temperature on Wilt Development under Greenhouse Conditions

The time taken to the onset of wilt symptoms and the rate at which the symptoms developed varied with the temperature to which the plants were exposed (Table 6). All the treatments had significantly different ($P \leq 0.01$) effects (Table 6; Appendix 8) on the development and expression of wilt symptoms. The symptoms appeared much earlier with increases in air temperature (Figure 3). The average time taken for the first wilt symptoms to appear was 136.5, 96.3, 76.0 and 57.3 days after inoculation for plants incubated at 17.7°, 21.5°, 25.0° and 30.0°C air temperatures respectively, with corresponding soil temperatures of 16.8°, 19.6°, 23.8° and 28.5°C.

High temperatures (25° and 30°C) resulted in death of plants within shorter periods of time than did low temperatures (Table 6). It took approximately 10 days for the plants incubated at 30°C to dry up completely after the first wilt symptoms, 12 days at 25°C and 20 days at 21.5°C. Plants exposed to 17.7°C air temperature had not dried up by the end of the experimental period (180 days).

Wilt symptoms became more pronounced as the temperatures increased (Plate 25). Plants incubated at 17.7° and 21.5°C after inoculation displayed mild symptoms, developing chlorotic margins, starting with the lower leaves and progressing to the upper ones. The chlorotic symptoms progressed towards the leaf petioles followed by necrotic symptoms.

Plants incubated at 21.5°C showed withering of leaves which eventually dried up , leaving behind pale green stems, unlike those incubated at 17.7°C which rarely withered and had not dried up by the end of the experimental period. Two plants incubated at 17.7°C were noted to be symptomless, although they were colonized by the fungus, *Fusarium oxysporum*, as evidenced by culturing the stem sections of those plants on PSA.

On plants incubated at 25° and 30°C after inoculation, wilt symptoms were more pronounced (Plate 25) and developed fully over shorter time periods in contrast to plants incubated at either 17.7° or 21.5°C (Table 6). Necrosis of leaves was also more rapid and occurred almost concurrently with chlorosis which was not clearly evident. Withering and drooping of leaves were noticed on all the plants incubated at these two temperatures. Withering was more severe at 30°C than at 25°C, with death of whole plants occurring without any appreciable necrosis at 30°C. Complete death of plants at 25° and 30°C resulted in drying up of stems unlike the pale green stems left behind after leaves dried up at 21.5°C.

All the inoculated plants incubated at 21.5°, 25° and 30°C exhibited wilt symptoms but none of the control (uninoculated) plants showed the disease (Plate 25). No fungal infection was noticed after culturing the stem sections of control plants on PSA.

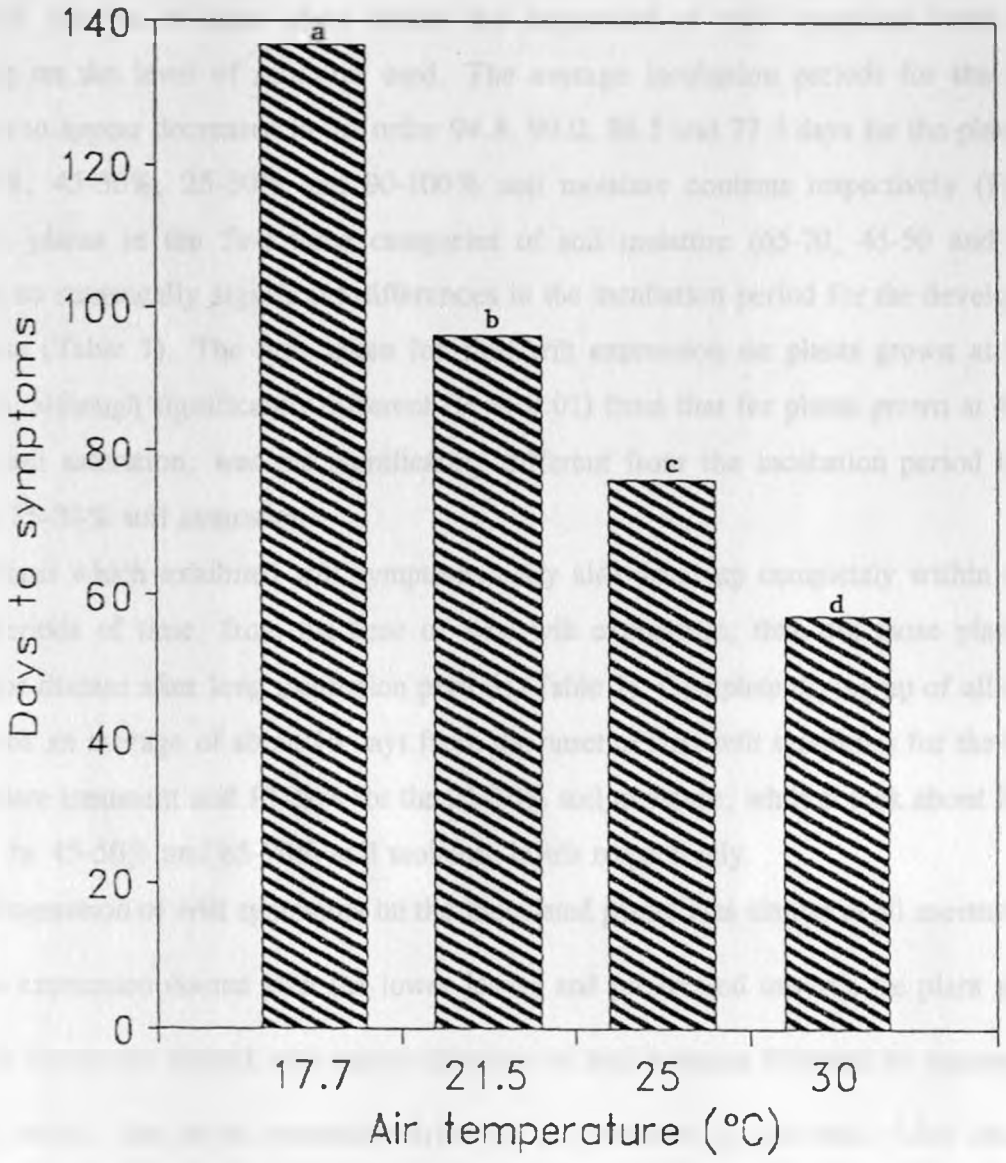
Table 6. Pyrethrum wilt development as affected by temperature under greenhouse conditions

	Air (ambient) temperature (°C)				CV%
	17.7	21.5	25.0	30.0	
Incubation period of wilt disease (days)*	136.5a	96.3b	76.0c	57.3d	6.02
Days to complete plant death after first wilt symptoms*	**	19.75a	11.5ab	9.5b	26.52

*Means followed by different letters in a row are significantly different at 1% level of significance by DMRT.
**Plants had not dried up completely by the end of the experimental period.
Inoculation was done on clone L/75/487 plants by root-dipping in a 1.5 x 10^6 conidia/ml suspension for 30 minutes.

Plate 25. Differential wilt expression by clone L/75/487 plants inoculated with *Fusarium oxysporum* and incubated at 30.0°C (T4), 25.0°C (T3), 21.5°C (T2) and 17.7°C (T1) ambient temperatures.

Fig 3. Influence of temperature on wilt development in pyrethrum.



Bars followed by different letters are significantly different at 1% level by DMRT.

4.4.2. Effect of Soil Moisture on Wilt Development under Greenhouse Conditions

The periods of time taken before the expression of wilt symptoms were different depending on the level of moisture used. The average incubation periods for the first wilt symptoms to appear decreased in the order 94.8, 90.0, 86.5 and 77.5 days for the plants grown at 65-70%, 45-50%, 25-30% and 90-100% soil moisture contents respectively (Figure 4). However, plants in the first three categories of soil moisture (65-70, 45-50 and 25-30%) exhibited no statistically significant differences in the incubation period for the development of the disease (Table 7). The time taken for first wilt expression on plants grown at 90-100% saturation, although significantly different ($P \leq 0.01$) from that for plants grown at 45-50 and 65-70% soil saturation, was not significantly different from the incubation period for plants grown at 25-30% soil saturation.

Plants which exhibited wilt symptoms early also dried up completely within relatively shorter periods of time, from the time of first wilt expression, than did those plants which showed the disease after long incubation periods (Table 7). Complete drying up of all leaves on a plant took an average of about 16 days from the onset of first wilt symptoms for the 90-100% soil moisture treatment and 13 days for the 25-30% soil moisture, while it took about 26 and 22 days for the 45-50% and 65-70% soil moisture levels respectively.

Progression of wilt symptoms on the inoculated plants was similar at all moisture levels. Symptom expression started with the lower leaves and progressed towards the plant apex with time. The symptoms started with initial chlorosis of leaf margins followed by necrosis of the chlorotic leaves. The leaves eventually dried up but remained on the stem. After two to four lower leaves had dried up, the other upper leaves could be seen drooping (withered) during the hot hours of the day but recovering towards the evening, with much more recovery during night time hours. Eventually, a point reached when the leaves could not recover from the withered condition and progressed to drying up, leaving behind pale green stems.

Table 7. Soil moisture effect on pyrethrum wilt development under greenhouse conditions

	Soil moisture levels (% saturation)				
	25-30	45-50	65-70	90-100	CV%
Disease incubation period (days)*	86.5ab	90.0a	94.8a	77.5b	5.86
Days to complete plant death after first wilt symptoms*	13.0c	25.5a	21.8ab	16.3bc	18.76

*Means followed by different letters in a row are significantly different at 1% level of significance by DMRT.

Inoculation was done on clone L/75/487 plants by root-dipping in a 1.5 x 10^6 conidia/ml suspension for 30 minutes.

Plate 26. Symptom expression on by clone L/75/487 plants inoculated with *Fusarium oxysporum* and grown at different soil moisture levels of 25-30% (M1), 45-50% (M2), 65-70% (M3) and 90-100% (M4).

Plants grown at 45-50% and 65-70% soil moisture showed inconspicuous symptoms which progressed up the plants more slowly than for plants grown at the other soil moisture levels (Plate 26, M2 and M3).

Of the sixteen inoculated plants at 45-50% soil moisture level, three were still symptomless by the end of the experimental period (180 days). On splitting the stems of the plants and plating some pieces on PSA, they were all found to be colonized by the fungus, *Fusarium oxysporum*, irrespective of the appearance of symptoms externally. All other inoculated plants at the various soil moisture levels developed wilt symptoms and they were found to be colonized by the fungus.

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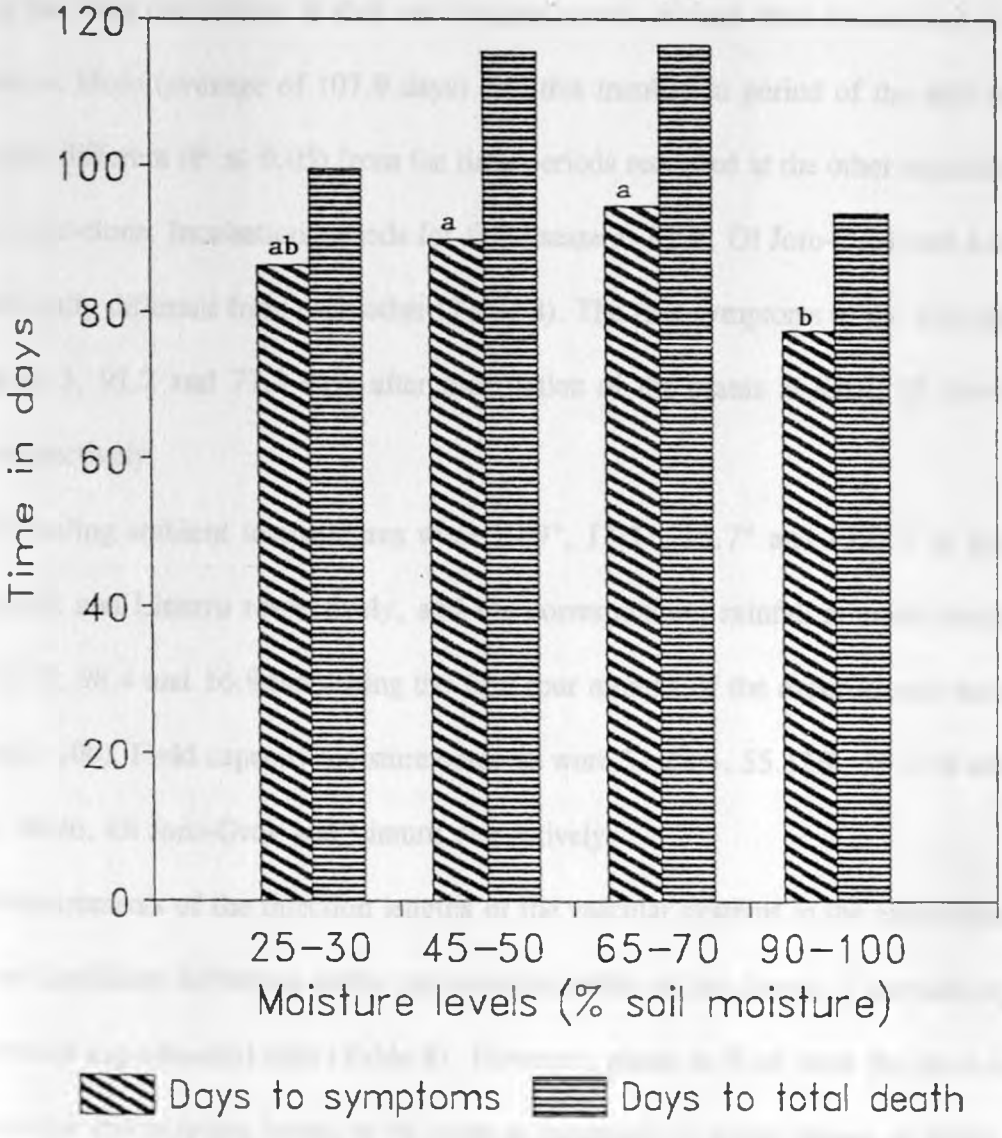
Fig 4. Influence of soil moisture levels on wilt development and expression.



Bars followed by different letters are significantly different at 1% level by DMRT.

4.4.3. Effect of Field Temperature and Rainfall on Wilt Development

The time taken for wilt symptoms to appear on clone L/75/487 was influenced to some extent by the field conditions. It took the longest period of time from inoculation to symptom expression at Molo (average of 107.9 days) and this incubation period of the wilt disease was significantly different ($P \leq 0.05$) from the time periods recorded at the other experimental sites with the same clone. Incubation periods for the disease at Kisii, Ol Joro-Orok and Limuru were not significantly different from each other (Table 8). The first symptoms of the wilt disease were observed 66.3, 91.2 and 77.3 days after inoculation of the plants at Kisii, Ol Joro-Orok and Limuru respectively.

Prevailing ambient temperatures were 19.9°, 13.1°, 13.7° and 13.0°C at Kisii, Molo, Ol Joro-Orok and Limuru respectively, and the corresponding rainfall amounts recorded were 164.9, 215.5, 98.4 and 16.9mm during the first four months of the experimental period (Table 8: Appendix 10b). Field capacity moisture contents were 52.25%, 55.12%, 56.51% and 54.60% for Kisii, Molo, Ol Joro-Orok and Limuru respectively.

Measurements of the infection lengths of the vascular systems in the experimental plants showed no significant difference in the colonization ability of the fungus, *Fusarium oxysporum*, at the different experimental sites (Table 8). However, plants at Kisii were the most colonized, with a vascular colonization length of 98.5mm as compared to plants grown at Molo, Ol Joro-Orok and Limuru which had average vascular colonization lengths of 58.0, 64.3 and 69.8mm respectively.

Natural infestation of soils by *Fusarium oxysporum* at the experimental sites showed that Kisii had the highest density of *Fusarium oxysporum* propagules (5600 spores/g of soil) followed by Limuru, Ol Joro-Orok and Molo in that order, with respective *Fusarium oxysporum* propagule densities of 4525, 4025 and 3800 spores per gram of soil (Table 8).

Table 8. Pyrethrum wilt development, plant infection and field conditions at four experimental sites

	Experimental Sites				CV%
	Kisii	Molo	Ol Joro-Orok	Limuru	
Average Temp (°C)	19.9	13.1	13.7	13.0	
Rainfall, 4 months (mm)	164.9	215.5	98.4	16.9	
Fusarium propagules (Spores/g of soil)	5600	3800	4025	4525	
Field capacity moisture (%)	52.25	55.12	56.51	54.60	
Days to symptoms*	66.3b	107.9a	91.2b	77.3b	8.80
Vascular colonization length (mm)*	98.5a	58.0a	64.3a	69.8a	28.96

*Means followed by different letters within a row are significantly different at 5% level by DMRT.

4.4.4. Field Hydrogen-ion Concentration and Effect of pH on Fungal Growth

4.4.4.1. Hydrogen-ion concentration of field soil samples

Determination of the pH of soil samples from experimental sites at Kisii, Molo, Mwongoris, Ol Joro-Orok and Limuru was carried out to determine if there would be need for conducting investigations on the effect of soil pH on wilt development. The results indicated that the soil samples had pH values in the range of 5.66 to 6.09. This is quite a narrow range even though there were significant differences among the samples from the various sites (Table 9a).

Soil at Limuru had the highest pH of 6.09 followed by Kisii soil pH of 6.01; these values were not significantly different from each other. Molo soil pH (5.85) was in turn not significantly different from both the Kisii and Ol Joro-Orok (5.68) soil pH values. Soil samples from Mwongoris were significantly different ($P \leq 0.01$) from all the others except the Ol Joro-Orok samples in terms of pH. In general, there was an overlap of lack of significant differences among the soil samples within the narrow pH range. However, the planting soil mixture used for greenhouse experiments had the highest pH of 6.6 which was significantly different ($P \leq 0.01$) from all the other soil sample pH values.

Table 9a. Hydrogen-ion concentration (pH*) values for soil samples from five sites and greenhouse soil mixture

Site (sample)	Soil pH values
Kisii	6.01bc
Molo	5.83cd
Mwongoris	5.66e
Ol Joro-Orok	5.68de
Limuru	6.09b
Greenhouse**	6.60a
CV%	1.41

Means followed by the same letter are not significantly different at 1% level of significance by DMRT.

*Measurements were obtained using a Metrohm 605 pH-meter (Swiss made).

**Samples were taken from a homogenous mixture of forest soil : peat moss : gravel mixed in the ratio of 20 : 8 : 5 (v/v).

4.4.4.2. Effect of pH of medium on growth of fungal isolates

Five *Fusarium oxysporum* isolates I, II, III, IV and V from Kisii, Molo, Mwongoris, Sotik and Limuru respectively were cultured on PSA at pH levels of 4.0, 5.0, 6.0, 7.0 and 8.0. There were no significant differences in the growth rates of the isolates at a given pH level of the medium (Table 9b). This implies that the isolates behaved in a similar manner at equal pH levels. indicating that they represented one organism species.

Colony diameters of the fungal isolates on the fourth day of incubation differed significantly ($P \leq 0.01$) from each other depending on the pH level used (Table 9b). The higher the pH of the medium, the more was the lateral growth of the colony (Figure 5). Interaction between the pH of the medium and isolates was not significant (Appendix 16). Growth was highest at pH 8 reaching a colony diameter of 5.2cm on the fourth day of incubation. This was followed by colony diameters for isolates grown at pH 7 (4.2cm). The slowest growth rate was observed at the lowest pH (4.0) tested with a colony diameter of 2.3cm at the fourth day of incubation.

Other aspects of the colonies which visually displayed differences at the different pH levels of the medium were colour development and mycelial growth. At pH 4 the colonies had the strongest pink colour on the reverse side of the plate marched with very sparse mycelial growth on the obverse side of the culture plates. The pink colour on the reverse side became lighter as the medium pH increased from 4.0 to 8.0. As the pink colour became more pale, mycelial growth on the obverse side also increased. Whereas there was only little mycelial growth at low pH values of 4.0 and 5.0, the cottony appearance commonly associated with *Fusarium oxysporum* cultures became more evident at higher pH levels. Cultures at pH 8 had the most conspicuous mycelial growth among the cultures grown under the various pH levels tested in this experiment.

Table 9b. Influence of medium pH on growth of five isolates of *Fusarium oxysporum*

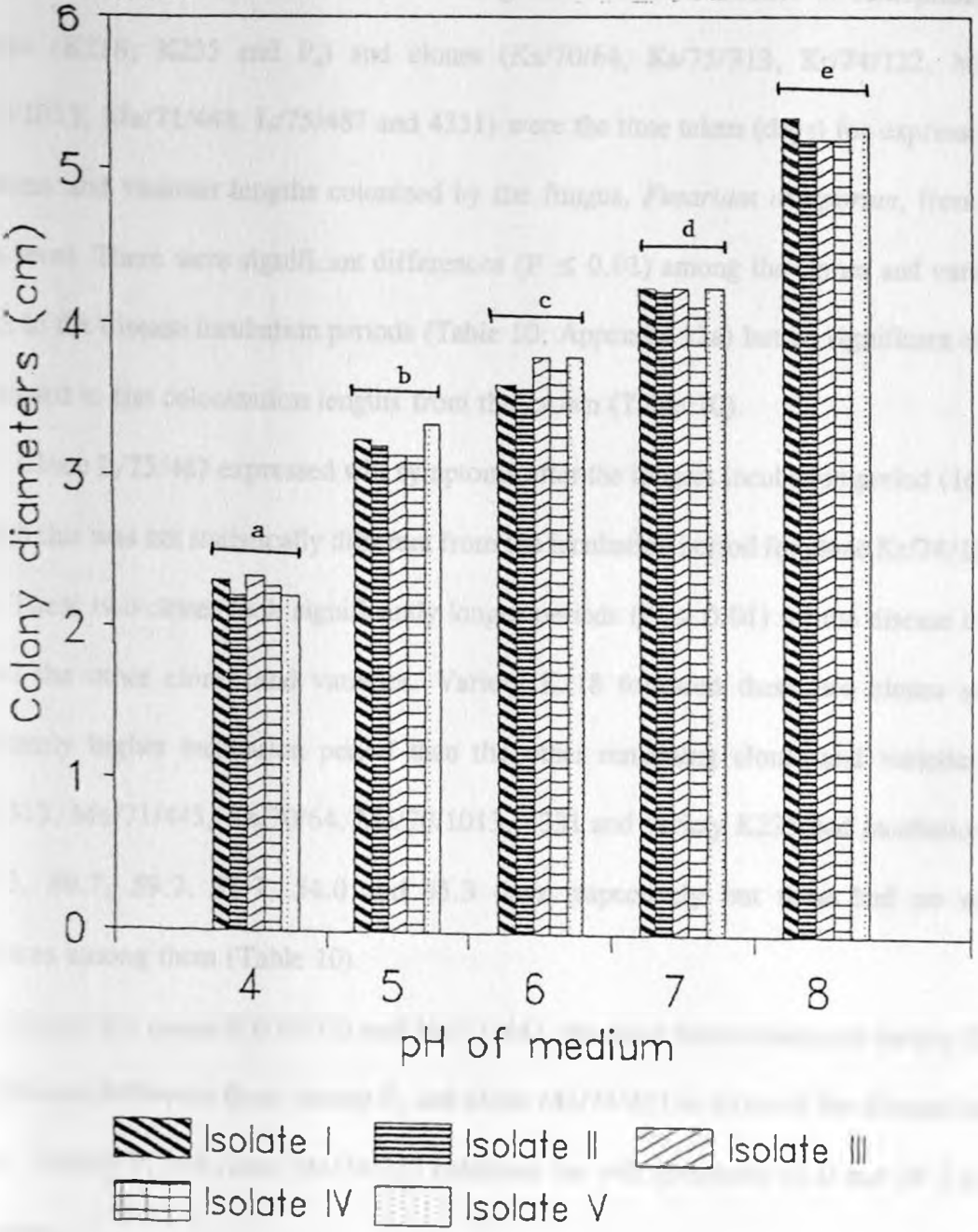
Medium pH	colony diameters** (cm) four days after incubation Isolates I	II	III	IV	V	pH means*
4.0	2.30	2.20	2.33	2.27	2.20	2.26a
5.0	3.23	3.20	3.13	3.13	3.33	3.21b
6.0	3.60	3.57	3.77	3.70	3.77	3.68c
7.0	4.23	4.20	4.23	4.10	4.23	4.20d
8.0	5.33	5.20	5.20	5.20	5.20	5.27e
Isolates means*	3.74a	3.67a	3.73a	3.68a	3.76a	

CV = 2.50%

*Means followed by different letters are significantly different at 1% level of significance by DMRT.

**Values are means of three replicates

Fig 5. Influence of medium pH on growth of *Fusarium oxysporum* isolates (4th incubation day).



Bar stacks followed by different letters are significantly different at 1% level by DMRT.

4.5. Screening for Resistance to Pyrethrum Wilt

4.5.1. Greenhouse experiment

The parameters considered in assessing the level of resistance or susceptibility of the varieties (K218, K235 and P_4) and clones (Ks/70/64, Ks/75/313, Kr/74/122, Mo/74/223, Ma/70/1013, Ma/71/443, L/75/487 and 4331) were the time taken (days) for expression of wilt symptoms and vascular lengths colonized by the fungus, *Fusarium oxysporum*, from the plant crown level. There were significant differences ($P \leq 0.01$) among the clones and varieties with respect to the disease incubation periods (Table 10; Appendix 12a) but no significant differences with regard to the colonization lengths from the crown (Table 10).

Clone L/75/487 expressed wilt symptoms after the longest incubation period (163.7 days) although this was not statistically different from the incubation period for clone Kr/74/122 (161.0 days). These two clones took significantly longer periods ($P \leq 0.01$) for the disease to develop than all the other clones and varieties. Variety K218 followed these two clones and had a significantly higher incubation period than the other remaining clones and varieties. Clones Ks/75/313, Ma/71/443, Ks/70/64, Ma/70/1013, 4331 and variety K235 had incubation periods of 76.3, 69.7, 59.7, 52.7, 54.0 and 55.3 days respectively but these had no significant differences among them (Table 10).

Except for clones Ks/75/313 and Ma/71/443, the latter three clones and variety K235 had no significant difference from variety P_4 and clone Mo/74/223 in terms of the disease incubation periods. Variety P_4 and clone Mo/74/223 exhibited the wilt symptoms 43.0 and 38.7 days after inoculation.

Although the length of vascular colonization by the fungus at the onset of wilt symptoms (or at the end of the experimental period - 180 days - for asymptomatic plants) displayed a ranking of the clones and varieties, there were no significant differences in this respect.

However, those clones/varieties which took longer periods before expressing wilt symptoms were also shown to have allowed less upward colonization of the vascular system by the fungus. For instance, clones L/75/487, Kr/74/122, variety K218 and clone Ks/75/313 which had long incubation periods of 163.7, 161.0, 133.7 and 76.3 days respectively also displayed increased length of vascular colonization as 112, 115, 123 and 129mm respectively (Table 10). The other clones/varieties did not display a similar trend when incubation periods of the wilt disease and colonization lengths were compared.

Apparently, those clones/varieties with greater lengths of colonized vascular bundles succumbed to the wilt pathogen infection by death of whole plants within relatively shorter periods of time (Table 10) and their expression of wilt symptoms was also more conspicuous (visual observations) than those clones/varieties which had been colonized to a lesser extent. The clones/varieties 4331, Ma/70/1013, Mo/74/223, P_4, Ks/70/64, Ma/71/443 and K235, with respective vascular colonization lengths of 151, 149, 144, 142, 137, 134 and 132mm, dried up completely 28, 35, 37, 46, 53, 59 and 67 days respectively after the first show of wilting symptoms (Table 10). However, Ks/75/313, K218, Kr/74/122 and L/75/487 had not dried up completely by the end of the experimental period; they also had been colonized to a lesser extent than those plants which had dried up completely.

Table 10. Pyrethrum wilt development, expression and plant infection for three varieties and eight clones inoculated with *Fusarium oxysporum* (Greenhouse experiment).

Clone/variety	Incubation period (days)	Time (days) to complete plant death	Length (mm) of vascular colonization
Ma/71/443	69.7c	59.0ab	134a
P_4	43.0d	46.7c	142a
Ks/70/64	59.7cd	53.0ab	137a
Mo/74/223	38.7d	37.3cd	144a
4331	54.0cd	28.3d	151a
K218	133.7b	___**	123a
Ma/70/1013	52.7cd	35.0cd	149a
K235	55.3cd	67.3a	132a
Ks/75/313	76.3c	___**	129a
L/75/487	163.7a	___**	112a
Kr/74/122	161.0a	___**	115a
CV%	12.25	13.07	15.89

Means followed by the same letters in a column are not significantly different from each other at 1% level of significance by DMRT.

**The plants had not dried up completely by the end of the experimental period.

4.5.2. Field Experiment

The time taken for the first appearance of wilt symptoms on various clones and varieties ranged from an average of 39.3 days for clone Mo/74/223 to 187.3 days for clone Kr/74/122 at the Mwongoris screening trial, with significant differences ($P \leq 0.05$) among the treatments (Appendix 13a). Clone L/75/487 had a wilt incubation period of 148.5 days but this was not significantly different from the wilt incubation period for clone Kr/74/122. All the other clones/varieties were significantly different ($P \leq 0.05$) from clones Kr/74/122 and L/75/487 but there were no significant differences among them (Table 11a).

Measurements of lengths of infected vascular bundles for various clones and varieties by *Fusarium oxysporum* did not show any significant difference among them (Table 11a; Appendix 13b). There was negative correlation between the time taken for the first wilt symptoms to appear and the vascular infection lengths for the Mwongoris trial (Appendix 14).

Effect of wilt on the yield of pyrethrum flowers for various clones and varieties was practically impossible to assess at Mwongoris since there was massive death of the plants even before flowering started in some clones/varieties. The percentage plant deaths are given in table 11a at the fifteenth week after inoculation.

Table 11a. Pyrethrum wilt development and plant infection for three varieties and eight clones in the field.

Clone/variety	MWONGORIS TRIAL			MOLO TRIAL
	Incubation period (days)	Vascular colonization length (mm)	Percentage dead plants (15th week)	Vascular colonization length (mm)
Ma/71/443	72.5b	125.3a	43.75	101.5cd
P_4	55.0b	140.5a	81.25	127.0abc
Ks/70/64	60.3b	129.0a	50.00	105.5bcd
Mo/74/223	39.3b	131.8a	81.25	100.8cd
4331	65.5b	143.0a	87.50	140.0a
K218	48.0b	114.0a	31.25	115.8abcd
Ma/70/1013	53.3b	139.3a	56.25	131.3ab
K235	41.5b	140.0a	75.00	138.3a
Ks/75/313	76.0b	131.3a	62.50	137.3a
L/75/487	148.0a	117.8a	18.75	88.5d
Kr/74/122	187.3a	113.0a	37.50	107.3bcd
CV%	56.14	14.85		15.48

Means followed by the same letters in a column are not significantly different from each other at 5% level of significance by DMRT.

Plants at the Molo screening trial appeared unaffected by the wilt disease. Very few plants, none in some replicates, had expressed wilt symptoms by the end of the experimental period (37 weeks) as depicted in appendix 15.

However, there were significant differences ($P \leq 0.05$) among the clones and varieties in terms of vascular colonization lengths (Table 11a; Appendix 13c).

There were significant differences ($P \leq 0.05$) among the clones and varieties in terms of the effect of wilt on their flower yields (Table 11b; Appendix 13d). The ratio of weight per flower decreased with time of flower picking (Figure 6), with significant differences ($P \leq 0.05$) among the flower picking dates (Table 11b). Uninoculated control plants of clone 4331 also showed this decrease but the decrease was not as pronounced as in the case of inoculated plants of the same clone.

The average flower weight decreased from 0.52g at 21 weeks to 0.19g at 35 weeks after inoculation of clone 4331 plants. During the same time period, uninoculated plants had their average flower weight reduced from 0.61g to 0.40g. The average flower weight (eight pickings) for inoculated clone 4331 plants was 0.38g which was significantly different ($P \leq 0.05$) from 0.51g for uninoculated plants of the same clone (Table 11b).

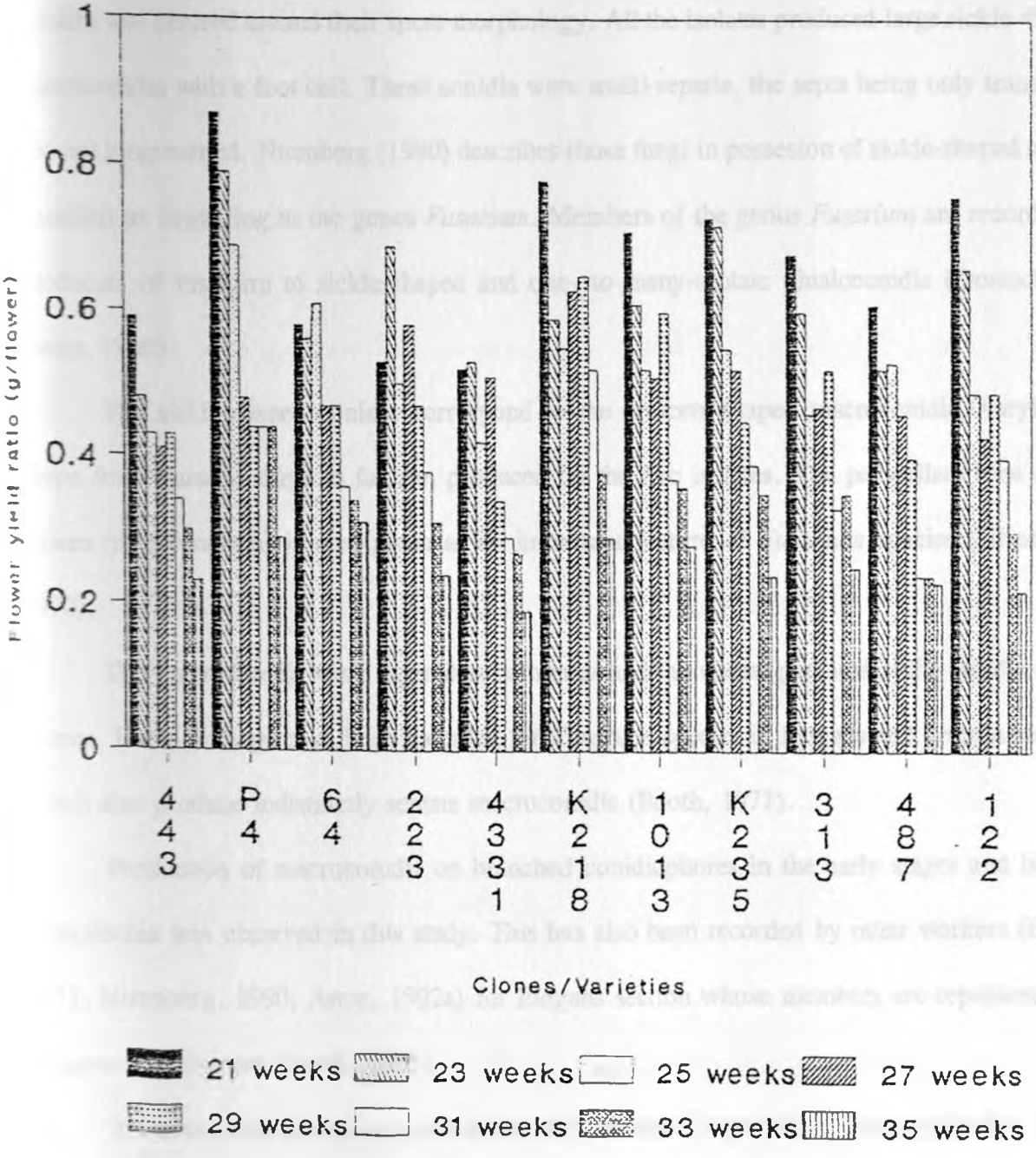
There was no interaction between the clones/varieties and picking dates in terms of the flower yield ratio.

Table 11b. Effect of wilt on flower yield ratio (Molo screening trial).

Clone/variety	Flower yield ratio (g/flower)								
	Picking dates (weeks after inoculation)								
	21	23	25	27	29	31	33	35	Clone means*
Ma/71/443	0.59	0.48	0.43	0.41	0.43	0.34	0.30	0.23	0.40cd
P_4	0.87	0.79	0.69	0.48	0.44	0.44	0.44	0.29	0.55a
Ks/70/64	0.58	0.56	0.61	0.47	0.47	0.36	0.34	0.31	0.46bc
Mo/74/223	0.53	0.69	0.50	0.58	0.47	0.38	0.31	0.24	0.46bc
4331	0.52	0.53	0.42	0.51	0.34	0.29	0.27	0.19	0.38d
4331 (Control)	0.61	0.58	0.56	0.54	0.50	0.44	0.47	0.40	0.51ab
K218	0.78	0.59	0.55	0.63	0.65	0.52	0.38	0.29	0.55a
Ma/70/1013	0.71	0.61	0.52	0.51	0.60	0.37	0.36	0.28	0.49ab
K235	0.73	0.72	0.55	0.52	0.45	0.39	0.35	0.24	0.49ab
Ks/75/313	0.68	0.60	0.46	0.47	0.52	0.33	0.35	0.25	0.46bc
L/75/487	0.61	0.52	0.53	0.46	0.40	0.24	0.24	0.23	0.40cd
Kr/74/122	0.76	0.66	0.49	0.43	0.49	0.40	0.30	0.22	0.47b
Date means*	0.67a	0.61b	0.52c	0.50c	0.48c	0.37d	0.33d	0.25e	

*Means followed by the same letters are not significantly different from each other at 5% level of significance by DMRT.

Fig 6. Effect of wilt on pyrethrum flower yield ratio with time.



CHAPTER FIVE

5. DISCUSSION

5.1 Identification of Fungal Isolates

Identification of the fungal isolates obtained from Kisii, Molo, Mwongoris, Sotik and Limuru was centred around their spore morphology. All the isolates produced large sickle-shaped macroconidia with a foot cell. These conidia were multi-septate, the septa being only transverse and not longitudinal. Nirenberg (1990) describes those fungi in possesion of sickle-shaped spores (conidia) as belonging to the genus *Fusarium*. Members of the genus *Fusarium* arc recorded as producers of fusiform to sickle-shaped and one- to many-septate phialoconidia (Domsch and Gams, 1980).

The sickle-shaped conidia correspond to the crescent-shaped macroconidia, varying in shape from sausage-shape to falcate, produced by the five isolates. The pedicellate base of the spores (macroconidia) is also given as an important feature of *Fusarium* species (Nirenberg, 1990).

The macroconidia were tapered at both ends and the widest part was at the middle of the spores. These findings have been recorded for *Fusarium* species in Elegans and Liseola sections which also produce indistinctly septate macroconidia (Booth, 1971).

Production of macroconidia on branched conidiophores in the early stages and later in sporodochia was observed in this study. This has also been recorded by other workers (Booth, 1971; Nirenberg, 1990; Anon, 1992a) for Elegans section whose members are represented by *Fusarium oxysporum* (Booth, 1971).

Macroconidial dimensions varied with the number of septa which is exemplified by Booth (1971); the length correspondingly increased with the number of septa. It was noticed that isolates I and V, which produced 7-septate macroconidia, also had the highest average conidial length

(about 40μm). Macroconidia from isolates II, III and IV had maximum lengths limited to 36.0, 32.4 and 36.0μm respectively as compared to maximum lengths of 46.8 and 43.2 μm for isolates I and V respectively.

Majority of the macroconidia from all the isolates were 3-septate. This is as documented by Booth (1971) who gives the 3-septate spore dimensions in the range 27-46x3-4.5μm. The 3-septate macroconidia for the five isolates had dimensions ranging from 28.8 to 36.0μm in length by 3.6μm in width. The 7-septate macroconidia had dimensions of 43.2-46.8x3.6-5.4μm in comparison to the published dimensions of 50-66x3.5-5μm for *Fusarium oxysporum* (Booth, 1971).

Fusoid-to-clavate, one- or non-septate microconidia were observed in cultures of all the five isolates. The microconidia were produced on monophialides and formed false heads. This has also been recorded by Singh *et al* (1991) and Anon. (1992a). They never form chains and are mostly non-septate, measuring 5-12x2.3-3.5μm (Domsch and Gams, 1980) for *Fusarium oxysporum*. However, the measurements for all the microconidia of the five isolates ranged from 7.2 to 10.8μm in length by 3.6μm in width. The small range in length may indicate that the isolates belonged to the same species, *Fusarium oxysporum*.

The colony diameters for all the five isolates on the fourth day of incubation at 25°C ranged from 4.3 to 5.6cm. Booth (1971) and Domsch and Gams (1980) observed similar growth rates of 4.5-6.5cm for *Fusarium oxysporum* after four days of incubation at 25°C.

Both terminal and intercalary, smooth-walled chlamydospores were formed in all the five isolates. *Fusarium oxysporum* group members are well known for production of chlamydospores (Booth, 1971; Domsch and Gams, 1980; Nelson *et al*, 1983; Anon, 1992a). Chlamydospores form more readily when cultures are very old or grown on a poor agar medium (Domsch and Gams, 1980) such as the SNA used in this study.

5.2. Identification of Nematodes

5.2.1. *Meloidogyne hapla*

The conspicuously rounded medium bulb observed on the specimens was as described by Jenkins and Taylor (1967) and Franklin (1965) for *Meloidogyne* species females. The faint visibility of body annules and their clear visibility around the neck and the posterior end of mature females was as recorded by various researchers (Goodey, 1963; Franklin, 1965; Jenkins and Taylor, 1967; Dropkin, 1980; Dropkin, 1989); these clear annulations (perineal patterns) around the posterior end of females is an important feature in identification of various *Meloidogyne* species. The slightly wavy, closely spaced and generally circular striae which were reported in this investigation (Plate 10) resemble the photomicrographs and diagrams given by Machon and Hunt (1991) and the descriptions by Williams (1974) for *Meloidogyne hapla*. The stippling of the areas between the anus and tail terminus by punctations is also reported by Jenkins and Taylor (1967), Williams (1974) and Machon and Hunt (1991) for *Meloidogyne hapla* perineal patterns. In deed, the use of the perineal pattern synoptic key, photomicrographs and drawings provided by Franklin (1965) offered an unquestionable basis for the identification of the nematodes as *M. hapla* Chitwood.

Jenkins and Taylor (1967) and Dropkin (1989) quote the perineal patterns of mature females as the basis for separating *Meloidogyne* species.

An important feature of *Meloidogyne* males was the semi-circular outline of the head with a well-defined cephalic framework (Goodey, 1963; Jenkins and Taylor, 1967; Heyns, 1971).

Mobile juveniles possessed a serpentine movement, with the body displaying some banding. This characteristic feature for *Meloidogyne* juveniles had been reported earlier (Heyns, 1971; Dropkin, 1989). This banding is explained to be due to utilization of stored lipids by the infective juveniles in the absence of the host (during starvation). Some lipid-filled cells are

opaque whereas others are clear, having lost their stored lipid through the utilization (Dropkin, 1989).

The irregular appearance of the tail tip for juvenile specimens had also been reported elsewhere for *Meloidogyne* species larval stages (Goodey, 1963; Jenkins and Taylor, 1967; Heyns, 1971; Machon and Hunt, 1991).

The measurements and ratios obtained in this study for the *Meloidogyne* specimens (Section 4.2.2.2; Appendix 5a) all fall within the ranges given by Williams (1974) for *M. hapla* although there is extensive overlap with other *Meloidogyne* species as provided by Williams (1972), Franklin (1973), Williams (1973), Cain (1974), Williams (1975) and Mulk (1976).

5.2.2. *Pratylenchus penetrans*

A common diagnostic feature for *Pratylenchus* species as documented by various authors (Goodey, 1963; Jenkins and Taylor, 1967; Dropkin, 1980; Dropkin, 1989) is the flat lip region on the anterior end, with a well-sclerotized cephalic framework. This was observed and described during this study (Plate 16; Section 4.2.2.1b).

Another feature noticed was the prodelphic outstretched ovary with no posteriorly directed ovary; this was only visible as a small undeveloped post-vulval sac. Corbett (1973) quotes this sac as short and undifferentiated for *Pratylenchus penetrans*.

The tail tip of the female was smooth and broadly rounded, similar to records by Goodey (1963), Heyns (1971) and Corbett (1973) for *Pratylenchus* species.

Similar to the findings in this investigation, Heyns (1971) and Corbett (1973) described the tail tip for *P. penetrans* males as smooth, conoid and enveloped by a bursa. Jenkins and Taylor (1967), Dropkin (1980) and Dropkin (1989) also quote the enveloping of the tail tip by a bursa, citing that the bursa extends anteriorly from the tail tip to the region opposite the distal

ends of the spicules.

By applying the descriptions given by Heyns (1971), Corbett (1973) and the identification key and compendium compiled by Handoo and Golden (1989), the *Pratylenchus* specimens were identified to be *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941.

5.2.3. *Tylenchus* species

The long filiform tail described in this study is characteristic of *Tylenchus* species (Heyns, 1971; Goodey, 1963).

The anterior part of these nematodes was narrowed down and had no cephalic framework. Heyns (1971) states that the cephalic framework is weakly developed or absent, while Goodey (1963) says that it is apparently absent in *Tylenchus* species.

There were no visible annulations on the body, although Heyns (1971) records the presence of transverse striae.

The esophagus ended with a pyriform basal bulb which did not overlap with the intestine. This is a tylenchoid type of esophagus, as described by Jenkins and Taylor (1967).

5.3 Wilt Expression in Pyrethrum

Wilt symptoms started with the lower leaves of the plant and there was gradual progression of the symptoms up the plant, with the upper leaves showing symptoms later. This observation accords well with recorded observations for herbaceous plants infected with *Fusarium oxysporum* (Mace *et al*, 1981).

Engelhard and Woltz (1973) reported that wilt pathogens of chrysanthemum, *Fusarium oxysporum* fsp. *chrysanthemi* and *Fusarium oxysporum* fsp. *tracheiphilum*, were isolated more frequently from the basal leaves than the top ones of symptomatic plants. This explains the steady symptom expression on pyrethrum plants in relation to the gradual upward colonization of plants from the stem bases. Garrett (1970) also describes progression of the wilt syndrome caused by vascular fungi as an acropetal succession towards the stem apex. Similar to the findings in the field observations and greenhouse experiments of this study, several plants have been reported to suffer leaf chlorosis before actual withering (Mace *et al*, 1981).

Fusarium oxysporum can penetrate a host plant either through wounds or directly. This fungus, being a vascular wilt pathogen, enters the xylem vessel elements and generally gets confined to the xylem vessel elements and tracheids initially. The pathogen then spreads to the top of the aerial shoots and even into the petioles and main veins of the leaves through the vascular xylem vessels (Garrett, 1970; Pennypacker and Nelson, 1972; Mace *et al*, 1981).

During the pathogenicity tests, low inoculum levels of $1.25x10^6$ conidia, $2.5x10^6$ conidia and $2.5x10^6$:500 conidia:nematode combination led to initial leaf chlorosis followed by dessication of leaves, starting from the leaf margins. At high inoculum levels of $5.0x10^6$ conidia, $1.0x10^7$ conidia, $2.5x10^6$:1500, $1.0x10^7$:500 and $1.0x10^7$:1500 conidia:nematode combinations, the plants withered and became necrotic without any appreciable initial chlorosis on leaves. Hence, the level of initial inoculum is an important factor in the type of symptoms expressed by a host plant

(Wardlaw, 1972).

Unilateral symptom development on some plants inoculated through the soil drenching method was noticed in this study. This has also been reported on other crops (Engelhard and Woltz, 1973; Mace *et al.* 1981).

Fusarium oxysporum, positively identified as the causal agent of wilt in pyrethrum during the pathogenicity tests in this study, falls under the category of tissue-specific pathogens (Krupa and Dommergues, 1979). This implies that the pathogen is restricted to certain tissues (in this case the xylem tissue) during the period of vegetative growth, and certain other tissues during the reproductive or senescent periods. The plant-pathogen relationship is prolonged and plants are not usually killed suddenly after infection. This is a characteristic feature of host-dominant diseases such as wilts (Krupa and Dommergues, 1979). This fact explains the failure of the pyrethrum plants to collapse completely; instead there was gradual necrosis which started with the leaves during this study. The roots were also not destroyed by the pathogen since they merely acted as avenues to the vascular systems of the host.

Vascular discolouration was observed on splitting open both naturally infected field plants and artificially inoculated pathogenicity test plants. Vascular browning has been reported in *Fusarium* wilts of sweet potatoes, tomatoes and pyrethrum (McClure, 1950; Walker, 1971; Ondieki and Anyango, 1980).

Fusarium species causing wilt diseases in plants have been shown to produce fungal phenoloxidases (Maraite, 1973). These enzymes catalyze the oxidation of mono- and dihydrophenols by moleclular oxygen to form brown or black polymeric substances (melanin pigments). Activity of these enzymes increases in diseased or wounded areas of plant tissues (Heiteffus and Williams, 1976).

Isolations from plants exhibiting a discoloured stele showed that *Fusarium oxysporum* fsp.

batatas, the causal agent of sweet potato wilt, was present only in the most severely discoloured areas and the discolouration preceded the pathogen up the stem (McClure, 1950; Chambers and Corden, 1963). The presence of vascular discolouration in advance of the pathogen is an indication that toxic by-products are produced and are carried in the transpiration stream (McClure, 1950). This arguement can be used to explain the higher intensity of vascular browning and the more rapid symptom expression observed on plants inoculated with higher levels of fungal inoculum than on those treated with lower inoculum levels during the pathogenicity tests of this study. Exposure to higher inoculum levels could have resulted in higher invasion incidence of plants leading to production of higher quantities of toxic factors.

The effect of plant-parasitic nematodes in enhancing the pyrethrum wilt disease is well exemplified by the results of the pathogenicity tests. Holding the fungal inoculum level constant but using different nematode populations to combine with the fungal inoculum resulted in significantly earlier wilt expression on plants inoculated with higher inoculum levels than on plants with lower nematode inocula exposures (Figures 2c and 2d; Tables 5c and 5d). This could have been due to inadequacy of the low nematode populations to cause any significant enhancement of the wilt development; this assertion is verified by the significant difference in the time required for wilt expression when combined inoculation using 500 and 1500 nematodes are compared at the same inoculum level of fungal propagules. Those plants inoculated using combined inoculum involving 1500 nematodes wilted significantly earlier than those inoculated with inocula involving 500 nematodes.

The mixed nematode populations used in the experiment consisted of *Meloidogyne hapla*, *Pratylenchus penetrans* and *Tylenchus* spp. which are sedentary root endoparasistes, migratory root endoparasites and sedentary root ecto- and endoparasites respectively (Anon, 1958). Due to their different feeding habits, these nematodes affect their hosts in different ways and cause

different damages (Linford, 1942; Christie, 1959; Klinkenberge, 1963; Jenkins and Taylor, 1967; Dropkin, 1989).

Among the species identified, *Meloidogyne hapla* and *Pratylenchus penetrans* are known to be major plant parasites (Melendez and Powell, 1967; Oyenkan and Mitchell, 1971). However, *Tylenchus* species are largely of little economic importance since they are not aggressive parasites, perhaps due to their delicate stylet (Heyns, 1971; Goodey, 1963). Although species of *Tylenchus* are commonly found in the soil and around plant roots, their food sources are largely unknown (Jenkins and Taylor, 1967) and, in general, they do not penetrate the cortex (Dropkin, 1980). *Tylenchus* spp. nematodes were only recovered from Sotik soil samples, in very small numbers, and not from the other sites (Table 1). This further implies that these nematodes are of little, if any, economic importance as pyrethrum parasites.

5.4. Effect of Environmental Conditions on Wilt Development in Pyrethrum

5.4.1. Effect of temperature on Wilt Development

From the results, it was obvious that symptom expression depends on the incubation temperatures used. The higher the incubation temperature, the more rapid was the symptom expression (Figure 3).

This observation is similar to the findings on carnation wilt caused by *Fusarium oxysporum* fsp. *dianthi*, in which case carnation variety Improved White Sim exhibited symptoms earlier at an air temperature of 70°F (21.1°C) than at 55°F (12.8°C). The plants grown at 70°F (21.1°C) expressed wilt symptoms 2-3 months earlier than those grown at 55°F (12.8°C). Experimental data from this investigation also show that soil and air temperatures influence

symptom expression and disease development (Nelson, 1964).

Soil temperature has been regarded as a very important factor in the infection of tulips by *Fusarium oxysporum* in the field. Investigations on field infection of tulip bulbs by *Fusarium oxysporum* fsp. *tulipae* Apt. indicated that many of the bulbs planted in early fall in relatively warm soil infested with the fungus were lost and flowering was decreased due to the fungal infection. This was unlike bulbs planted later in the fall when soil temperatures were lower; fungal invasion was arrested and plants emerged and flowered normally the following spring (Bergman and Noordermeer-Luyk, 1973).

The high temperature treatments and the high inoculum concentration used during the greenhouse experiment (Section 3.4.2) have an epidemiological significance. Possibly, these conditions are not always met in the field. For instance, pyrethrum is known as a high-altitude crop, being grown in areas experiencing low average air temperatures of around 15°C (Wanjala, 1991; Parlevliet, 1971). This fact combined with the lower inoculum densities in the field (Table 8) could explain, at least in part, why the wilt disease has not been known to limit pyrethrum production in the high-altitude areas. However, this observation has an important bearing in the low-altitude areas such as Kisii which experience higher atmospheric temperatures of about 20°C.

The optimum temperature for wilt symptom expression in pyrethrum clone L/75/487 was not attained within the air temperature range tested (17.7°C to 30.0°C). A similar observation was made with some chrysanthemum cultivars whereby no optimum temperature for wilt development was reached with a temperature test range of 24-35°C (Gardiner *et al*, 1987). Similarly, progressively shorter incubation periods were required for symptoms to develop with increase in incubation temperatures.

By using a single clone, L/75/487, it is imperative to infer that a clone might mistakenly be rated tolerant or resistant to the pyrethrum wilt disease if screening is done at low

temperatures of about 17°C or below. This assertion holds true considering the fact that some inoculated plants of clone L/75/487 showed no symptoms although they were infected at an incubation temperature of 17.7°C.

5.4.2 Effect of Soil Moisture on Wilt Development

In this study, soil moisture levels used increased through the ranges 25-30%, 45-50%, 65-70% and 90-100% soil saturation. For the first three soil moisture levels, the pyrethrum wilt disease expressed itself earliest at the lowest soil moisture level and latest at the highest soil moisture level. The increase in the length of incubation period thus observed could well be attributed to the increase in anaerobic conditions with increase in soil moisture. The anaerobic conditions could have contributed to a decline in the original populations of the *Fusarium oxysporum* inoculum leading to decreased ability of the fungal units left behind to incite disease; hence the wilt disease expressed itself much later at high soil moisture levels than at low moisture levels where the required oxygen was readily available for the fungal metabolic activities.

The increases in soil moisture content might have had an important bearing in the amount of aeration of the soils which was likely to affect the availability of oxygen needed for multiplication of the initially introduced fungal inoculum. A decrease in the survival of *Fusarium oxysporum* fsp. *cubense*, the causal agent of banana wilt, with reduced aeration has been demonstrated (Stover, 1953b; Stover, 1955).

Stover *et al* (1953) demonstrated that flooding of banana land to eradicate *Fusarium oxysporum* fsp. *cubense*, the causal organism of Panama disease, led to a reduction of at least 50% of the numbers of indigenous soil fungi during the first 35 days of submergence and at least 85% of the indigenous *Fusaria* were eradicated during the first 40 days of flooding. These

findings further articulate the fact that anaerobic conditions are not favourable for most *Fusaria* and other soil fungi.

It is difficult to explain the relatively short incubation period of the pyrethrum wilt disease for soil moisture levels at or near the field capacity (90-100% saturation) compared to the other soil moisture levels. This is especially in consideration of the fact that *Fusarium* spp. are generally most active in quite dry soils (Graham *et al*, 1957).

However, rapid germination of spores might occur at or near the soil field capacity (Griffin, 1972). The rapid conidial germination phenomenon possible at or near the field capacity of the soil might not have been possible at the lower soil moisture levels, leading to a possible reduction in initial infection incidence of the host plants by the fungus. Some soil-borne pathogens may attain high virulence when conditions become unfavourable for their hosts in some way (Bateman, 1961). Davis *et al* (1976) also suggested that soil moisture levels could influence the availability or absorption of nutrients such as potassium, phosphorus and calcium by host plants from the soil. Some of these nutrients are important in resistance mechanisms of plants against pathogens. Therefore, it can be speculated that the high soil moisture levels led to malfunctioning of the pyrethrum plants, making them more vulnerable to attack by the wilt pathogen, *Fusarium oxysporum*.

These two factors (rapid germination of conidia and the likely increase in host vulnerability) combined might have been possible contributory factors to the short incubation period of the wilt disease for plants treated at 90-100% moisture level.

5.4.3. Effect of Combined Field Conditions on Wilt Development in Pyrethrum

Plants grown and inoculated at Kisii (average ambient temperature of 19.9°C) expressed wilt symptoms earlier than plants grown at the other experimental sites and had incidentally the greatest lengths of vascular colonization. This is an indication that temperature plays a significant role in enhancing wilt development in pyrethrum.

There were more *Fusarium oxysporum* propagules at Kisii (with average daily temperature of 19.9°C) than any of the areas Molo, Limuru and Ol Joro-Orok with average daily temperatures of 13.1°, 13.0° and 13.7°C respectively (Table 8). This observation is similar to the findings of Banihashemi and Dezeeuw (1973) who found that the population of *Fusarium oxysporum* fsp. *melonis* (Leach & Currence) Snyd. & Hans., the causal agent of wilt of watermelons, was highest at 15°C and lowest at 5°C, after 14 months of incubation, in both autoclaved and non-autoclaved soils. Inoculum build-up in high-temperature areas may lead to higher pyrethrum wilt incidences than in low temperature areas. This has the implication that the pyrethrum industry stands to lose greatly from the effect of the wilt disease on various clones especially at high-temperature areas unless the situation is saved in good time.

Although the temperatures recorded at Molo, Ol Joro-Orok and Limuru were almost the same (around 13°C), plants at the Molo trial wilted significantly later than those at the other trial sites (Section 4.4.3). This may be attributed to the higher rainfall experienced at Molo (215.5mm) than at the other two experimental sites (Table 8). High rainfall is a pre-requisite to high soil moisture contents which have the effect of reducing soil aeration. The strained aerobic conditions in the soil are detrimental to *Fusarium oxysporum*, with the result that the fungal inoculum potential is reduced (Stover, 1953b; Stover, 1955). The Molo trial was also conducted on an area with low initial *Fusarium* propagules (Table 8).

There were significant differences in pH levels for the field soil samples. However, the

pH values were within a very narrow range of 5.66 to 6.09. The range was on the acidic bias and the difference between the highest and the lowest values was less than half a pH unit. Therefore, it is worthwhile asserting that soil pH values for the sites tested could not account for any significant differences in the development and expression of wilt symptoms in the respective sites. The greenhouse planting soil mixture was also acidic (pH 6.6). This has the implication that soil pH may not be a significant factor in pyrethrum wilt investigations.

5.4.4. Effects of Hydrogen-ion Concentration on Fungal Isolate Growth

The five *Fusarium oxysporum* isolates grown on PSA did not differ significantly from each other at equal pH levels (Table 9b). This indicates that the isolates represented one and the same organism, *Fusarium oxysporum*. However, as the medium pH increased from 4.0 to 8.0 colony diameters also increased (Figure 5). This shows that growth of the fungal isolates was favoured by increased alkalinity of the medium. The behaviour of an organism in culture, although different in many ways from its behaviour in a complex medium such as soil, is an important guide to the true characteristics of the organism. The result of this experiment leads to the suggestion that alkaline soils may be more prone to high infestations by *Fusarium oxysporum* than acidic soils.

Fusarium oxysporum isolates were found to develop more intense pink colour at low pH than at high pH levels in this study. This accorded well with the report by Sebek (1952) that the most abundant pigment formation by *Fusarium oxysporum* fsp. *lycopersici* was at low medium pH levels between 2.7 and 4.5.

5.5. Screening for Resistance to Pyrethrum Wilt

All the eight clones and three varieties screened for resistance became diseased but they took varying periods of time before expressing the wilt symptoms (Tables 10 and 11a). The longest incubation period of the wilt disease under greenhouse conditions was an average of 163.7 days (equivalent to 5½ months) for clone L/75/487. The longest time period before wilt symptoms were noticed under field conditions at Mwongoris was exhibited by clone Kr/74/122 which was recorded as wilted after 187.3 days (equivalent to 6 months). Considering the fact that pyrethrum is a perennial crop, this period is too short for the crop to have produced any economic yields.

As the natural inoculum propagule counts indicated, there are always some levels of soil infestation in the fields where pyrethrum is grown (Section 4.4.3). Therefore, highly drastic losses could be anticipated if a certain area has a sufficiently high soil infestation level by the wilt pathogen. These facts call for serious investigations into control strategies of pyrethrum wilt in order to avoid a reduction in the crop productivity due to wilting and death of the plants.

Failure of most clones/varieties at the Molo screening experiment to express wilt symptoms (Appendix 15) can be attributed to the low temperatures prevailing at the site (13.1°C) combined with the high rainfall recorded (215.5mm) during the experimental period (Table 8). This was contrary to the Mwongoris trial plants where the area is relatively warmer than Molo. Plants at the Mwongoris trial succumbed to wilt and the plot stands were seriously disrupted by death of the plants (Table 11a).

The differential incubation periods exhibited by the clones and varieties tested are an indication that pyrethrum plants possess at least some inherent ability to tolerate the wilt disease. Some clones/varieties expressed wilt symptoms quite early compared to others. This argument is further supported by the fact that those clones/varieties which took considerably long periods

before expressing the wilt symptoms also required longer periods of time before drying up completely. In fact, clones L/75/487, Kr/74/122, Ks/75/313 and variety K218 had not succumbed by dying by the end of the experimental period. This shows that even though these plants exhibited wilt symptoms, they were able to tolerate the disease, at least under the greenhouse test conditions.

There was no consistent trend of relating the time taken for the first wilt symptoms to appear and the length of colonized vascular system for all the clones and varieties screened under both greenhouse and field conditions. Therefore, the length of vascular colonization could not concretely be used as a measure of resistance or susceptibility of pyrethrum clones and varieties to the *Fusarium* wilt.

Measurements of vascular colonization by the pathogen, *Fusarium oxysporum*, for the clones and varieties tested were not significantly different (Tables 10 and 11a). Therefore, the efficiency of the fungus in colonizing the plants did not differ with the clones and varieties.

In general, there was a more rapid development of the wilt symptoms in some clones/varieties than in others as exemplified by the different periods of time required for the various clones/varieties to dry up completely (Table 10). The clones/varieties allowing slow wilt symptom development could be in possession of some horizontal resistance which contributed to the slowed down symptom development. Conversely, those plants which succumbed to the wilt disease faster could have been displaying little or lack of horizontal resistance to the wilt disease.

There were significant differences among the clones and varieties tested under field conditions in terms of their flower yield ratios (Table 11b). Inherent differences in their productivity were, therefore, displayed even in the presence of the wilt disease. The varieties (P_4, K218 and K235) had higher flower yield ratios than the clones (Table 11b). This implies that the varieties possess relatively dense flowers or their genetic base is broad enough to counteract

adverse effects of the disease and the environment.

An important observation was the reduction of the flower yield ratios for the clones and varieties with time (Figure 6; Table 11b). This is an indication that the wilt disease resulted in reduced weight per flower for the clones and varieties tested. The ability of the plants to absorb water and minerals from the soil could have been limited as a result of vascular colonization by the fungus, *Fusarium oxysporum*. This argument is supported by the fact that there was a more rapid reduction in the average weight of flowers of inoculated than uninoculated plants of clone 4331 with time (Table 11b). This fact, in addition to increased number of tillers whose nutritional needs could probably not be met as the plants grew, could have resulted in the reduction of the flower yield ratio with time.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Pyrethrum plants were seriously damaged by the wilt disease in the field. There was a reduction in flower weight and, in some cases, plants dried up completely.

Fusarium oxysporum was identified as the causal agent of the wilt disease in pyrethrum. Inoculation of pyrethrum plants with this fungus resulted in wilt development.

Interaction between *F. oxysporum* and the nematodes, *Meloidogyne hapla* and *Pratylenchus penetrans*, resulted in increased wilt severity. Inoculated plants wilted much earlier when the fungus was combined with the nematodes than in cases where fungal inoculum was used alone. Wilt symptoms were also more pronounced. Inoculation with nematodes alone did not result in any wilt symptoms.

Disease severity varied with inoculum levels and method of inoculation used. High inoculum levels resulted in earlier onset of wilt symptoms than low inoculum levels. The disease developed much earlier in root-dip-inoculated plants than in plants inoculated through soil drenching.

Atmospheric temperature and soil moisture affected the incubation period of the wilt disease. The disease developed progressively early as temperature increased. In general, low soil moisture levels led to earlier expression of the disease than high moisture levels.

Growth of *Fusarium oxysporum* on PSA was affected by pH. Low pH levels restricted growth of this fungus. High pH levels are likely to promote high multiplication rates of *F. oxysporum* in the soil.

None of the eight clones and three varieties screened had a high resistant response to the

wilt disease. All of them succumbed to the disease within such short time periods that economic yields would be unlikely before total plant death under the test conditions used.

6.2. Recommendations

The damage due to the wilt disease should be put into quantitative terms and losses accompanying this damage should be put to a halt in pursuit of sustainability of the pyrethrum industry.

In addition to the role of *Fusarium oxysporum*, the role of the nematodes recovered from rhizospheres and roots of wilted plants should be fully established. Attempts should also be made to establish if other nematodes, apart from *Meloidogyne hapla*, *Pratylenchus penetrans* and *Tylenchus* species, could be parasitic to pyrethrum plants. The levels of damage caused by nematodes should also be established.

Although very little may be done to alleviate the problem posed by some environmental conditions such as temperature and soil moisture in the enhancement of wilt development in pyrethrum, the most conducive conditions for wilt development should be exploited in screening pyrethrum germplasm for resistance to the wilt disease and for efficacy testing of any wilt management measures.

Application of alkaline fertilizers to pyrethrum fields should be done with great caution. This is because the causal agent of wilt, *Fusarium oxysporum*, is favoured by high pH levels as indicated by the results from the test on effect of pH on growth of the fungus. As a long-term wilt control strategy, all pyrethrum germplasm should be screened for resistance to the wilt disease. Clones and/or varieties noted to possess resistance or tolerance to the disease should be used for breeding even more durable planting materials.

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APPENDICES

Appendix 1: Potato Sucrose Agar (PSA)

Potato extract	500ml
Sucrose	20.0g
Agar	15.0g
Trace metal solution (see below)	1.0ml
Distilled water	500ml

Adjust the pH of the solution to 6.7 +/- 0.1 before autoclaving.

Trace metal solution

Zinc sulphate ($ZnSO_4.7H_2O$)	1.0g
Copper sulphate ($CuSO_4.5H_2O$)	0.5g
Distilled water	100ml

Appendix 2: Spezieller Nahrstoffarmer Agar (SNA)

Agar	15g
Sugar solution (see below)	10ml
Salt solution (see below)	10ml
Distilled water	1000ml

When the medium has set, place a sterile filter paper strip (10x30mm) in each Petri dish.

Sugar Solution

Glucose (anhydrous)	10.0g
Sucrose	10.0g
Distilled water	500ml

Salt Solution

Potassium dihydrogen orthophosphate (KH_2PO_4)	50.0g
Potassium nitrate (KNO_3)	50.0g
Magnesium sulphate ($MgSO_4.7H_2O$)	25.0g
Potassium chloride (KCl)	25.0g
Distilled water	500ml

Appendix 3: Kerr's Medium Composition and Preparation

-Used to isolate *Fusarium* spp. from soil.

Sodium nitrate	2.0g
Potassium dihydrogen phosphate	1.0g
Potassium chloride	0.5g
Magnesium sulphate	0.5g
Iron (II) sulphate	0.01g
Sucrose	30.0g
Yeast extract	0.5g
Agar	15.0g
Distilled water	1000ml

After autoclaving, the cooled molten basal medium is amended with 60mg rose bengal, 100ml quintozene and 50mg streptomycin.

Appendix 4: TAF Fixative Preparation

Formalin (40% formaldehyde)	7ml
Triethanolamine	2ml
Distilled water	91ml

Double strength fixatives are prepared using half the amount of water indicated.

TAF has the advantage of enabling the retention of nematode life-like appearance for several hours.

ppendix 5. Measurements and ratios for some representative nematode specimens
part from the ratios a, b, b', c, and V all the other values are in micrometres (μm)

ey to the abbreviations used
L → Body length
B_w → Greatest body width
a → Length/Greatest body width
H_l → Head length
S_l → Esophagus length to esophago-intestinal valve
b → L/Esophagus length to esophago-intestinal valve
b' → L/Distance from anterior end to middle of median bulb
S_p → Stylet (spear) length
S_w → Stylet base width
S_k → Stylet knobs length
B_l → Median bulb length
B_w → Median bulb width
T_l → Tail length
c → Body length/tail length
S_p → Spicules length (measured on a chord)
G_u → Gubernaculum length
(M) → Specimen D was a male e.g for the *Meloidogyne* specimen
(J) → Specimen A was a juvenile e.g for the *Meloidogyne* specimen
(F) → Specimen B was a female e.g for the *Pratylenchus* specimen

(a) Measurements for *Meloidogyne* species specimens

	Characters															
	L	B_w	a	H_l	S_l	S_w	S_k	B_l	b	b'	M_l	M_w	T_l	c	S_p	G_u
A (J)	349.2	14.4	24.25	-	10.8	1.8	-	-	-	7.46	7.2	3.6	39.6	8.82	-	-
B (J)	349.2	14.4	24.25	-	10.8	1.8	-	-	-	6.93	7.2	3.6	36.0	9.70	-	-
C (J)	377.0	18.0	20.94	-	10.8	1.8	-	-	-	6.55	10.8	7.2	46.8	8.06	-	-
D (M)	1131.0	29.0	39.00	7.2	18.0	3.6	3.6	79.2	14.28	18.48	21.6	10.8	5.4	209.44	25.2	10.8
E (J)	356.4	14.4	24.75	-	10.8	1.8	-	-	-	7.07	7.2	3.6	36.0	9.90	-	-
F (M)	1174.5	28.8	40.78	7.2	18.0	3.6	3.6	82.8	14.18	19.19	25.2	10.8	7.2	163.13	25.2	10.8
G (J)	349.2	14.4	24.25	-	10.8	-	-	-	-	8.08	7.2	3.6	39.6	8.82	-	-
H (J)	352.8	14.4	24.50	-	10.8	-	-	-	-	7.00	-	-	-	-	-	-
J (M)	1145.5	25.2	45.46	7.2	21.6	3.6	3.6	79.2	14.46	18.72	21.6	10.8	7.2	159.10	25.2	10.8
K (M)	1131.0	28.8	39.27	7.2	18.0	-	-	75.6	14.96	18.48	25.2	10.8	7.2	157.08	21.6	10.8
L (J)	360.0	18.0	20.00	-	10.8	-	-	-	-	7.14	-	-	43.2	8.33	-	-

(b) Measurements for *Pratylenchus* species specimens

	Characters														
	L	B_w	a	H_1	S_1	S_w	S_k	M_1	M_w	E_1	b	b'	T_1	c	V
A (F)	623.5	29.0	21.50	18.0	14.4	1.8	5.4	14.4	9.0	108.0	5.77	11.55	-	-	81.40
B (F)	522.0	21.6	24.17	18.0	14.4	1.8	5.4	14.4	7.2	97.2	5.37	9.67	33.4	16.11	83.33
C (M)	551.0	25.2	21.87	16.2	14.4	1.8	3.6	10.8	7.2	93.6	5.89	10.93	36.0	15.31	-
D (F)	638.0	29.0	22.00	18.0	16.2	-	5.4	14.4	10.8	104.4	6.11	11.81	36.0	17.72	81.82
E (J)	493.0	21.6	22.82	12.6	10.8	-	-	9.0	3.6	-	-	11.41	-	-	-
F (F)	594.5	25.2	23.59	18.0	14.4	-	5.4	14.4	10.8	104.4	5.69	11.01	-	-	82.93
G (F)	623.5	28.8	21.65	18.0	16.2	-	7.2	14.4	10.8	100.8	6.19	11.55	39.6	15.74	83.72
H (J)	478.5	21.6	22.15	-	10.8	-	-	9.0	3.6	-	-	11.08	39.6	12.08	-
J (M)	536.5	25.2	21.29	16.2	14.4	-	3.6	10.8	7.2	90.0	5.96	10.64	32.4	16.56	-
K (J)	493.0	21.6	22.82	12.6	12.6	-	-	9.0	3.6	-	-	10.53	39.6	12.45	-

(c) Measurements for *Tylenchus* species specimens

	Characters										
	L	B_w	a	H_1	S_1	S_w	E_1	b	T_1	c	V
A (J)	855.5	32.4	26.40	-	-	-	226.8	3.77	72.0	11.88	-
B (J)	870.0	43.2	20.14	-	46.8	-	-	-	79.2	10.98	-
C (F)	1725.5	57.6	29.96	72.0	79.2	3.6	420.5	4.10	449.5	3.84	51.26
D (F)	1870.5	72.5	25.80	68.4	75.6	3.6	464.0	4.03	288.0	6.49	51.16
E (J)	855.5	58.0	14.75	10.8	14.4	3.6	208.8	4.10	165.6	5.17	47.46
F (J)	928.0	58.0	16.00	10.8	14.4	3.6	237.6	3.91	68.4	13.57	-
G (F)	1754.5	61.2	28.67	72.0	79.2	3.6	435.0	4.03	449.5	3.90	52.07
H (F)	1827.0	68.4	26.71	75.6	79.2	3.6	464.0	3.94	338.4	5.40	51.59
J (F)	1725.5	61.2	28.19	68.4	72.0	3.6	420.5	4.10	406.0	4.25	52.94
K (J)	957.0	58.0	16.50	10.8	14.4	-	237.6	4.03	82.8	11.56	-
L (F)	1754.5	72.0	24.37	72.0	79.2	-	449.5	3.90	420.5	4.17	52.89
M (F)	1783.5	72.5	24.60	72.0	75.6	-	435.0	4.10	420.5	4.24	52.03

Appendix 6a. ANOVA table for the effect of isolates, inoculum levels and inoculation methods on pyrethrum wilt incubation period

Source	SS	df	MS	F
Main Effects				
Inoculum level	27991.117	7	3998.731	331.158***
Isolate	1134.183	4	283.546	23.482***
Inoculation method	6762.817	1	6762.817	560.068***
Interaction				
Level x Isolate	286.883	28	10.246	0.848ns
Level x Method	319.517	7	45.645	3.780***
Isolate x Method	62.850	4	15.713	1.301ns
Level x Isol x Meth	219.817	28	7.851	0.650ns
Error	1932.000	160	12.075	
Total	38709.183	239		

Appendix 6b. Tables for multiple regression analysis of days to wilt symptoms against inoculum level

i) Fungus inoculum alone using root-dip inoculation

Source	df	Sum of squares	Mean sum of squares	F-value
Regression	2	7978.46	3989.23	268.82***
Isolates	1	11.41	11.41	0.77ns
Inoculum levels	1	7967.05	7967.05	536.87***
Error	57	845.87	14.84	
Total	59	8824.33		

Regression equation: $Y = 126.01 + 0.31X_1 + -10.31X_2$

Coefficient of determination (R^2) = 0.904

Y refers to days to symptom expression

X_1 refers to isolates and X_2 refers to inoculum levels

ii) Fungus inoculum alone using soil-drench inoculation

Source	df	Sum of squares	Mean sum of squares	F-value
Regression	2	6192.16	3096.08	179.08***
Isolates	1	72.08	72.08	4.17*
Inoculum levels	1	6120.08	6120.08	353.98***
Error	57	985.49	17.29	
Total	59	7177.65		

Regression equation: $Y = 131.41 + 0.78X_1 + -9.03X_2$

Coefficient of determination (R^2) = 0.863

Y refers to days to symptom expression

X_1 refers to isolates and X_2 refers to inoculum levels

iii) Nematode-fungus inoculum using root-dip inoculation

Source	df	Sum of squares	Mean sum of squares	F-value
Regression	2	6396.13	3198.07	272.13***
Isolates	1	48.13	48.13	4.10*
Inoculum levels	1	6348.00	6348.00	540.16***
Error	57	669.87	11.75	
Total	59	7066.00		

Regression equation: $Y = 115.10 + 0.63X_1 + -9.20X_2$

Coefficient of determination (R^2) = 0.905

Y refers to days to symptom expression

X_1 refers to isolates and X_2 refers to inoculum levels

iv) Nematode-fungus inoculum using soil-drench inoculation

Source	df	Sum of squares	Mean sum of squares	F-value
Regression	2	4863.25	2431.64	96.93***
Isolates	1	182.53	182.53	7.28**
Inoculum levels	1	4680.75	4680.75	186.58***
Error	57	1429.97	25.09	
Total	59	6293.25		

Regression equation: $Y = 121.30 + 1.23X_1 + -7.90X_2$

Coefficient of determination (R^2) = 0.773

Y refers to days to symptom expression

X_1 refers to isolates and X_2 refers to inoculum levels

Appendix 7. Anova tables for the effect of isolates and inoculum levels on pyrethrum wilt development

a) Root-dip inoculation using fungus alone

Source	df	SS	MS	F
Main effects				
Inoculum levels	3	8106.333	2702.111	229.641***
Isolates	4	190.500	47.625	4.047**
Interaction				
Levels x Isolates	12	56.833	4.736	0.402ns
Error	40	470.667	11.767	
Total	59	8824.333		

b) Soil-drench inoculation using fungus alone

Source	df	SS	MS	F
Main effects				
Inoculum levels	3	6246.983	2082.328	120.715***
Isolates	4	136.233	34.058	1.974ns
Interaction				
Levels x Isolates	12	104.433	8.703	0.504ns
Error	40	690.000	17.250	
Total	59	7177.650		

c) Root-dip inoculation using Nematode-fungus inoculum

Source	df	SS	MS	F
Main effects				
Inoculum levels	3	6400.267	2133.422	361.597***
Isolates	4	327.833	81.958	13.891***
Interaction				
Levels x Isolates	12	101.900	8.492	1.439ns
Error	40	236.000	5.900	
Total	59	7066.000		

d) Soil-drench inoculation using nematode-fungus inculum

Source	df	SS	MS	F
Main effects				
Inoculum levels	3	4971.917	1657.306	123.833***
Isolates	4	674.000	168.500	12.590***
Interaction				
Levels x Isolates	12	112.000	9.333	0.697ns
Error	40	535.333	13.383	
Total	59	6293.250		

Appendix 8. ANOVA tables for the effect of temperature on pyrethrum wilt development in the greenhouse

a) Wilt Incubation period

Source	df	SS	MS	F-value
Main effects				
Temperature	3	9214.929	3071.643	117.238***
Error	10	262.000	26.200	
Total	13	9476.929		

b) Time to complete plant death after first wilt symptoms

Source	df	SS	MS	F-value
Main effects				
Temperature	2	236.167	118.083	9.103***
Error	9	116.750	12.972	
Total	11	352.917		

Appendix 9. ANOVA table for the effect of soil moisture on pyrethrum wilt development

Source	SS	df	MS	F
Main Effects				
Moisture	37.688	3	212.563	8.156**
Error	312.750	12	26.063	
Total	950.438	15		

Appendix 10a. ANOVA table for the effect of field conditions on pyrethrum wilt development

Source	df	SS	MS	F-value
Main effects				
Site	3	3890.24	1296.747	22.84***
Reps	3	5314.18	1771.393	31.20***
Error	4	227.10	56.775	
Total	10	9431.52		

Appendix 10b. Temperature and Rainfall data for temperature-moisture experimental sites

PERIOD 1994	EXPERIMENTAL SITES							
	Kisii		Molo		Ol Joro-Orok		Limuru	
	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)
June	19.6	183.3	13.2	136.0	13.5	89.7	13.2	31.2
July	19.2	157.9	13.1	372.8	13.9	188.3	12.5	26.2
August	19.7	147.1	12.8	303.8	13.6	109.1	12.6	6.4
September	21.0	171.1	13.3	49.5	13.8	6.4	13.6	3.8
Mean monthly	19.9	164.9	13.1	215.5	13.7	98.4	13.0	16.9

Appendix 11. ANOVA table for the effect of field conditions on plant vascular infection length

Source	df	SS	MS	F-value
Main effects				
Site	3	3847.25	1282.417	2.90ns
Reps	3	598.25	199.417	0.45ns
Error	9	3982.25	442.472	
Non-additivity	1	380.44	380.440	0.85
Residual	8	3601.81	450.226	
Total	15	8427.75		

Appendix 12. ANOVA tables for the effect of pyrethrum wilt development on three varieties and eight clones in the greenhouse.

a) Wilt Incubation period

Source	SS	df	MS	F
Main Effects				
Clone/variety	66042.909	10	6604.291	64.594***
Error	2249.333	22	102.242	
Total	68292.242	32		

b) Vascular infection lengths

Source	SS	df	MS	F
Main Effects				
Clone/variety	5079.394	10	507.939	1.129ns
Error	9894.667	22	449.758	
Total	14974.061	32		

c) Time taken to complete drying of plants

Source	SS	df	MS	F
Main Effects				
Clone/variety	7928.952	6	1321.492	7.954**
Error	2326.000	14	166.143	
Total	10254.952	20		

Appendix 13a. Anova Table for days to wilt symptoms for Mwongoris resistance screening trial

Source	df	SS	MS	F-Value
Clone	11	89103.00	8910.30	4.77***
Replications	3	1515.64	505.21	0.27ns
Error	33	56053.36	1868.44	
Non-additivity	1	1.43	1.43	
Residual	32	56051.93	1932.83	
Total	47	146672.00		

CV = 56.14%

Appendix 13b. Anova Table for vascular colonization of Mwongoris plants used in screening for resistance

Source	df	SS	MS	F-Value
Clone	11	4742.23	474.22	1.28ns
Replications	3	112.80	37.60	0.10ns
Error	33	11101.95	370.07	
Non-additivity	1	249.48	249.48	0.67ns
Residual	32	10852.47	374.22	
Total	47	15956.98		

CV = 14.85%

Appendix 13c. Anova Table for vascular colonization of Molo plants use in screening for resistance

Source	df	SS	MS	F-Value
Clone	11	12943.41	1294.34	3.91**
Replications	3	775.09	258.36	0.78ns
Error	33	9938.41	331.28	
Non-additivity	1	14.99	14.99	0.04ns
Residual	32	9923.42	342.19	
Total	47	23656.91		

CV = 15.48%

Appendix 13d. Anova Table for flower yield ratio of Molo plants used in screening for resistance

Source	df	SS	MS	F-Value
Blocks	3	0.3177	0.1059	7.711***
Main effects				
Picking date	7	6.1949	0.8850	64.431***
Clone/variety	11	0.9911	0.0991	7.216***
Interaction				
Date x Clone	77	0.9681	0.0138	1.007ns
Error	285	3.5849	0.0137	
Total	383	12.0568		

CV = 12.64%

Appendix 14. Correlation analysis for days to wilt symptoms and vascular colonization lengths for plants screened for wilt resistance at Mwongoris

Clone or variety	Reps	Correlation factor (r)	Slope (b)	Y-intercept (mm)
All	All	-0.3439	-0.1134	138.258
443	All	-0.1682	-0.0683	130.204
P_4	All	-0.6988	-0.1547	149.012
64	All	0.4108	0.3412	108.446
223	All	-0.3471	-0.4398	149.011
4331	All	-0.2690	-0.1671	153.946
K218	All	-0.0526	-0.0159	114.762
1013	All	-0.9932	-0.4504	163.236
K235	All	0.6112	0.7168	110.251
313	All	-0.6862	-0.1823	145.107
487	All	-0.0308	-0.0045	118.420
122	All	-0.2751	-0.1991	150.278
All	1	-0.6469	-0.1973	147.629
All	2	-0.0990	-0.0377	133.550
All	3	-0.3661	-0.1578	141.925
All	4	-0.1465	-0.0348	129.275

Appendix 15. Table of values for the time taken to first wilt symptoms at Molo screening trial

Clone/variety	Days to first wilt symptoms			
	Replications			
	I	II	III	IV
443	**	**	**	**
P_4	82	75	54	68
64	180	242	**	**
223	**	54	**	**
4331	**	**	**	**
K218	54	96	**	**
1013	**	**	**	222
K235	**	**	**	103
313	**	96	**	242
487	54	**	**	180
122	**	**	**	**

** None of the plants had exhibited wilt symptoms by the end of the experimental period (37 weeks).

Appendix 16. ANOVA table for the effect of pH of medium on colony growth of five *Fusarium oxysporum* isolates.

Source	SS	df	MS	F
Main Effects				
Isolate	0.089	4	0.022	2.562*
pH	74.062	4	18.516	2136.408***
Interaction				
Isolate x pH	0.223	16	0.014	1.610ns
Error	0.433	50	0.009	
Total	74.807	74		