STUDIES ON THE BURROWING NEMATODE RADOPHOLUS SIMILIS (COBB, 1893) THORNE 1949 ON PEPPER (PIPER NIGRUM L.) AND ITS ROLE IN SLOW WILT DISEASE

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To

My Mother and Father

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C E R T I F I C A T E

This is to certify that the thesis entitled STUDIES ON THE BURROWING NEMATODE *RADOPHOLUS SIMILIS* (COBB, 1893) THORNE 1949 ON BLACK PEPPER (*PIPER NIGRUM* L.) AND ITS ROLE IN 'SLOW WILT' DISEASE, submitted by Mr. T.S.Venkitesan for the degree of DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY of the University of Agricultural Sciences, Bangalore, is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Dated: 7th October, 1976

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INTRODUCTION

I. INTRODUCTION

Black pepper, Piper nigrum L. is one of the most ancient and historic crops in India. The forest ghats of Kerala and the Kanara region of Karnataka are the original home of pepper (Abraham, 1959). The cured berries form the black pepper of commerce. The production of pepper as per official estimates in 1974-75 was 28,150 tonnes from an area of 121,820 hectares which is nearly 25 per cent of the total world production. During this period 26,341 tonnes of pepper were exported from India earning a foreign exchange worth 344.8 million rupees (George, 1976). Kerala state covers over 90 per cent of India's pepper area and production. The rest is contributed by Karnataka and Tamil Nadu.

Black pepper is known to be affected by two types of "wilt diseases", one is called as "quick wilt" and the other as "slow wilt". These diseases are prevalent in the pepper growing tracts in our country. Occurrence of "slow wilt" was first reported by Menon (1949) from South Wynad (Kerala). Recent studies indicated that "quick wilt" was caused by Phytophthora sp. a fungal pathogen (Nambiar and Sarma, 1974). But the etiology involved in "slow wilt" has remained unexplored hitherto. The disease is attributed to various causes. Recent reports on the presence of the burrowing nematode, Radopholus similis (Cobb, 1893), Thorne 1949 in South India and the association of this nematode with banana

(Nair, et al., 1966) and other plantation crops like pepper and arecanut (D'Souza, et al., 1970) and coconut (Koshy, 1975) has caused great concern with regard to its role in certain diseases affecting these crops. Eventhough 23 species of Radopholus have been reported (Sher, 1968; Colbran, 1970) only, R.similis has been recognized as a major nematode pathogen causing economic damage to several crops.

In recent years, the "slow wilt" of black pepper has been observed to be affecting large numbers of vines in the major pepper growing regions. The typical symptoms of the disease are, partial to complete yellowing of leaves, leaf drop and withering of vines (Figs. 1, 2, 3 and 4). The affected area covers about 10 per cent of the total pepper growing area. The same nematode was also reported to be responsible for the "yellows disease" of pepper in the Island of Bankpo during 1950's which resulted in the decline of pepper industry there (van der Vecht, 1950). Only a few investigations have been carried out relating to this nematode particularly, on pepper in our country.

Therefore to gather some preliminary information on the role of this nematode in the "slow wilt" disease incidence, the following investigations were carried out.

1. Survey of some important black pepper growing areas suspected to be affected by "slow wilt" in Kerala and

Fig. 1. Healthy pepper vines

Fig. 2. Diseased pepper vines on areca palms with partial yellowing

Fig. 3. Diseased pepper vines showing all leaves turned yellow, with sparse foliage

Fig. 4. Diseased pepper vines in final stage of the disease, showing all leaves dropped

Karnataka, to obtain information on the occurrence and distribution of R.similis in these areas.

2. The pathogenicity of R.similis to black pepper, including the nature of damage to host.

3. To establish by experimental evidence, the biotype status of the pepper isolate of R.similis and the cross infective behaviour of the populations of this nematode isolated from pepper, banana, coconut and arecanut, among them.

4. Screening of some common cultivars of black pepper from Kerala and Karnataka including wild Piper spp. against R.similis.

5. Conducting of pot culture studies to test the efficacy of three nematicides namely, aldicarb sulfone, fenbulfothion, D-D-C-T and neem cake, in controlling R.similis on black pepper and their effect on growth and vigour of the pepper vines.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

R.similis (Cobb, 1893) Thorne, 1949 popularly known as the burrowing nematode has now become a widespread nematode pathogen in tropical and subtropical parts of the world. During Cobb's investigations on the diseased banana root materials, received from Fiji in 1891 he observed male specimens of a parasitic nematode, which he described in 1893 as Tylenchus similis. In the same year he described the female specimens as Tylenchus granulosus obtained from banana roots. Again while studying roots of diseased sugarcane in Hawaiian Islands he came across both males and females of a species of nematode, which in 1909, he described as Tylenchus biformis. Subsequent investigations carried out by Cobb, with diseased banana rhizomes, convinced him, that the above three new species described by him were actually one and the same and therefore he retained Tylenchus similis as the type species in the detailed redescription published in 1915. Though it was included under various generic names, by several subsequent workers, the present generic name was proposed by Thorne (1949) and considered till now as a valid name. Sher (1968) regards T.granulosus, as an earlier synonym of R.similis and confirmed as the type species of the genus.

Distribution of the Pathogen and the Disease Incidence

R.similis is found now associated with several important crops and ornamental plants. Sher (1968) considered the genus

Radopholus to be indigenous to Australia. Zimmermann (1898) found it associated with coffee in Java. It was established as a potential parasite of tea in Java by Steiner and Buhrer (1933). Van der Vecht (1950) reported this nematode from Banda Islands, Indonesia causing "Pepper yellows". In Florida, the "Spreading decline of citrus" was known since 1928, but not linked with this nematode until, 1953 (Suit and DuCharme, 1953). Though Cobb (1915) reported this nematode as the cause of the root disease of sugarcane and banana, only recently, it was recognised as the major banana root pathogen, replacing the fungus Fusarium (Stover, 1972). In banana, the disease caused by R.similis is known throughout the world as Radopholus root rot, black-head, black-head toppling disease and decline. It is reported as associated with banana from Pacific Islands (Cobb, 1893), South East Asia (Carter and Allen, 1953), Central and South America (Stover and Fielding, 1958), the Caribbean Islands (Leach, 1958), Ivory coast (Luc and Vilardebo, 1961) and Australia (Lamb, 1961). It is also reported to be present in France (Scotto La Massese, 1967), Surinam (Anon, 1968b), Ceylon associated with tea (Sivapalan, 1968), in Low veld of Rhodesia (Anon, 1969a), Mosambique (Cvaristo, 1969), Germany (Sturhan, 1970), South Africa (Kuchane and Milne, 1969), Ghana (Addoh, 1971), West Indies (Anon, 1971b), Malawi (Anon, 1972) and in Taiwan (Huang, 1972). It is present in Tansania and Kenya (Ngunda and Taylor, 1973) and Zambia (Raemaekers and

Patel, 1973). Tarjan (1971) reported its occurrence on coffee in Western hemisphere for the first time. It is associated with betle vine in Kenya (Anon, 1974b).

The occurrence of this nematode in South India has been recorded as early as 1933 by T.Goodey. Nair et al. (1966) reported it on banana for the first time, from Kerala. Recent surveys indicated its association with banana, black pepper, betle vine, cardamom (D'Souza, et al., 1970) and arecanut (Kumar et al., 1971). Though Van Weerdt et al. (1960) established coconut as a host of R.similis in Florida, Weischer's (1967) studies revealed its occurrence for the first time in the "coconut wilt" affected palms in Southern Kerala. Venkitesan (1972) reported its association with black pepper in Northern Kerala. The extent to which this nematode is distributed in different states in South India has not been investigated so far.

Economic Importance

Crops affected by R.similis result in unprofitable returns, by reducing vigour of host crops and subsequent yield. Yield increases of 30-60 per cent were obtained by adopting control measures in banana (Blake, 1972). Wehunt and Edwards (1968) reported that uninfested banana plots yield upto 17,000 lbs per acre per year more fruits, than

from infested plots. In Surinam, Mass (1969) reported that in 100 per cent infested plots banana yield was 37 tonnes compared with 73 tonnes per hectare per year in less than 30 per cent uninfested fields. In Florida, the spreading decline of citrus disease causes a yield reduction of 50-80 per cent in grape fruits and 40-70 per cent in oranges, in infested groves (Poucher et al., 1967). Healthy groves produced 535 boxes per acre compared to 25 boxes in infested groves. In the Bangka Island, Indonesia where over 22 million "pepper trees" (vines) were flourishing well, at one time, the "yellows" disease caused by X.similis reduced the vines standing to a fewer than 2 million vines in the course of two decades, i.e. between 1930 and 1950. When a garden produced for several decades, a planting now hardly survives for 3 to 5 years (Thorne, 1961). In a survey on coconut in Kerala, X.similis was recovered from roots of palms affected by root-wilt disease (Nayar, 1974a).

Host Range

X.similis is a highly polyphagous nematode and has a wide range of hosts (Poucher et al., 1967) including, mainly banana, citrus, black pepper, coffee, tea, coconut, avocado sugarcane, maize, several vegetables, grasses, tree crops and cereals. They reported mango, lantana, marigold, crotalaria, litchi, turnip, lettuce, wild lime, Narcissus sp. as some important non-hosts. New hosts reported were potato, peanut,

rice,and wheat (Martin et al., 1969), Kallstroemia maxima (DuCharme, 1972), Momordica charantia (O' Bannon, 1973), sorghum, sword bean, lima bean, sesbania, rabiza bean and Tephrosia vogelii and 4 weed species (Edwards and Wehhunt, 1971). In India Piper betle, Elattaria cardamomum (D'Souza et al., 1970) and arecanut (Kumar et al., 1971) are reported as new hosts of this nematode. Even though large number of plant species have been reported as hosts of R.similis, this nematode has been observed to have host preferences, among different populations in different regions or tracts. Moreover no exhaustive studies have been conducted to determine the host range.

Symptomatology

In black pepper due to the infection by the burrowing nematode, symptoms appear at one or more spots in a pepper garden where one or few vines are affected and gradually spreads until large areas are involved. The vines stop growing, the foliage turns yellow and there is die-back and leaf drop (Christie, 1957). According to Van der Vecht (1950) the disease usually starts in isolated spots. Due to the active dispersal of nematodes in soil these spots extend gradually, so that large bald areas develop, surrounded by plants in various stages of deterioration and yellowing. Such areas are with apparently healthy plants, roots of which, however

on examination show already light infestation. Early growth stagnation in a few plants points to, already existing infestation of the soil. Usually, the typical yellowing symptoms followed by leaf drop show up not earlier than during first ripening of fruits. Older plants may also suffer heavily from the nematode infection and perish within a short period. Apparently this is due to relatively slight lignification of main roots, which may be killed completely by nematodes penetrating into wide medullar rays. Van der Vecht observed that in this case also, nematodes penetrate into "hair" roots. They bore tunnels into parenchyma tissues, mainly longitudinal ones. The pierced cells die and soon tunnels are visible as dark spots, starting from thin roots. They often penetrate into parenchymatous cortical tissues and also into medullar rays of thicker roots, which in case of heavy infestation are cut off completely. Thus roots show numerous lesions and pronounced discoloration. The main roots are devoid of small feeder roots and extensive necrosis of the larger laterals gradually develops. Growth ceases, soon after the yellowing of leaves, becomes apparent and production of pepper rapidly declines. Severe die-back and death of the vines eventually follow. The first indication of yellows disease of pepper is the appearance of occasional yellowed leaves on vines. This increases in numbers until within a year, large portion or even all of the foliage may become involved. Nambiar (1976)

reported that slow wilt disease makes its appearance after south west monsoon. The symptoms are characterised by initial yellowing of lower leaves, which gradually progress upwards resulting in complete yellowing of the foliage and simultaneous defoliation. The affected vines die gradually after the appearance of external symptoms. The affected stem and roots show vascular browning though not consistently. Ting (1975) reported, Meloidogyne spp. as the most important group of nematodes in Malaysia causing gradual decline of black pepper characterised by unthrifty growth and yellowing of leaves.

Biology

The burrowing nematode spends virtually its entire life within host root-lets. They attack only healthy young succulent feeder root-let tips. Loos (1962) studied the life history and habits of the nematode attacking banana. The females laid on an average 3.5 to 4.6 eggs per day and continued to lay for two weeks. The eggs hatched within 5 to 8 days. Larval period was 10 to 15 days, but matured to adults in 11 days. Adults laid eggs in two days after the last moult. All larval stages and females were infective but males were unable to infect roots. The life cycle took 20-25 days to complete at 75-90°F temperature. According to McCharme and Price (1966), in citrus the life cycle was completed in 18-20 days at 75.2 to 80.6°F. The number of eggs laid ranged from one to six eggs

per day. Under controlled conditions in axenic root culture they found the largest population in a colony started from one female reached to 47,000 numbers in 85 days. In some colonies, second generation females laid viable eggs and produced active colonies of males and females although no males have been introduced into the colony. Van der Vecht (1950) studied the influence of R.similis infestation on 2 month old pepper seedlings in pots with sterile soil inoculated with 20 females. After six months, he could obtain about 2,500 nematodes from the roots and soil of inoculated pot. He observed that eggs are laid singly in the roots, after a few days the larvae hatch, which develop into adults in four to five weeks. Males are found in the root tissues only at spots where at least one generation has developed. The nature of reproduction, embryology and post-embryonic development of R.similis have been studied in detail by Van Veerdt (1958, 1960). Brooks and Perry (1963) apparent Parthenogenetic reproduction upto third generation.

Invasion, Root Injury and Histopathology

Blake (1966) studying the invasion of R.similis on Musa ornata seedlings observed that fourth stage larvae or adult females had invaded roots, 12 hours after inoculation, within 1 cm of the root tip, although few invaded along the whole length. Longitudinal and transverse sections of infection courts showed that both adults and larvae took up a

feeding position between parenchyma cells in the cortex, one to four cells beneath epidermis. The presence of these nematodes caused contiguous cells to separate. From an intercellular position they inserted their stylet through primary cell wall, depressed cytoplasm and caused it to invaginate around their stylet tip. Within 12 hours of their entry, size of nucleus and nucleolus in a few cells immediately surrounding them had increased significantly. By about 24 hours the cytoplasm had begun to retract from cell wall and after 36 hours, usually occupied less than one third volume of the cell. These observations suggest that they feed directly on cell cytoplasm. Similar changes occurred in two or three cells around the head of each nematode, suggesting that they feed in turn on these cells. The amount of cytoplasm in cells on which they fed continued to be reduced until only nucleus and a small amount of cytoplasm remained. Then nucleus disintegrated, primary cell wall ruptured, a cavity formed and the nematodes usually moved into this space. Several nematodes were associated with each infection court and these cavities formed by individual nematodes later coalesced. The nematodes enlarged these cavities by feeding on peripheral cells and tunneling laterally in cortex and towards endodermis. Hyperplasia and hypertrophy were rare and necrosis was usually confined to cells lining cavities, tunnels and to epidermal cells injured during invasion. The

surface of root did not show evidence of invasion during the first week, except a small puncture on epidermis, which could be detected with difficulty. After 10 to 14 days, incipient lesions could be detected by necrosis of cells around infection court. In 21 to 28 days extensive cavities formed in the cortex and deep longitudinal cracks with raised margins were evident on root surface overlying the lesion. Invasion of stele was not observed, even where the innermost cells of cortex were destroyed. In banana roots, the endodermis is strongly developed which probably prevents R.similis from entering the stele through the cortex.

In citrus DuCharme (1959) observed that females and larvae enter growing feeder roots near tips in the region of cell elongation and root hair production. In penetration they feed on cortical parenchyma cells and gradually burrow towards stele, creating tunnels and cavities in the tissue. They accumulate in phloem and cambium ring region. This part of root is often completely destroyed, leaving a cavity filled with them. Hypertrophy, hyperplasia of pericycle cells, tumour formation and accumulation of wound gum result in parasitised tissues. Infested roots bear numerous lesions. Starch grains disappear from cells in and adjoining lesion area. Gum accumulated tissues impart a tan-to-amber colour in older portion of lesions. Pericycle tumours are also attacked by them. Suit and DuCharme reported (Cited by Poucher et al., 1967)

that the population in individual lesions varied from 1-739. Cassidy (1930) obtained 2532 specimens of R.similis from one linear inch of sugarcane root in Hawaii. The female nematode can usually enter a root in citrus in less than 24 hours. The optimum temperature for root invasion and reproduction is 24°C, the minimum 12°C - 15°C and the maximum 29.5 - 32.5°C (DuCharme, 1969).

In pepper roots they bore tunnels into parenchyma tissue. The pierced cells die and soon tunnels are visible as dark spots. They also penetrate into medullary rays of thicker roots leading to blocking of the vessels. The histological changes brought out by their penetration and feeding in pepper roots have not been studied and clearly understood. Information is also lacking in respect of injuries caused by the nematode to other host crops like coffee, tea, coconut and arecanut.

Biotypes of the Pathogen

The first report on the possibility of biological races in R.similis was made by Bally and Keydon (1931) when they were unable to infect Gigantochloa apus with specimens obtained from Coffea robusta. Later DuCharme and Birchfield (1956) identified two physiological races of R.similis, one, the "citrus race" which parasitizes both citrus and banana, recorded only in Florida and second the "banana race" which parasitizes banana but not citrus. They got the first

evidence indicating the existence of physiological races, from the observations that roots of banana (Musa nana) adjacent to a citrus grove were heavily parasitized but not the citrus roots. They reported the possibility of existence of a third race in the field infecting citrus only. They also studied 100 adult female specimens of both the races and measurements taken, but could not fix up any differences between them by their morphological characters. They did not study characters of larvae or adult males. Loos and Loos (1960) studied the black head disease of banana (Musa accuminata) in Jamaica and have given some evidence of the existence of races of R.similis from different locations. Van Weerdt (1957) reported that the populations of R.similis obtained from Buxus microphylla variety Japonica, Calathea lietzei, Hedychium coronarium, Musa nana, Scindapsus aureus and Citrus sinensis on C.limon root stock were used for various cross inoculation studies. Inoculations on to the hosts from which specimens were originally extracted, were successful. From citrus they readily infected Lycopersicon esculentum variety Rutgers and Zea maya variety Bantam, but reinoculation to C.limon from these hosts were not successful. Specimens from Musa nana and Zea maya readily infested both hosts. He stated that citrus should be called a poor host for R.similis. He could not also find any morphological variations between the different populations. Sher (1968) also studied

in detail, the morphology of specimens of R.similis from several hosts and from different locations and was not able to find marked variations in their measurements and characters to differentiate them as to two or more separate species. Ayala and Roman (1963) observed in Puerto Rico that R.similis widely distributed in banana and plantain soils. They further reported that the coffee was infected by R.similis, but the plantains and citrus roots intermingled with R.similis infected coffee roots were free from the parasite, indicating that a non-banana strain is present in Puerto Rico. R.similis was not found infecting sugarcane and citrus in the areas they surveyed. Laboratory studies conducted by them did not result in cross infections. Thus they postulated that within banana race there appeared to be several strains as indicated by host responses. Martin et al. (1969) observed in Rhodesia that R.similis infected groundnut, maize, tobacco, potato, rice, wheat, cotton, soyabean, sugarcane and tea, but not lemon. They planted seedlings of these crops close to the pseudostems of banana variety, dwarf cavendish, roots of which were heavily infested with R.similis. Except lemon seedlings the nematode was recovered from roots of all crops grown in the trial. They considered the race of R.similis present there to be a most serious parasite of groundnuts, as most of the underground parts were heavily invaded. Edwards and Wehunt (1971) tested thirty six crops and sixty four weed species against two populations (Panama and Honduras) of R.similis infecting

banana areas, in central America. They found that both populations, infected in common only Rabiza bean, Tephrosia candida, Sorghum bicolor, Calapogonium muscunoides and had differential infectivity to all other plant species tested by them, showing the different infective behaviour of the two populations. In Natal (South Africa) Meetch (1972) reported his findings on the reaction of R.similis on over 40 commercially grown plant species. According to him the race of R.similis attacking dwarf cavendish banana in Natal caused moderate root injury on aubergine, coffee, tomato and potato cultivars. It caused severe to very severe injury on peanut, soyabean, plantain banana, sorghum, maize and sugarcane varieties. Tea, tobacco, lemon and several vegetables were resistant on which the nematode did not markedly increase and only slight root damage occurred. Eragrostis curvula variety ermelo, radish, shallot, Phaseolus atropurpureus, and spinach appeared to be immune to attack. In Mozambique (Fein, 1974) analysis of over 1000 samples showed that the banana population was associated with peanuts, cotton, maize and tomato crops. D'Souza et al. (1970) reported the occurrence of R.similis on Musa spp., Piper nigrum, Piper betle and Elletaria cardamom in the coffee tracts of south western India. They could not find coffee roots infested with R.similis. In addition, Kumar et al. (1971) recorded it on Areca catechu from coffee tracts. In Kerala R.similis has been observed to infect coconut and banana. Studies con-

ducted at Kayamkulam revealed that the population of R.similis from coconut, attacks roots of areca, banana, sweet potato, sugarcane, groundnut, but not Citrus spp. It has been identified as "the banana race" (Koshy, 1975). The host range of R.similis populations infecting other crops in India has not been studied.

Extraction of R.similis from Roots

The burrowing nematode being a migratory endoparasite, special techniques were followed for extracting them from roots. A method of incubating moist roots by placing them in closed jars containing a small amount of water was the popular technique followed in earlier years (Young, 1954). Du Charme (1954) followed this method for extracting the burrowing nematode in his preliminary studies conducted for determining the cause and nature of spreading decline of citrus. Yeast (1957) reported that incubating citrus roots in water instead in a moist chamber gave quicker and higher recovery rates for the burrowing nematode. Roots were washed free of soil and cut into length of 5 - 10 cm. Diameter of fleshy roots can be split longitudinally and kept in containers with a small quantity of water at 20 - 25°C to enable nematodes emerge. After 24 hours of incubation the water was collected and retained for examination. Fine sand extraction continued by adding fresh water and reclosing containers. Most of the nematodes were recovered within 4 - 7 days. Tarjan (1960a) found that

polythene bags, which are permeable to oxygen, kept at 75°F gave better extraction than glass jars. Tarjan (1967) obtained enhanced recovery of R.similis from roots of orange trees and rough lemon root stock at temperature between 69 - 71°F with addition of 1 - 3 per cent hydrogen peroxide. He stated that this technique enhanced the chances of detecting R.similis in roots that contain only few numbers. Gowen (1973) reported that higher recovery of R.similis from banana roots, when they are cut into 1 - 3 cm bits, macerated for 10 seconds and incubating at 20.5 to 23.5°C in pie-pans in a solution containing 1 per cent hydrogen peroxide to which is added commercial detergent at the rate of 1 ml per litre.

Control

Chemical methods

Work carried out for the control of R.similis has proved infinitely more difficult, due to its true endoparasitic habit, its ability to survive on various host plant species and its concentration in deeper soil layers. In case of spreading decline disease in Florida, due to limited occurrence, made it possible to devise more uniform control measures applicable to local conditions (Cohn, 1972). Whereas its world wide distribution with banana has made it a difficult problem to completely eradicate the nematode, only resulting in its spread to new frontiers. Several nematicides have been tried against

R.similis to obtain satisfactory control. E D B, D D and D B C P were tried initially for control of R.similis in citrus groves. Plots treated with D B C P were kept free of R.similis for 9 months only (Suit and Feldman, 1961). D B C P applied along with sprinkler diminished infection of R.similis in citrus, but trees still suffered from spreading decline. When applied as technical material it was less nematotoxic than emulsifiable formulation (Feldman, et al., 1963). D B C P when applied through overhead irrigation, R.similis was absent in roots till 14 months only, after treatment, but returned to the level of untreated trees after 32 months (Suit and Feldman, 1964). Collins and Feldman (1966) reported that D B C P applied at the rate of 100 U.S. gallons per acre did not give control of nematodes below 6 feet in soil. But D B C P was successful as a preplant treatment at the rate of 10 U.S. gallons per acre and infection was controlled over four years (Suit, 1961). Use of systemic nematicides in the groups of organophosphates and carbamates are being tried recently. These chemicals are tried as bare root dips for nursery seedlings, soil drench, sub-surface drench, injections and granular forms. Some methods have given promising results. The reports of various authors have been summarised in Table I. Use of various nematicides for control of R.similis on banana have been tried. But in most cases the soil fumigation poses problems due to edaphic conditions and terrain under which banana

TABLE I

Control of Radopholus similis on citrus

Chemical tried	Dosage	Duration and type of treatment	Effect on host crop/ plants	Reference
Thionazin, Fensulfothion Prophos	1000 ppm	30–60 minutes bare root dips	Eradicated R.similis in roots, no phytotoxic effect.	O'Bannon and Taylor, (1967)
Aldicarb and Oxamyl	0.6 g/m^2	Monthly application around calathea plant	Complete eradication of R.similis in glass house.	Heungens, (1971)
Ethyl 4 (Methylthio) m-tolyl-isopropyl phosphoramidate	250–600 ppm	30 minutes dips	No phytotoxic effect, eradicated R.similis in roots.	O'Bannon and Taylor, (1967)
Phenamiphos Fensulfothion Thionazin Prophos		In solutions as leaf or root dips	No toxicity, number of R.similis effectively, reduced on seedlings. Nemacur foliardip was most effective, acted as repellent	O'Bannon and Tomerlin, (1971)
Hot water treatment	122°F(50°C)	10 minutes nursery stock dipping or rough lemon seedlings. Roots to note 5 hours dip.	Kills R.similis in roots.	Birchfield, (1954), Spears,(1955)
Organic mercurial (Aaventa)	1%	Dipping of roots of ornamentals for one hour	Found to kill nematodes in roots.	Spears (1955)

TABLE 1 -(Contd.)

Chemical tried	Dosage	Duration and type of treatment	Effect on host crop/ plants	Reference
Thionazin	8, 16 & 32 lbs per acre a.i. 50 lbs/ acre 16 lbs/acre	3 applications to citrus seedlings in green house, in soil columns 3 field applications	Controlled R.similis from infection - killed L.similis upto 9' depth controlled for 3 months, increased twig growth.	Suit and Feldman, (1961)
D C P(Dichloropropene)	45, 60 & 75 lbs g/acre	Preplanting soil treatment in groves	Controlled infection for 4 years	Suit, (1961
D B C P(Technical)	10 lbs g/acre		-do-	
E D B (-do-)	93 - 15 lbs g/acre			
D B C P	4 lbs g/acre	Triple treatment in field for sprinkler application or injection or granules	Better control of infection	Suit,et al. (1961)
Thionazin	32 lbs/acre in 50 lbs g/water 3 treatments	Soil drench around trees + Post-treatment water drench	Better increased feeder roots in infected trees.	Collins and Feldman, (1965)
Fensulfothion, Thionazin, prophos	100, 200, 400 p m	Sub-surface drench mixed with water in nursery containers.	L.similis was eradicated in seedlings (roughlemon)	Taylor and O'Bannon, (1968)
Ethion	750-1000 lbs/acre	Soil drenches on citrus seedlings, infected by L.similis	Treated plants produced taller heavier aerial parts; heavier root system.	Tarjan and Wouts,(1965)

plantations are raised in most parts of the world (Blake, 1961). But still nematicides in other forms namely, systemic ones in granules and those which can be applied along with irrigation water are becoming popular. Use of nematode free planting materials, by disinfestation has been practiced in a large way. Bougnon and Vilardebo (1973) observed initial delay of growth due to toxicity of young root system when D B C P is used. Guerout (1970) observed Nemacur P was found most effective than Teracur P, mocap and D B C P, in a field experiment in ivory coast for control of R. similis in banana. The recommendations for chemical control of R.similis in banana, based on the results obtained by various workers are presented in Table II. In case of citrus, for control of R.similis, Henninger et al. (1958) used hydrogenated fish oil and studied its effectiveness. When infected seedlings of various ages were either sprayed or drenched with 1 or 1.5 per cent solutions at a dosage of 100-150 ml for each plant, 2-3 times at 2 week intervals, it was observed that no phytotoxic effect occurred on the plants and there was significant reduction of nemas in infected roots and no nematodes were found in fresh roots.

Non-chemical methods

Among the non-chemical methods used for control of R.similis, use of hot water treatment of root-stocks of citrus

TABLE II

Control of Radopholus similis on Banana

Chemical tried	Dosage	Duration and type of treatment	Effect on host crop/ plants	Reference
D B C P E C	0.5 ml a.i./plant		Decreased infection	Peachy & Hooper (1963)
D B C P (70%)	Solution based on suspension	Root dips	Control of R.similis	Beccari and Soavasson,(1966
D B C P	1.0% solution	5 minutes dip	Elimination of infection	Casamayor,et al (1966)
Phorate Phenamiphos (40%)	8 - 11 ml/plant 50 ppm (v/v)	15 - 30 minutes dip	Plants completely free from infestation	Taboado and Caballero, (196
D B C P Pranilage	550 ml + 40 l clay soil + 50 l water	Dip in the solution for few seconds	No attack or spread or multiplication till 14 months	Vilardebo and Robin, (1969).
D B C P	0.5% emulsion	Five minutes dip (soil kept moist 24 hours)	Total elimination of infection	Decker, et al. (1971)
Phenamiphos	100 ppm (40% emulsion) a.i.	5 minutes dip	Total elimination	Decker, et al. (1971)
Phenamiphos, Aldicarb methomyl, D B C P		3 applications at 6 monthly intervals.	Give better results and control, decreased toppling	Coates, (1972)
D B C P	39.9 l/ha (75% E C)	Once in 12 months applied to 12 weeks old "orse" plantation	More growth and heavy bunches, complete elimination of infection.	Hutton and Chung (1973).
D B C P	3 ml/plant	Twice a year in 8 injections around the plant	No infestation, 40% increase in crop growth and weight of banana	Beugnon and Vilardebo,(1973)

and pared banana sets are very popular. The thermal death point of R.similis (from citrus) in vitro was determined to be 10 minutes exposure at 122°F (Birchfield, 1954). This was found to be the same for R.similis from banana (Blake, 1961). Various practices of heat therapy adopted by different workers have been summarised for citrus in Table I and for banana in Table III. For citrus nursery stock, hot water treatment is given before planting in new areas, such seedlings used for heat therapy must have only stem and root thickness less than 1½ inches. The roots are cooled in cold water for 10 minutes immediately after heat-therapy and they are cared for in the planted sites by frequent watering (Cohn, 1972). Heat-therapy for banana is successful only where cheap labour is available, as paring of sets becomes a laborious process. Special hot water tanks to maintain the requisite temperature have been designed (Blake, 1963).

A special control programme "push and treat" method, applicable for Florida has been programmed for benefit of citrus growers (Pencheon et al., 1967). Flooding and fallowing for 5-6 months eliminated L.similis from banana fields in Honduras and Panama (Loos, 1961). Flood-fallowing is practiced in Surinam for control of the burrowing nematode, but bare fallowing was unsuitable (Maas, 1969). Colbran (1964) recommended a two year fallow period with a cover crop of Panicum maximum variety trichoglume in Queensland, Australia. In

TABLE III

Hot water treatment of "banana setts" for control of R.similis

Temperature range	Duration of treatment	Type of treatment	Effect on host	Reference
55°C	5 minutes	Immersion	Treated setts free from infection	Pereira et al., (1960).
55°C	30 minutes	Immersion	Minimum loss of crop. Plants were free from R.similis for over 5 years	Blake, (1961)
55°C	25 minutes	Immersion	Effective control of infection	Blake, (1963)
55°C	20 minutes	Immersion of setts (with 13 cm dia-meter)	Control spread and damage	Casamayor, et al. (1966)
55°C & 50°C	15 minutes 10 minutes	Immersion + dip in organo mercurial solution	Control of infection, elimination of crop loss	Senanayake, (1969)
52 - 53°C	20 minutes	Immersion	General control from infection	Taylor, (1969)
55°C	20 minutes		R.similis was totally eliminated	Decker, et al.(1971)

New South Wales R.similis was not detected in soils six months after infested stools were mechanically uprooted (Blake, 1969). Rotation of 5 months with sugarcane, immediately removing bananas from fields has met with some success (Loos, 1961). Attempts at biological control, either by the use of nematode trapping fungi (Tarjan, 1962) or by growing Tagetes erecta as a cover crop have not met with any success under field conditions (Tarjan, 1960b).

MATERIALS AND METHODS

III. MATERIALS AND METHODS

Survey

Collection of soil and root samples

A survey was undertaken to know the occurrence of the burrowing nematode R.similis associated with the slow wilt disease suspected pepper vine gardens in Kerala and Karnataka States. A proforma with relevant details on field symptoms, source of soil and root samples, was developed (Appendix I). An information sheet was also prepared, detailing the procedure to be followed, in collection, package and despatch of the soil and root samples (Appendix II). These proformae and information sheets were supplied to Junior Agricultural Officers at various NES blocks in the two districts of Cannanore and Kozhikode in Kerala State and to the Taluk Horticultural Officers of the various districts in Karnataka State, to collect and send the soil and root samples from the slow wilt suspected pepper gardens for laboratory processing and examination.

Processing of soil and root samples

Immediately after the receipt of the soil samples, 100 ml of soil from each lot representing diseased and healthy areas were processed by Cobb's sieving and sifting technique, using a 400 mesh sieve at the final sieving. The fine debris in the nematode suspension was cleared through a modified Baermann's funnel technique. From the root samples received, the feeder

roots were sorted out, weighed and nematodes were extracted by chopping them into bits of 0.5–1 cm length and placing them over a modified Baermann's funnel.

Estimation of nematode population in soil and root samples

After forty eight hours of setting up of the Baermann's funnel, the suspension containing the nematodes was carefully transferred to clean 100 ml glass beakers, allowed to settle for about three hours and concentrated to five ml. The total population of *P.similis* and other genera of plant parasitic forms were counted and estimated. The nematodes in suspension are then killed and fixed by adding equal quantity of boiling 5 per cent formalin and preserved for further studies. Nematodes emerged from the root samples were collected every 24 hours and counted. The daily counts of nematodes emerging from root-samples were continued for 10–12 days till no nematodes were observed to emerge from the roots consecutively for three days. The total population of nematodes was computed to per gram root weight of the samples. The nematode suspensions, from roots were also preserved after killing and fixing. The collection and processing of samples from Kerala were carried out during the period from September 1974 to March 1975 and July 1975 to September 1975, and from January 1975 to March 1975 and September to October 1975 from Karnataka.

Establishment of Pure Culture of R.similis

Root and soil samples infested with R.similis from pepper vines collected from the Pepper Research Station, Panniyur formed the original nucleus culture material. Few specimens of adult females and males were measured and confirmed that the nematode in question is R.similis (Cobb, 1893), Thorne, 1949. Further multiplication of the nematode was done and maintained either on pepper seedlings raised from seeds or rooted cuttings of variety Kalluvally. About 50 culture pots of 15 cm size were maintained for the experimental work. They were periodically examined for population build up and kept isolated from other pots in the glass-house to maintain purity. Same population was also maintained on local banana suckers. Likewise R.similis populations from banana and coconut collected from Central Plantation Crops Research Institute, Kayamkulam and population from arecanut collected from Main Research Station, Hebbal, were maintained separately on banana suckers.

Sterilization and Preparation of Pot soil

Red loam soil from the fields of Main Research Station, Hebbal, free from clumps and stones was obtained and mixed with compost in the proportion of 6:1. This soil-compost-mixture was sterilized (denematized) by methyl bromide fumigation at the rate of 1 lb per 100 cft and stored in clean concrete tanks, to avoid nematode contamination and for subsequent use in

experimental work. Whenever this soil mixture was used for potting, it was periodically examined and confirmed that no other nematodes had contaminated the soil mixture.

Preparation of Nutrient solution

Nutrient solution as prescribed by Arnon and Hoagland (1940) for providing minor and trace elements to pot culture plants was prepared and stored in polythene carboys, ready for use whenever required.

Raising and maintenance of rooted cuttings of pepper vine

Stem cuttings of matured vines of cultivar - Kalluvally with 5 - 6 nodes obtained from Pepper Research Station, Panniyur and Horticultural Research Station, Ambalavayal, were planted after treating them for one minute with 50 ppm indole butyric acid solution, in wide earthern pots containing sterilized soil. They were transplanted after 50 days into single pots when they struck roots and allowed to grow. Stem cuttings with 2 nodes were taken from these vines and further planted for rooting. Likewise large number of two nodes rooted cuttings were multiplied for pot culture experiments.

Pathogenecity of R.similis on Black Pepper

Experimental

A pot culture experiment was conducted to establish the

pathogenecity of R.similis on black pepper, under glass-house conditions. The treatments included inoculation of the nematodes in logarithmic levels of 10, 100, 1000 and 10000 numbers per pot of 22.5 x 15 cm size, holding 1500 ml soil, including a check pot without the nematodes. Fifty five days old, two nodes rooted pepper cuttings with uniform growth, stem girth, node lengths, 2-3 emerged leaves and with 5-6 lateral roots were selected and transplanted singly into the pots. 4 week after planting, the cuttings were inoculated with nematodes. The exact number of leaves, length of sprouts of each plant were recorded before adding the nematode inoculum. Eight replicates were maintained for each treatment. The pots were kept under the glass house benches to allow partial shade, in randomised way. The temperature in the glass house was maintained at 24 ± 3°C throughout the experiment.

Nematode inoculum

Nematode inoculum was prepared by extracting R.similis from the roots of the pure culture plants. Nematode inoculum obtained within a period of 72 hours and stored under 5 ± 1°C in the low temperature incubator was used for the experiment. The inoculum was added to the pots by making five holes around the stem about three cm away, with seven cm in depth, by pouring the suspension in equal proportions in all the holes. Pots were inoculated with five ml aliquot suspension of 10, 100, 1000

and 10000 nematodes of all stages. The holes were then covered with loose soil and pots watered with 750 ml of water just to moisten the soil in the whole pot. The check pot was also given an equal quantity of plain water.

Maintenance of plants and recording of the observations

The pots were given all agronomic requirements. Nutrient solution was added at the rate of 100 ml to each pot at intervals of four weeks. Two sprays with Dithane Z 78 at 0.2 per cent concentration were given during the period of the experiment to check any fungal attack. Observations on the growth of the shoots, emergence of side branches, and number of leaves produced were recorded at intervals of 30 days from the date of inoculation of nematodes. The final additional growth parameters were calculated subtracting the observations recorded at the time of adding the inoculum.

The experiment was concluded after 150 days of nematode inoculation. The shoot portions (vines) were cut at the ground (soil) level and fresh shoot weight, number of leaves and shoot length were recorded. On the same day the root system from each pot was carefully lifted by gentle tapping of the pot on all sides and bottom and removing the loose soil. The roots were cleaned of adhering soil particles by gentle washing in water.

These washings were collected, passed through 400 mesh sieve and preserved for nematode count. The clean root system was pressed between folds of bloting paper and fresh root weight, length of main laterals were recorded. Nematodes from the root system were extracted following the procedure described earlier. The soil from each pot was thoroughly mixed and a representative sample of 100 ml, was measured out and processed for extracting the nematodes following the procedure mentioned earlier.

The nematode counts from the roots and soil were recorded as per the procedure mentioned earlier. The final soil population in each pot was computed from the count in 100 ml sample x 15. The total final population per pot was thus estimated by addition of the counts from soil, root system and from the washings collected at the time of washing each root system.

Root Invasion and Nature of Injury

To study the invasion of R.similis to pepper roots, a new technique was developed (Fig.5). Rooted pepper cuttings with 4-5 lateral roots were lifted carefully from the nursery pans and the roots were washed free of adhering soil particles. Small earthen pots of 6.5 x 5.0 cm size with holes sideways at bottom were taken. Keeping the stem cutting portion inside the pots, the root ends of feeder roots were brought outside through the holes and the pot filled with acid washed fine sand. These root ends (two cm length), after surface sterilisation with 0.1

Fig. 5. Technique developed for inoculation of *R. similis* under laboratory conditions to study root invasion and development of lesions on pepper feeder roots

per cent $HgCl_2$ were introduced with least injury into plastic cases (usually used as cover glass boxes) 2.5 x 3 cm size, through small holes (enough to pass the roots) drilled on their sides. These cases were filled with acid washed sand, to support the root ends. The sand in the plastic case was moistened with sterile water. The pot was watered with sterile water. About 50 adult well developed female specimens of R.similis were surface sterilized with 0.1 per cent $HgCl_2$ for one minute and introduced near each root tip and the root portions were then covered with just enough sand and moistened with sterile water. The pots along with the cases were placed in petriplates and left on the laboratory bench, where the temperature during the studies was 27 $\pm$ 2°C. Observations were recorded at interval of every 24 hours by cutting the root bits and examining them for invasion of the nematodes by staining with acid fuchsin lactophenol. The roots were observed till the formation of lesions on them.

Root bits from the boxes were lifted and were thoroughly washed free of soil adhering to them. Healthy and infected portions of roots (0.5 - 1 cm) were separated and free hand cut sections were taken. Observations on the nature of root injury by the nematodes were recorded.

Control of R.similis on black pepper

An experiment was carried out to find out the effects of neemcake and three nematicides namely, D B C P, fensulfothion and aldicarb sulfone on R.similis infecting pepper.

Host plant material

Pepper cultivar - Kalluvally was used for these studies. Two nodes stem cuttings were used from the pure stock maintained, as described earlier. The rooted cuttings were planted in 20 x 15 cm size earthen pots filled with denematized soil-compost mixture of 1500 ml capacity. The cuttings were planted and one week period was allowed for their establishment.

Nematicides

The chemical nomenclature of the nematicides, method of application and source of supply are given in Table IV. All the dosages of nematicides are expressed in terms of active ingredient per hectare. Only common names of the chemicals have been used in the text. The dosage per pot was worked out based on the surface area of the pot.

Dosages and methods of application

Various dosages used were, neemcake 1250, 2500 and 5000 kg per hectare, D B C P 22, 33 and 44 litre a.i. per hectare fensulfothion 2, 4 and 8 kg a.i. per hectare and aldicarb sulfone 2, 4,

TABLE IV

Chemical composition, formulation, method of application and the source of supply of the materials used for control of *h.similis* on pepper

Common name	Trade name	Chemical composition	Formulation	Method of application	Source
Neem cake	-	-	Cake powder	Soil application	Non-edible oil and soap, Industry, Khadi and Village Industries Commission, Poona.
D B C P	Nemagon	1, 2 - dibromo 3 - chloropropane	E C 75% v/v	Soil drench	Shell chemical company.
Fensulfothion	Dasanit	Diethyl 4-(methylsulfinyl) phenyl-phosphorothiate	5% G	oil application	Bayer India Ltd.,
Aldicarb sulfone	UC 21865	2 methyl-2-(methylsulfonyl) propionaldehyde O-(methyl carbamoyl) oxime	75% W.P	oil drench	Union Carbide, India Ltd.

E C = Emulsifiable concentrate G = Granules W.P = Wettable powder

and 8 kg a.i. per hectare. Two methods of application i.e. pre and post inoculation of the materials were adopted. In the case of pre-inoculation treatments, the neemcake was applied to soil surface in the pots 10 days before the inoculation of nematodes and mixed with the soil thoroughly. D B C P was applied seven days before fensulfothion and aldicarb sulfone two days prior to inoculation of the nematodes. In the case of D B C P and aldicarb sulfone, the calculated amount of nematicide for each dosage was mixed with 200 ml of water and then added to clay pots as soil drench. The quantity of water (200 ml) required for drenching the soil was determined earlier. Fensulfothion (Dasanit granules) was applied to the soil surface and mixed well into the soil. The pot was immediately irrigated with 200 ml of water. Nematode inoculation was given ten and seven days after application of neemcake and D B C P and two days after application of aldicarb sulfone and fensulfothion in the case of pre-inoculation treatment and seven days before application of the nematicides in the case of post-inoculation treatments. Thus the date of nematode inoculation was kept same both in the case of pre and post-inoculation treatments. Three replicates of each treatments were maintained. There were 25 such treatments including the check pots.

Maintenance of plants and recording of observations

The plants were placed under the glass house benches in partial shade. The temperature in the glass house throughout

the experimental period was maintained at 24 ± 1°C. The pots were arranged in a simple randomised way. Observations on growth characters viz. shoot growth, number of leaves produced, were recorded at intervals of every 30 days and the experiment was concluded after 150 days from the date of application of the test materials. The final observations on shoot and root length, fresh shoot and root weight and soil and root population were estimated following the same procedures described earlier.

Determination of the race status of R.similis from Panniyur pepper population

An experiment was carried out to study the race status of the population of R.similis from pepper collected from Panniyur and its capability to infect citrus, coffee and banana. Three citrus species viz., Citrus sinensis (sweet orange) C.reticulata (Mandarin orange), C.aurantifolia (lime), Coffea arabica, local variety of banana and pepper cultivar - Kalluvally were used as differential hosts for comparative infectivity by R.similis. About 35 days old seedlings/suckers of banana/rooted cuttings of each host were potted in 10 x 10 cm size earthen pots filled with denematized soil. Each pot was inoculated with 500 nematodes (all stages) obtained from pure culture. Five replicates of citrus species and three replicates of banana and pepper were maintained. The nematodes were inoculated seven days after planting. Ninety five days after inoculation of the

nematodes, the plants were depotted and nematodes were extracted from the roots and soil. The final population in each pot was estimated following similar procedure mentioned earlier.

Cross infectivity of nema isolates from pepper, banana, coconut and arecanut hosts

An experiment with R.similis population isolated from pepper (Pepper Research Station, Panniyur), banana, coconut (Central Plantation Crops Research Institute, Kayamkulam) and arecanut (Main Research Station, Hebbal) was conducted to study their ability to infect and reproduce on the other three hosts, Clay pots of 30 x 25 cm size were filled with R.similis infested soil to seven litre volume. The source of infested soil was from the previously built up pure culture pots of the respective population. Thus each pot contained approximately 5000 R.similis. In one set of three such pots containing the same population, an infected host plant and the suscept host plant were planted very close together so that the roots of the two host plants came into close contact and intermingled. The suscept host plants were raised in sterile soil separately. In case of pepper (Kalluvally cultivar) banana (local) coconut (west coast tall variety) and arecanut (South Kanara variety) were used. In case of coconut isolate population, R.similis infested coconut seedlings obtained from the Central Plantation Crops Research Institute, Kayamkulam were used as such and planted in the pots. Thus four groups of pots containing three pots in each group with the twin planted

hosts in each pot formed the treatments (Figs. 6, 7, 8 and 9). The coconut seedlings were planted close to the other infected hosts after removing the husk portion, so that the fast growing fleshy roots will come into contact with roots of R.similis infected hosts. The plants were depotted, 140 days after planting in case of pepper, banana and arecanut populations, and 200 days in case of the coconut population. The root systems of the original infected hosts and suscept host plants were carefully lifted and thoroughly washed with a strong continuous spray of water to remove all adhering debris and soil particles. The nematodes were extracted from the roots, following the procedures as mentioned earlier. The roots were examined for lesions. A few lesions were cut and teased in few drops of water on a slide and examined under a binocular stereomicroscope, to see whether they contained all the stages of the nematode. Based on these observations, the nature of the infectivity by different populations (isolates) was recorded.

Morphological characters of R.similis from pepper, banana, coconut and arecanut

Ten numbers of selected (picked out) adult females and males of the four populations were killed and relaxed by gentle heat. Temporary mounts of these nematodes were prepared in water. Measurements were taken following the method of de Man 's formula (Southey, 1970) using ocular micrometer scale. They were also critically examined for their differences in morphological characters.

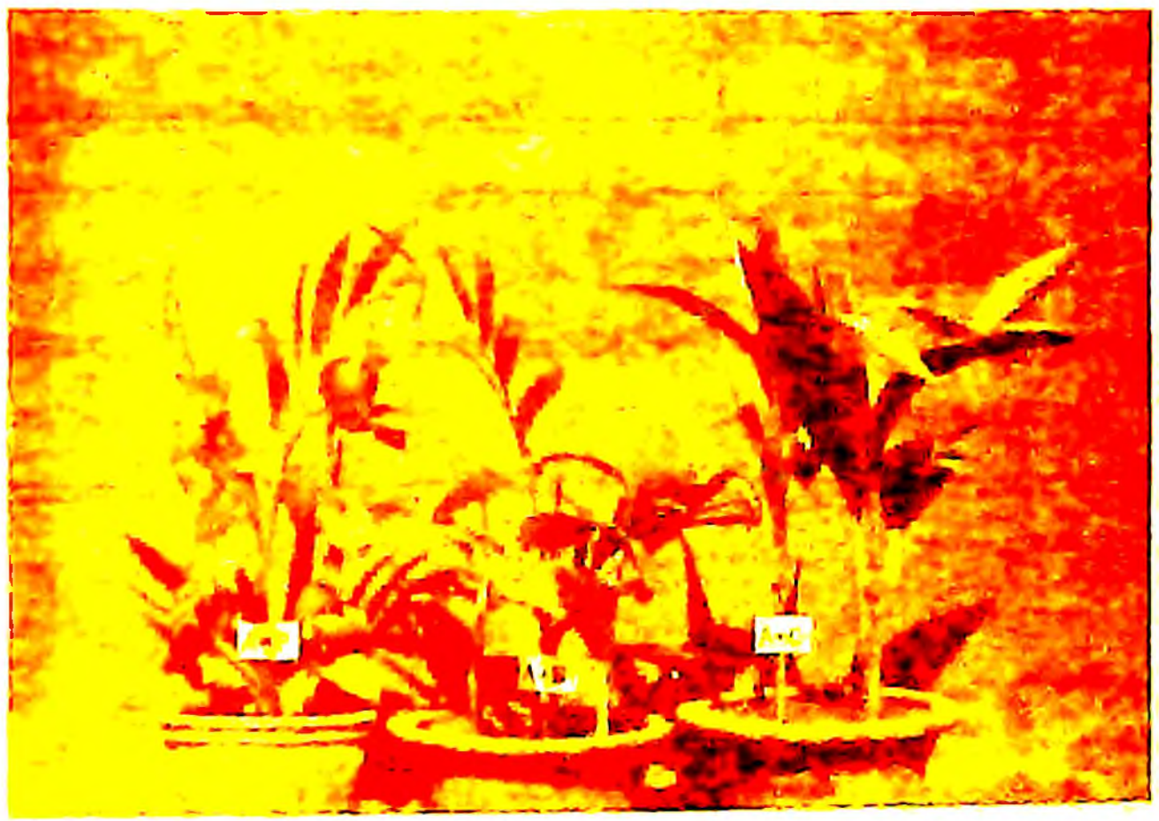
Fig. 6. Pots planted together with banana, coconut and arecanut plants and pepper, containing the 'pepper isolate' of *R.similis* by "double plant method"

Fig. 7. Pots planted together with arecanut, pepper and coconut plants and banana, containing the 'banana isolate' of *R.similis* by "double plant method"

Fig. 8. Pots planted together with banana, pepper and arecanut plants and coconut containing 'coconut isolate' of *R.similis* by "double plant method"

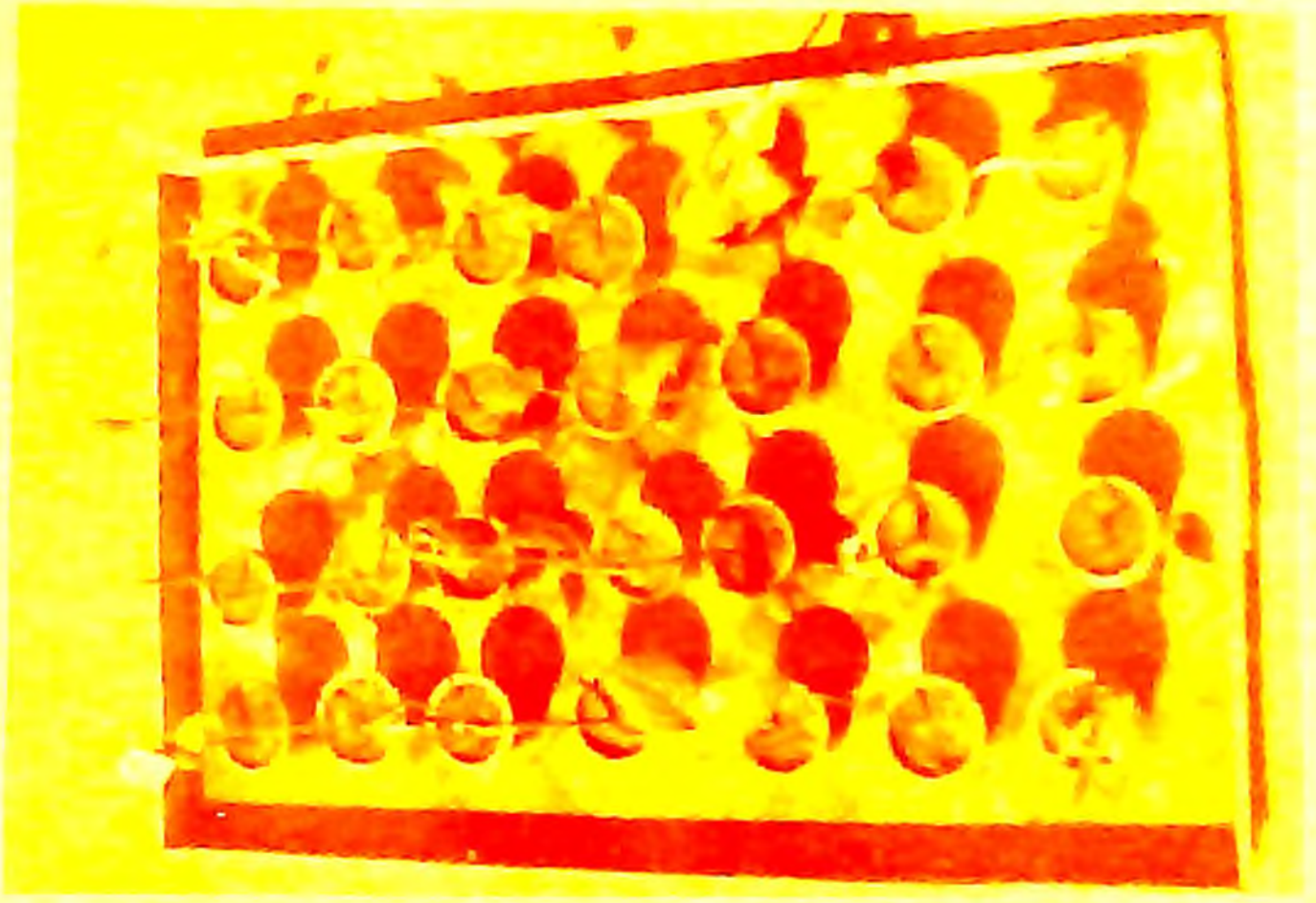
Fig. 9. Pots planted together with pepper, banana and coconut plants and arecanut containing 'arecanut isolate' of *R.similis* by "double plant method"

Fig. 7



Screening of pepper cultivars and wild species for their resistance against R.similis

Nine cultivars, each from Kerala and Karnataka, five wild collections and four species of Piper (including two cultivated species) were screened for their resistance to R.similis. Rooted cuttings of these cultivars and collections were raised following the procedure reported earlier. Rooted cuttings of uniform growth as standardised earlier were planted in small earthern pots (6 x 5.0 cm size) with 75 ml soil capacity, filled with sterilised soil. These (Fig.10) pots were inoculated with 100 nematodes (all stages) of R.similis collected from original culture of pepper population from Panniyur. Inoculation was done by pouring the nematode suspension in three ml water, equally into the three holes of two cm depth, about 1.5 cm away from the stem of the cutting in the pot. The holes were covered immediately after inoculation with soil and pots dronched with water. Two replicates for each cultivar/wild collection were maintained. Check pots without nematodes were also kept. The nema inoculation was done seven days after planting of the cuttings. After 90 days of inoculation, the plants were carefully lifted from the pots and washed free of soil and debris adhering to them. The roots were dried in between the folds of blotting papers and fresh weight of the roots were recorded. The entire soil from the pots was processed for extraction of the nematodes. The nematodes from roots were extracted to determine the final population per pot following the procedures detailed earlier.



EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

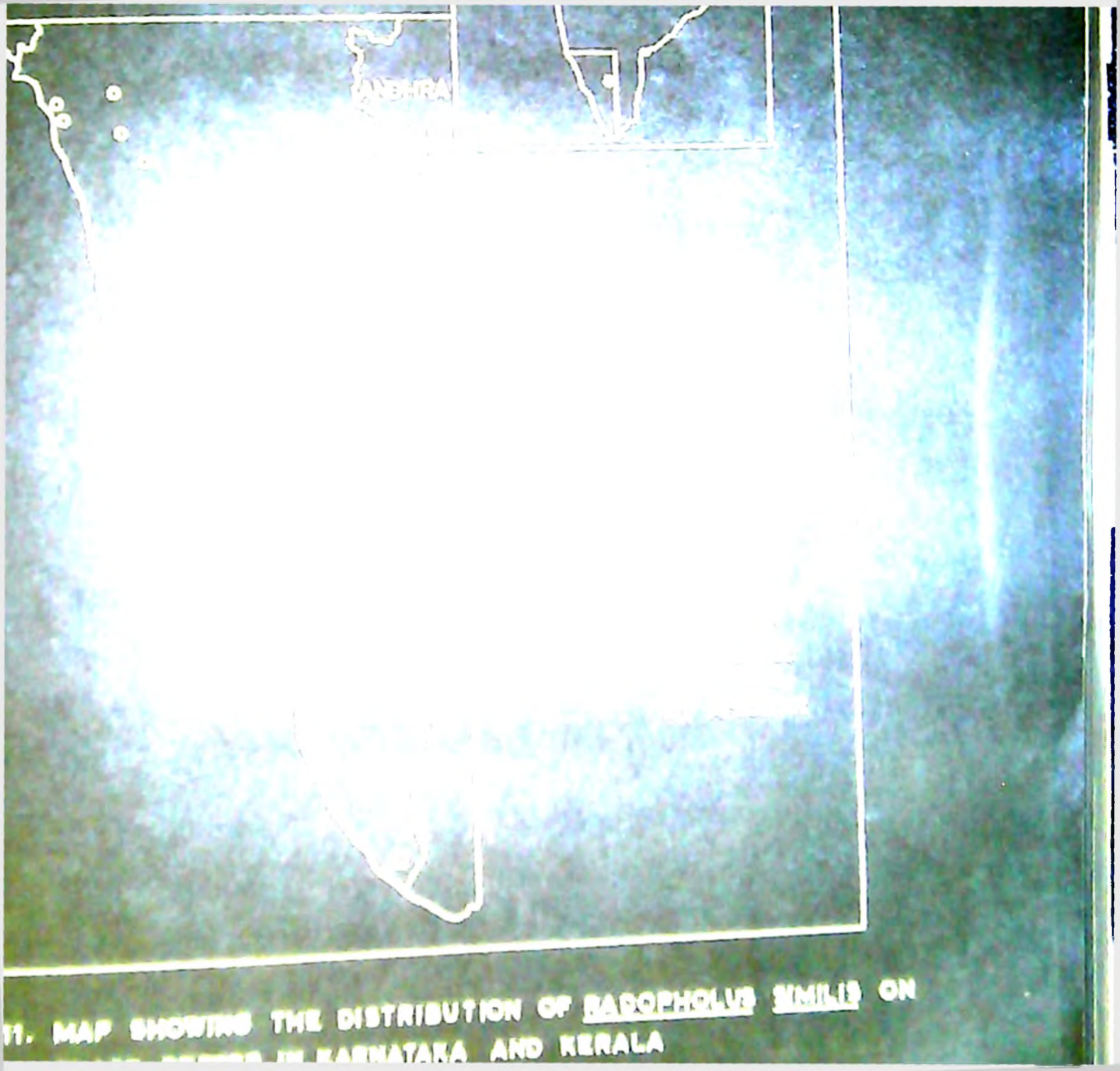
Survey

The location surveyed and the distribution of R.similis in the pepper growing regions are shown in Fig.11.

Incidence of R.similis population in the samples

Soil and root samples from healthy and diseased vines were collected from 19 and 6 locations in Cannanore and Kozhikode districts of Kerala state respectively. In Karnataka altogether 16 samples were collected from locations spread over six districts. Thus samples from a total number of 41 locations in the disease suspected pepper growing tracts of Kerala and Karnataka were examined for nematode populations. Root samples from five locations in Kerala and two locations in Karnataka were not received. The presence of R.similis and its counts in the samples collected from various locations in both the states are furnished in Table V.

Out of the 25 soil samples collected from Kerala, seven under healthy vines and 14 under diseased vines revealed the presence of R.similis. The soil population of R.similis ranged from 4 - 22 per 100 ml of soil (average 11.1) in healthy vines and from 3 - 48 per 100 ml soil in diseased vines. Only one root sample from healthy vine and nine out of 20 root samples from diseased vines revealed the presence of R.similis. The number of R.similis recovered from roots of healthy vines was 10 per g of root and in case of diseased vines from 4 - 237 (average 53) per g of root.



T1. MAP SHOWING THE DISTRIBUTION OF BADOPHOLUS SIMILIS ON
IN KARNATAKA AND KERALA

TABLE V

Density of R.similis in soil and root samples collected from healthy and diseased pepper vines from different locations in Kerala and Karnataka

State	District	Locations	Nematode population					
			Numbers per 100 ml soil		Numbers per g root		Total	
			Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Kerala	Cannanore	Kallody	-	-	-	-	-	-
		Edikkara	-	-	-	-	-	-
		Anjukunnu	-	-	-	-	-	-
		Vellamunda	-	-	-	-	-	-
		Chemperi I	-	-	-	-	-	-
		Chemperi II	-	-	-	-	-	-
		Chemperi III	-	3	-	-	-	3
		Payatuchal	-	8	-	6	-	14
		Chundukunnu	-	-	-	-	-	-
		West Kari	12	28	-	4	12	32
		Karindalam	-	-	-	-	-	-
		Kinnanur	-	14	-	12	-	26
		Manjeswar	-	22	-	7	-	29
		Cheeripady*	-	16	-	-	-	16
		Uliyil	-	-	-	-	-	-
		Panniyur	15	33	-	118	15	151
		Karimbam	6	18	-	48	6	66
		Perinthitta	6	48	10	237	18	285
		Takkad	-	-	-	-	-	-
	Calicut	Anikampoil	-	6	-	23	-	29
		Chambalamannu*	-	13	-	-	-	13
		Chalayad*	4	11	-	-	4	11
		Chakkittamma	11	14	-	22	11	36
		Kaduvayal	-	-	-	-	-	-
		Mundayayal*	22	39	-	-	22	39
Karnataka		Siddapur*	13	19	-	-	13	19
		Kalkhandy	12	10	-	46	12	64
		Buntwal	6	22	12	326	18	348
		Sarleshpur	-	-	-	-	-	-
		Puttur	49	61	42	121	91	182
		Sagar*	4	16	-	-	4	16
		Thirthahally	12	54	-	18	12	72
		Uddipi	8	13	7	16	15	29
		Mudigere	-	-	-	-	-	-
		Ankola	4	15	-	13	4	28
		Karkala	-	-	-	-	-	-
		Kumta	7	9	-	96	7	105
		Koppa	-	-	-	-	-	-
		Sringeri	3	16	-	42	3	58
		Sirsi	5	7	-	21	5	28
		Somwarpet	-	-	-	-	-	-

* Root samples were not received and examined

TABLE V - (Contd.)

State	District	Locations	Nematode population					
			Number per 100 ml soil		Numbers per g root		Total	
			Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Observed mean			11	21	17	65	15	68
Statistical mean ($\sqrt{X}$ + 0.5 transformation)			1.8	2.9	1.03	3.44	2.8	6.4
S.Em ±				0.407		0.9766		0.81
CD at 1% level				1.05		1.97		2.089

In Karnataka state 11 out of 16 locations revealed the presence of the nematode. In soil samples from healthy vines the population ranged from 3 - 49 (average 21) per 100 ml of soil. In root samples from healthy vines 3 out of 16 contained R.similis, while in case of diseased vines 9 out of 16 contained the nematode. The number of R.similis recovered from root samples varied between 7 and 42 (average 20) per g of root in healthy vines and 13 to 326 (average 77.5) per g of root in diseased vines.

In Kerala the total area covered by sampling was 29.3 hectares, while in Karnataka it was 28.5 hectares. The disease incidence reported from various locations accounted for 11.51 per cent of the surveyed area in Kerala and 12.5 per cent in Karnataka.

Incidence of other plant parasitic nematodes

Plant parasitic nematodes other than R.similis frequently encountered in soil samples in the surveyed areas were Meloidogyne spp., Rotylenchulus spp., and Helicotylenchus spp. Occasionally Criconemoides spp., Tylenchorhynchus spp., and Hoplolaimus sp were also encountered. Only in one location i.e. in Siddapur, both soil and root samples contained Pratylenchus spp. The number of locations in which the different genera of plant parasitic forms were encountered is presented in Table VI. It was observed that samples from Kerala contained Meloidogyne spp. and

TABLE VI

Number of locations with frequencies on the occurrence of different plant parasitic nematodes

Nematode genera	Number of locations											
	Kerala				Karnataka				Total			
	Healthy		Diseased		Healthy		Diseased		Healthy		Diseased	
	S.	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.	R.
Radopholus	7	1	14	9	11	3	11	9	18	4	25	18
Meloidogyne	19	7	16	6	12	4	9	3	31	11	25	9
Rotylenchulus	15	4	15	4	4	-	3	-	19	4	18	4
Helicotylenchus	17	-	16	-	6	-	8	-	23	-	24	-
Tylenchorhynchus	-	-	1	-	2	-	4	-	2	-	5	-
Pratylenchus	-	-	-	-	1	1	1	1	1	1	1	1
Hoplolaimus	1	-	2	-	-	-	-	-	1	-	2	-
Criconemoides	1	-	-	-	1	-	2	-	1	-	2	-

S - In soil R - In roots

Rotylenchulus spp., both in healthy and diseased vines without great variation in their number. Karnataka samples also contained Meloidogyne spp. both in healthy and diseased vines. But the presence of Rotylenchulus spp. was observed only in few samples. The presence of R.similis was more in soil and root samples collected from the diseased vines. The population of other plant nematode genera were not found varying in diseased vines when compared with healthy vines.

Occurrence of R.similis in different soil types

The data on soil types in which R.similis was present showed that red laterite soil contained more followed by forest black soil. Loamy soils contained the nematode in few locations, and was not encountered in clayey soils. The details are presented in Table VII.

Occurrence of R.similis in relation to intercropping pattern

The survey revealed that pepper was mostly intercropped with either one or more crops like banana, coconut, arecanut, coffee, ginger, yam and cassava. The details of locations where R.similis was encountered in relation to intercrops grown are summarised in Table VIII. Arecanut palms form the major intercrop with pepper and are used as supports for trailing the vines whereas others are planted as mixed or as border

TABLE VII

Presence of R.similis in different soil types

Soil types		Number of locations		
		Present	Absent	Total
Red laterite	..	10	3	13
Forest black soil	..	7	7	14
Red loam	..	4	3	7
Black loam	..	2	0	2
Sandy loam	..	2	0	2
Laterite clay	..	0	1	1
Clay loam	..	0	2	2
	Total	25	16	41

TABLE VIII

Occurrence of R.similis population in relation to the intercrops grown in pepper gardens

Intercrops	Number of locations		
	Present	Absent	Total
Arecanut ..	11	2	13
Coconut ..	7	3	10
Banana ..	6	2	8
Coffee ..	0	6	6
Ginger ..	0	2	2
Yam/Cassava	1	1	2
Total	25	16	41

crops in pepper plantations. The occurrence of R.similis was more in pepper when planted with arecanut followed by coconut and banana. In locations where coffee or ginger were grown R.similis was not encountered. The pattern of intercropping is given in Fig.12.

Pepper cultivars affected by disease incidence

The survey data in respect of the pepper cultivars affected by the disease showed that out of 14 cultivars,11 were found associated with R.similis. The details are presented in Table IX. The common cultivars affected by R.similis were Karimunda and Kalluvally. Out of 14 cultivars four were from Karnataka and nine belonged to Kerala. One cultivar (local) was found infected with R.similis in five out of six locations.

Root invasion and lesion formation

Observation of the stained roots revealed that most of the nematodes penetrated into roots within 24 hours of inoculation. They preferred root tip region just above the elongation zone (Fig.13). They were also observed to penetrate 1 - 1.5 cm above this region. Though nematodes penetrated individually, three to six nematodes were found in one region inside the root (Fig.14). The cells around the site of penetration were found with brown discolouration. Lesions were observed as minute dots within 72 hours of inoculation (Fig.15). The cells below the epidermal layers were dark brown in colour near the anterior region of the nematodes.

TABLE IX

R.similis population encountered with different cultivars of pepper

Name of cultivars	Number of locations		
	Present	Absent	Total
Kalluvally ..	6	3	9
Karimunda ..	5	5	10
Panniyur-I ..	1	2	3
Arakulam munda ..	2	0	2
Thekkenadan* ..	1	0	1
Balankotta* ..	0	2	2
Valiyakaniyakadan*	0	1	1
Poonharan Munda ..	0	1	1
Mundikodi ..	1	0	1
Mallegessera ..	1	1	2
Karimallegessera*	1	0	1
Uddakkare ..	1	0	1
Cavathi ..	1	0	1
Local ..	5	1	6
Total	25	16	41

* Root samples were not received and examined

Fig. 13. *R. similis* in the process of penetration into feeder root tip region of black pepper

Fig. 10. Technique used for screening of rooted cuttings of different pepper cultivars inoculated with *R. similis* for their resistance

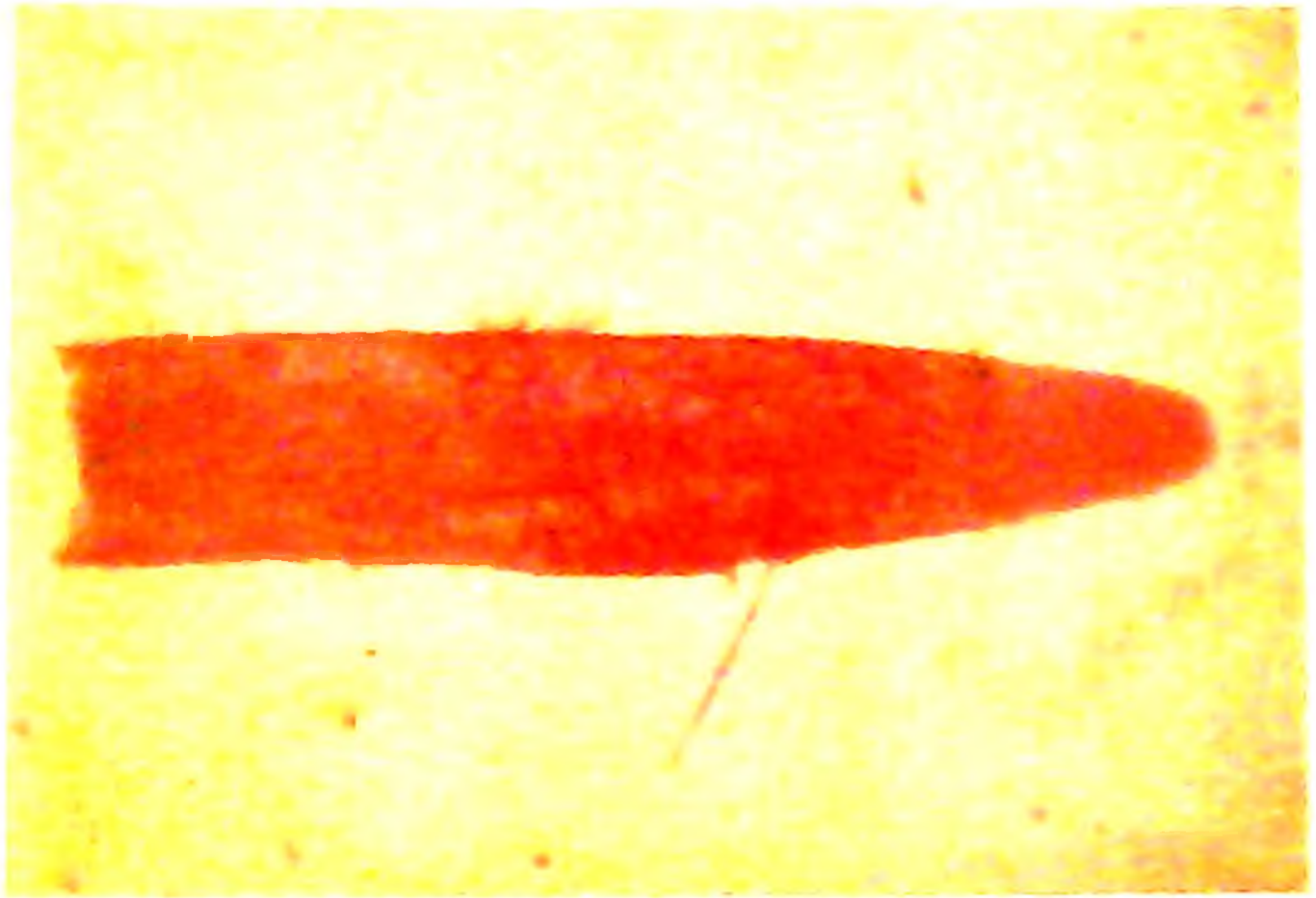


Fig. 14. R.similis inside the root of black pepper in cortical region

Fig. 15. Feeder root of black pepper showing lesions with blackened parenchyma cells by R.similis invasion within 72 hours after nematode inoculation

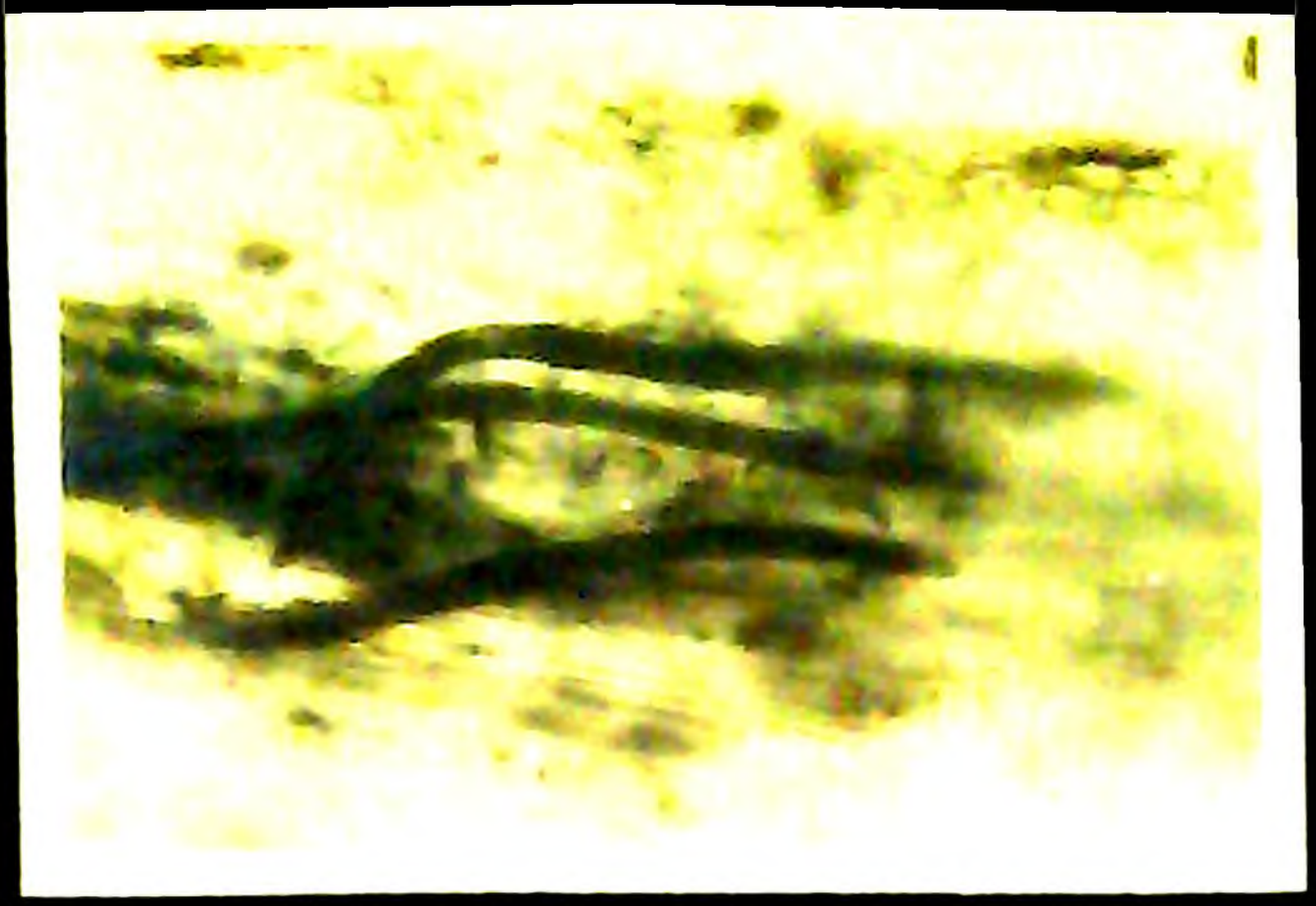
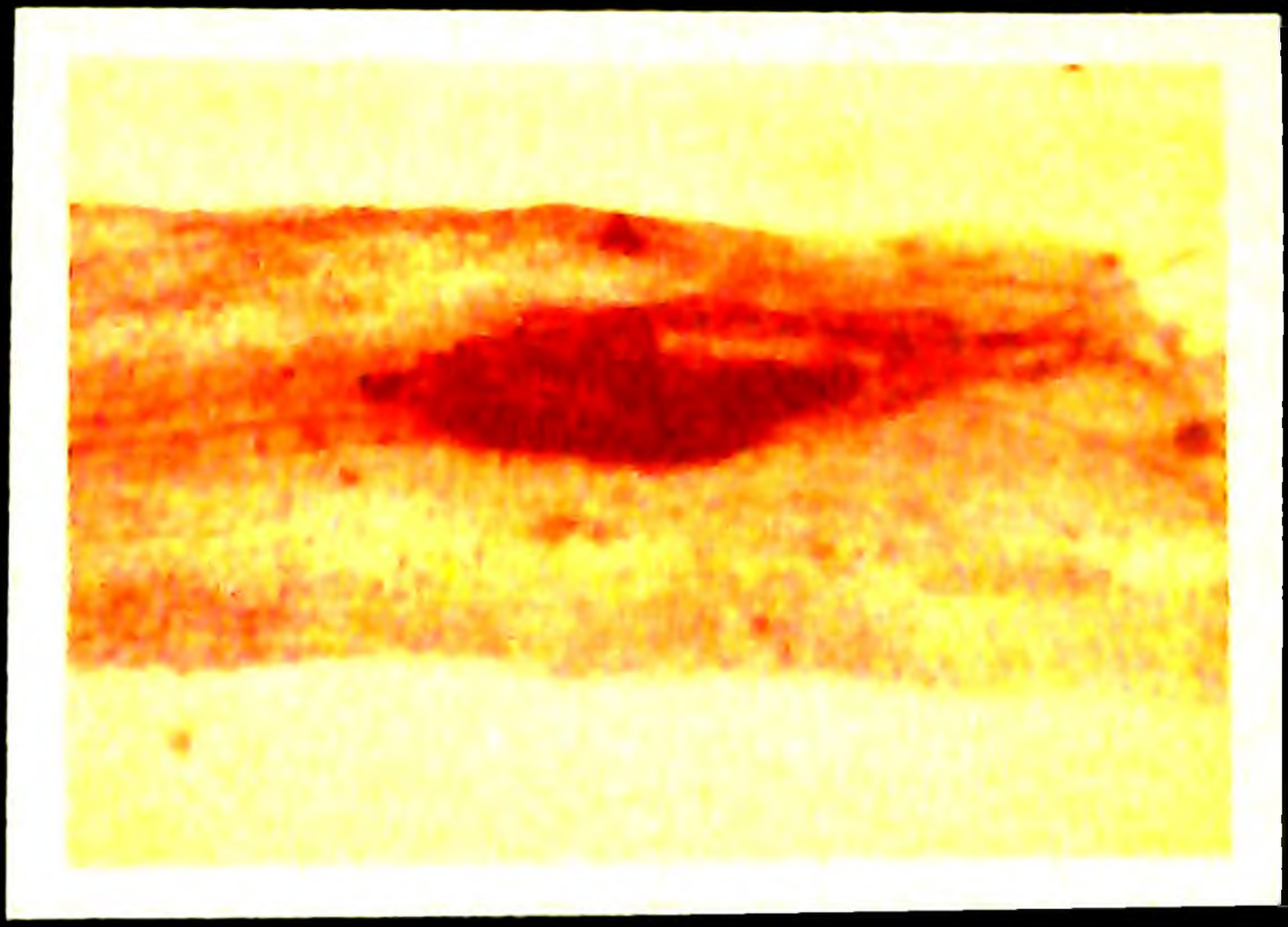


Fig. 14



Nature of root injury caused by the nematode

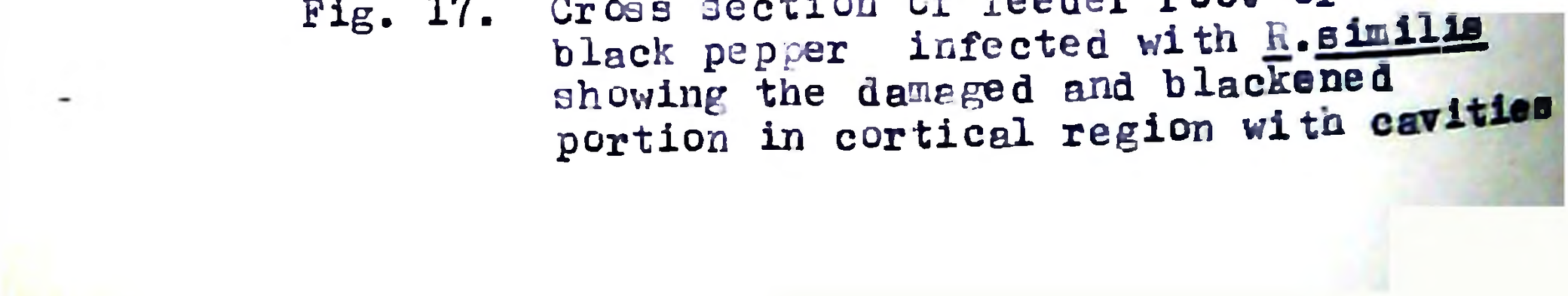
Free hand transverse sections of healthy and infected roots are shown in figures 16 and 17. In the infected root, the parenchyma cells in the region surrounding the infection site were blackened and in a disarray. In advanced stages, these became necrotic and by a gentle touch with a fine needle they got separated very easily from healthy portions. The cells in the region of the nematode advancement were darkened and granulation of cell contents were observed (Fig.18). The stelar portions were not affected. It was also observed that eggs were laid within the tissues within six days of their entry into the roots (Fig.19).

Pathogenecity of *T.gingilis* to black pepper

The object of this experiment was to gain information on the role of this nematode in causing damage, as expressed in terms of plant growth characters. The growth characters like shoot length, fresh shoot weight, leaf production, root length and fresh root weight were recorded. Out of eight replicates, plants in two replicates, inoculated with 1000 nematodes per pot and in three replicates inoculated with 10,000 nematodes per pot showed wilting symptoms after 90 days, and they died after 118 days of nematode inoculation. Due to the death of the plants in the above treatments, the statistical analysis was carried out following Yates (1933) and Taylor (1949) methods.

Fig. 16. Transverse section of healthy feeder root of black pepper

Fig. 17. Cross section of feeder root of black pepper infected with R.similis showing the damaged and blackened portion in cortical region with cavities



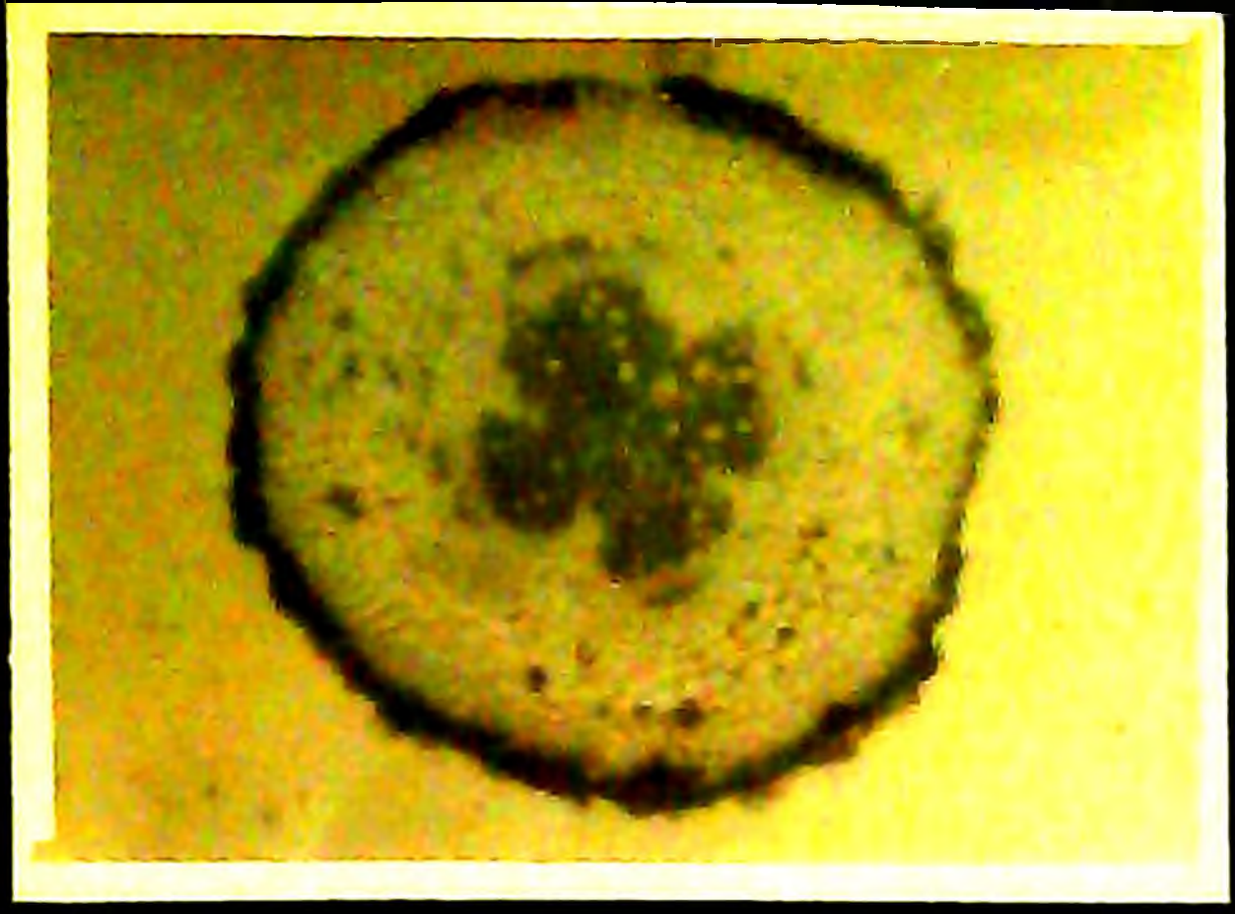


Fig. 16

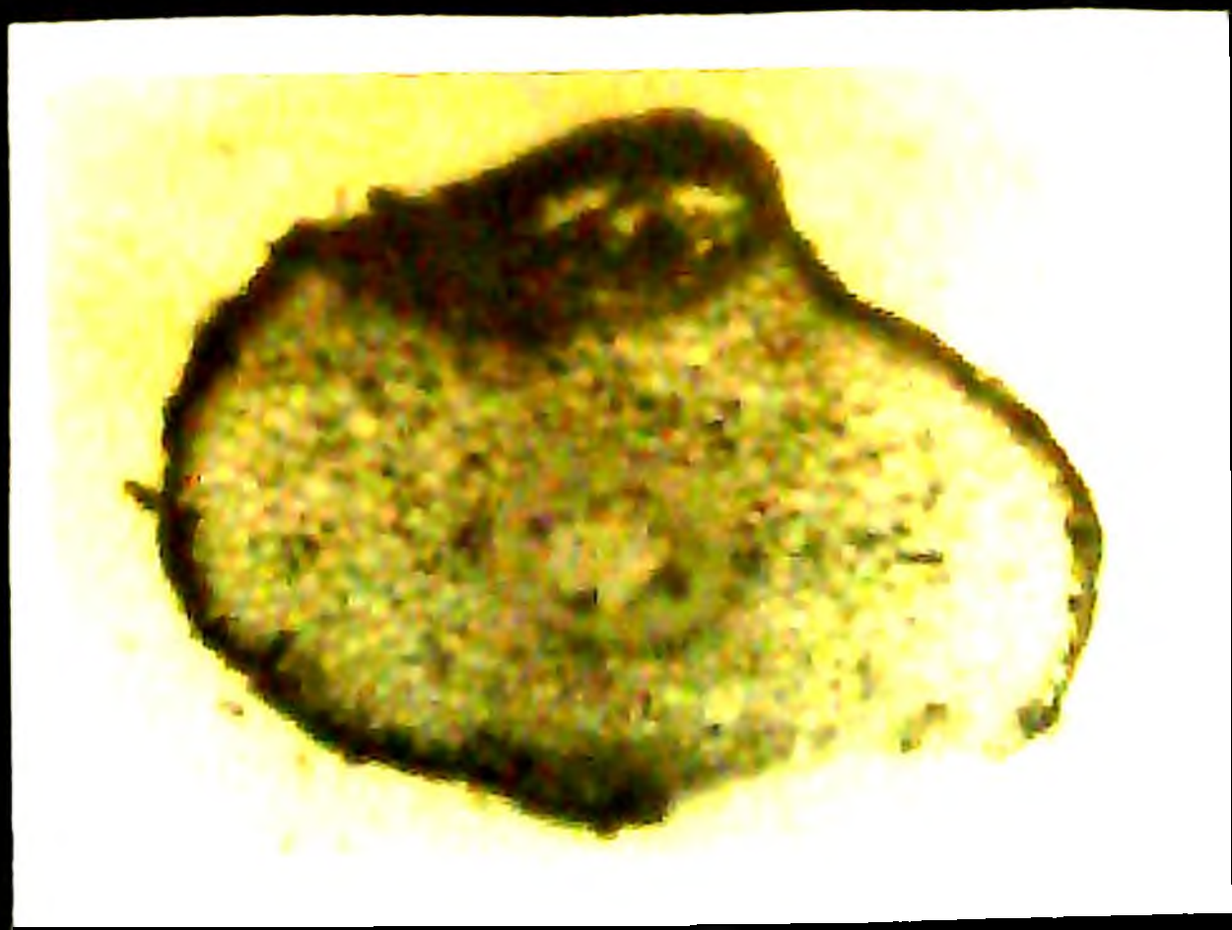


Fig. 18. Longitudinal section of feeder root of black pepper infected with R.similis showing the nematode head fixed in parenchyma cells and damaged cells around the head of the nematode

NH = Nematode head N = Nematode

Fig. 19. Longitudinal section of black pepper feeder ro showing nematodes and eggs laid inside root tissues by R.similis within six days of nemato inoculation

N = Nematode E = egg laid inside tissue

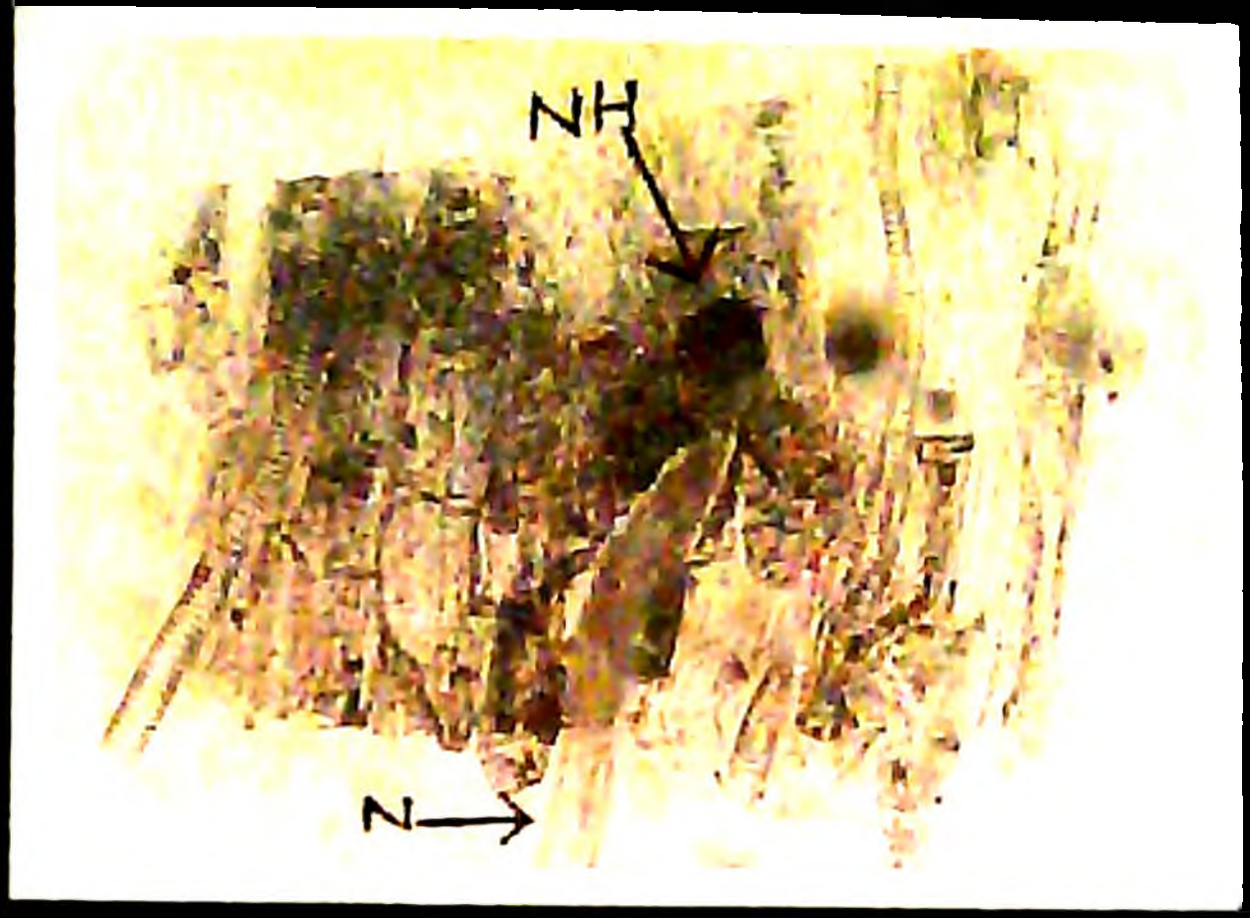
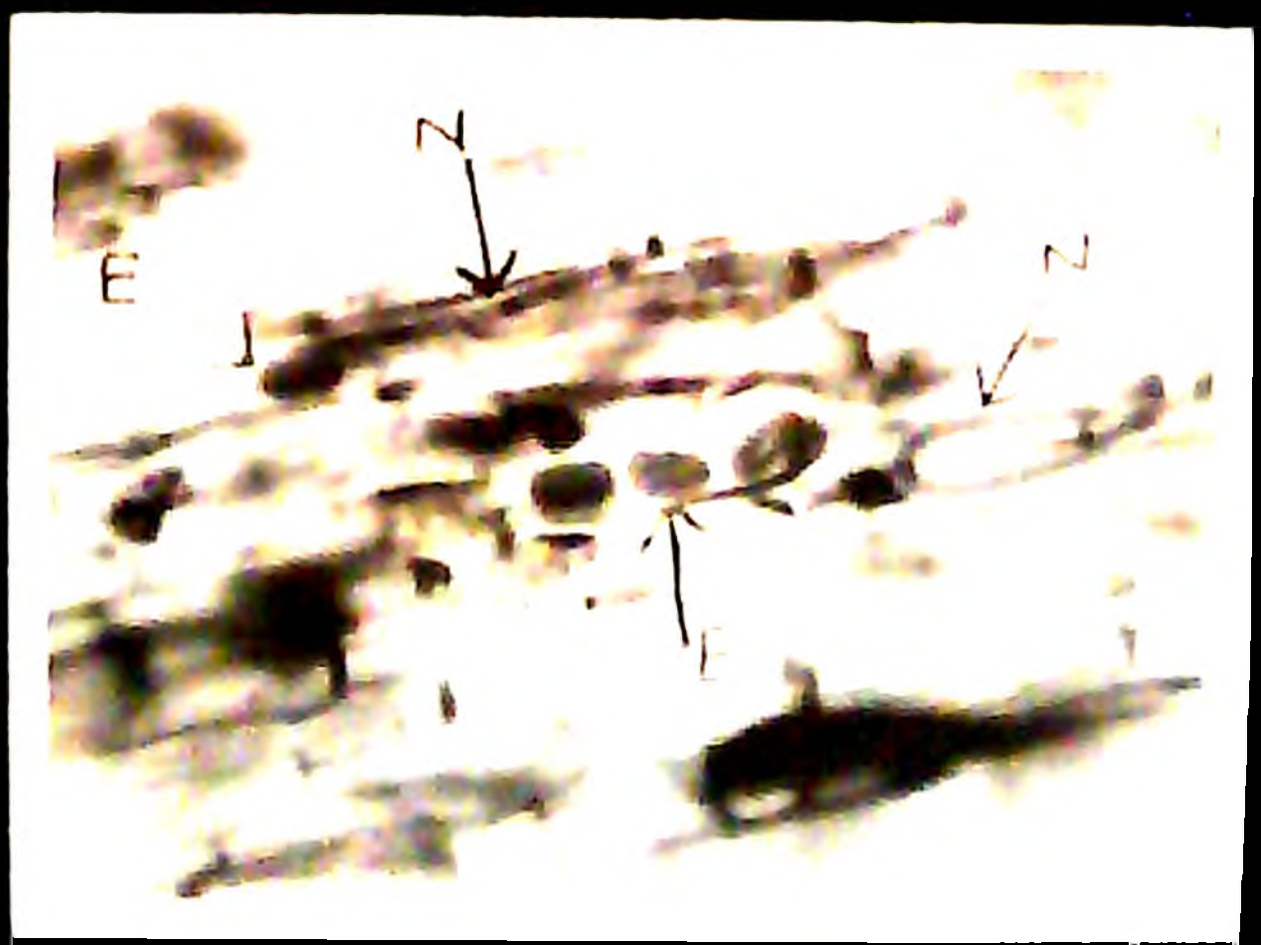


Fig. 18



Effect on shoot length

The stunting of the plants increased as the inoculum level of the nematodes increased from 100 to 10,000 per pot. In case of plants inoculated with 10,000 nematodes, shoot growth was practically arrested (Figs.20 and 21). The difference in shoot lengths of the plants at varying inoculum levels recorded at 30 days interval from 30 to 150 days were highly significant and the data is summarised in Table X (Fig.22). It was found that plants inoculated with 10,000 nematodes put-forth shoot length of 2.96 cm compared with 59.26 cm produced by the check plants. Thus there was 20 fold decrease in shoot growth of plants inoculated with 10,000 nematodes which was corresponding to a reduction of 94.97 per cent in shoot growth as compared to control plants at the end of 150 days. Reduction in shoot growth due to other inoculum levels ranged from 22.3 to 93.8 per cent. The effects on shoot length between 100 and 10000 nematodes inoculation levels, were not significant at 30, 90 or 150 days, but at 60 and 120 days intervals they exhibited significant difference. The results indicated that when the nematode inoculum level was 100 per 1500 ml soil, it could cause over 70 per cent reduction in growth in course of 150 days on 55 days old pepper cuttings. When the inoculum was over 1000 nematodes per 1500 ml soil, over 90 per cent reduction in shoot growth, near death or stoppage of growth of plant in the same period were observed.

Pathogenecity of *R.similis* to black pepper

Fig. 20. Effect on growth of vines by *R.similis* infection, with four inoculum levels at 120 days after nematode inoculation

Fig. 21. Effect on growth of vines and the development of root system by *R.similis* infection with four inoculum levels at 150 days after nematode inoculation

Fig. 20

TABLE X

Pathogenecity of R.similis to black pepper. Shoot length(cm) of pepper cutting at 30 days interval after nematode inoculation

(Mean of eight replicates)

Inoculum level	Days				
	30	60	90	120	150
Check	4.36 (0.6349)	8.10 (0.9020)	17.00 (1.2192)	37.92 (1.5788)	59.26 (1.7728)
10 Nematodes per pot	3.20 (0.4955)	4.27 (0.6365)	12.25 (1.0874)	32.17 (1.5074)	48.46 (1.6853)
100 -do-	2.31 (0.3429)	3.39 (0.4845)	6.16 (0.8379)	8.67 (0.9385)	21.60 (1.3383)
1000 -do-	1.91 (0.3157)	2.50 (0.4023)	2.90 (0.4546)	3.34 (0.5205)	3.73* (0.5748)
10,000 -do-	1.51 (0.3043)	1.89 (0.2474)	2.25 (0.3327)	2.48** (0.3921)	2.98** (0.4759)
S.Em. ±	0.1344	0.0552	0.3035	0.0187+ 0.0219&	0.0481$ 0.0533& 0.0618@
CD at 1% level	0.0912	0.1531	0.2287	0.0521+ 0.0611&	0.1350$ 0.1496& 0.1275@

Values in parentheses are log transformed means.

* Mean of six replicates. ** Mean of five replicates.

● Significant at 5 per cent level

+ Between check and 100 nematodes inoculated pots
$ -do- 100 -do- -do-
& -do- 100 1000 -do- -do-
@ -do- 1000 10000 -do- -do-

FIG. 22. PATHOGENECITY OF R. SIMILIS TO BLACK PEPPER. EFFECT ON GROWTH OF SHOOT LENGTH AT 30 DAYS INTERVALS AFTER NEMATODE INOCULATION

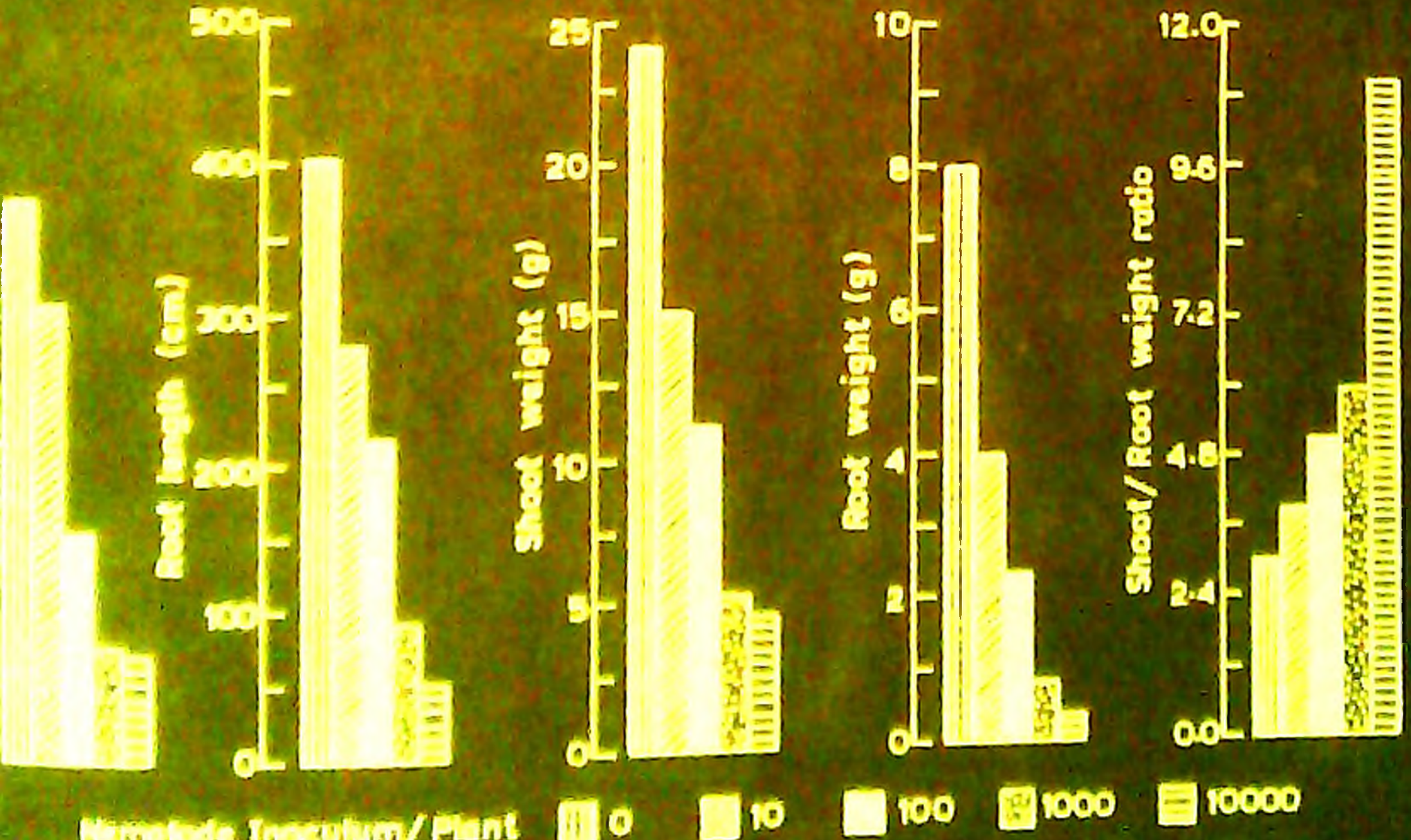


FIG. 23. PATHOGENECITY OF R. SIMILIS TO BLACK PEPPER. EFFECT ON VARIOUS GROWTH FACTORS AT 150 DAYS AFTER NEMATODE INOCULATION

Effect on fresh weight of shoots

The effect of different inoculum levels of the nematode on fresh shoot weight was recorded and the data are summarised in Table XI (Fig.23). The average shoot weight of check plants was 24.76 g as compared to 15.3 g, 11.51 g, 5.15 g, and 4.56 g of the plants inoculated with 10, 100, 1000, 10000 nematodes respectively. Highly significant reduction in the shoot weight was observed between the 100 and 1000 nematodes inoculated plants and the reduction in the shoot weight was 53.5 per cent and 79.2 per cent compared to check plants (Fig.24). However no significant difference in shoot weight was observed between plants inoculated with 1000 and 10000 nematodes.

Effect on leaf production

The number of leaves produced by the plants at 150 days after nematode inoculation was recorded to ascertain the effect of nematode infection on leaf production. The data are presented in Table XII (Fig.23). The check plants produced on average 7.5 leaves, while the plants inoculated with 1000 and 10000 nematodes produced only less than two leaves during the same period. The leaves of these plants were smaller compared to the leaves of check plants. No typical yellowing of leaves was observed. The plants inoculated with 10 and 100 nematodes produced 6.1 and 3.0 leaves respectively and the differences were significant between check and plants inoculated with 10 and 100 nematodes. Highly significant difference was observed

TABLE XI

Pathogenecity of R.similis to black pepper. Fresh weight of shoots (g) at 150 days after nematode inoculation

(Mean of eight replicates)

	Check	Nematodes per pot			
		10	100	1000	10000
Weight of shoot(g)	24.76 (1.3912)	15.30 (1.1835)	11.51 (1.0604)	5.15* (0.7057)	4.56** (0.6537)
S.Em. ±		0.0259		0.0277&	0.0321£
C D at 1% level		0.0702$		0.0778&	NS

Values in parentheses are log transformed means

* Mean of six replicates. ** Mean of five replicates.

$ Between check and 100 nematodes inoculated pots.

& Between 10 and 1000 -do- -do-

£ Between 1000 " 10000 - do- -do-

TABLE XII

Pathogenecity of R.similis to black pepper. Number of leaves per plant produced at 150 days after nematode inoculation.

(Mean of eight replicates)

	Check	Nematodes per pot			
		10	100	1000	10000
Number of leaves produced	7.500 (2.902)	6.125 (2.643)	3.000 (1.984)	1.500* (1.564)	1.400** (1.541)
S.Em. ±		0.1266$	0.1403&	0.1626£	
C D at 1% level		0.3554$	0.3938&	NS	

Values in parentheses are mean of $\sqrt{X}$ + 1 transformation

* Mean of six replicates. ** Mean of five replicates.

$ Between check and 100 nematodes inoculated pots

& -do- 100 " 1000 -do- -do-

£ -do- 1000 " 10000 -do- -do-

among plants with inoculum levels of 100 and 1000 nematodes per pot. However no significant difference was observed among plants inoculated with 1000 and 10000 nematodes per pot. The results indicated that effective production of leaves were affected by nematode infection at the level of 100 nematodes per 1500 ml soil and more markedly when this inoculum level increased to 1000 nematodes per 1500 ml soil.

Effect on the root length

The root system of plants inoculated with R.similis showed patchy brown discolouration, whereas it was creamy white in colour in the check plants, with plenty of feeder roots and lateral roots. The root system of plants inoculated with 100 and 1000 nematodes was devoid of feeder roots and where feeder roots were present, they were black in colour and almost decayed. The root system of plants inoculated with 10000 nematodes was having practically no feeder roots and had only matured lateral roots. The length of roots recorded under different treatments are summarised in Table XIII (Fig.23).

The maximum root length of 406.06 cm was recorded in case of check plants as compared to 54.44 cm in plants inoculated with 10000 nematodes. The differences between the treatments, as affected by the varying inoculum levels were highly significant. The percentage reduction in root lengths

TABLE XIII

Pathogenecity of R.similis to black pepper. Length of roots (cm) at 150 days after nematode inoculation.

(Mean of eight replicates)

	Check	Nematodes per pot			
		10	100	1000	10000
Length of roots(cm)	406.06 (2.6083)	278.70 (2.4450)	213.05 (2.3282)	90.26 (1.9554)	54.44 (1.7352)
S.Em ±		0.0114$		0.0126&	0.0146£
C D at 1% level		0.0319$		0.0355&	0.0409£

Values in parentheses are log transformed means.

* Mean of six replicates. Mean of five replicates

$ Between check and 100 nematodes inoculated pots

& -do- 100 and 1000 -do- -do-

£ -do- 1000 and 10000 -do- -do-

due to different inoculum levels were 31.4, 47.6, 67.8 and 86.6 respectively in the plants treated with 10, 100, 1000 and 10000 nematodes, compared to the check plants (Fig.24). The above results indicated that even an inoculum level of 10 nematodes per 1500 ml soil could cause retardation in root development up to 31 per cent in course of 150 days in 55 days old freshly planted rooted pepper cuttings. With increase in the inoculum level in soil, the plants were devoid of a well developed root system. This also showed that the root growth was adversely affected with increasing nematode inoculum in the soil.

Effect on fresh root weight

Observations recorded on the fresh root weight of the plants in five treatments are given in Table XIV (Fig.23). The maximum root weight of 8.006 g was recorded by check plants compared with the least weight of 0.412 g in plants inoculated with 10000 nematodes. The ratio of root weight to root length of the plants in the five treatments were found to be 1:50, 1:70, 1:93.5, 1:100 and 1:132 respectively. The differences in the root weight of plants among the five treatments were highly significant. Among plants inoculated with 10 and 100 nematodes, the difference was significant only at five per cent level. Compared to check plants the per cent root weight reduction in case of plants inoculated with 10, 100,

TABLE XIV

Pathogenecity of R.similis to black pepper. Fresh root weight (g) at 150 days after nematode inoculation

(Mean of eight replicates)

	Check	Nematodes per pot			
		10	100	1000	10000
Fresh root weight(g)	8.006 (0.9542)	3.990 (0.6947)	2.280 (0.5148)	0.896 (0.2778)	0.412** (0.1493)
S.Em ±		0.0826$		0.0967&	0.0106£
C D at 1% level		0.2318$		0.2714&	0.0297£
at 5% level		0.1708$			

Values in parentheses are log of x + 1 transformed mean

* Mean of six replicates. ** Mean of five replicates

$ Between check and 100 nematodes inoculated pots

@ -do- 10 " 100 -do- -do-

& -do- 100 " 1000 -do- -do-

£ -do- 1000 "10000 -do- -do-

PER CENT

Shoot length (cm) a

weight (g)

Root weight (g)

Inoculum/Plant

Nematode

1000 and 10000 nematodes were 50.4, 71.6, 88.8 and 94.5 respectively (Fig.24). The results suggested that the increase in nematode inoculum in the soil reduced the root weight and was detrimental to host plant.

Effect on shoot and root weight ratio

The ratio between shoot and root weight was calculated from the observations recorded on fresh shoot and root weight, and the data are summarised in Table XV (Fig.23). Highly significant differences were observed between the shoot and root weight ratios of the plants inoculated with nematodes and check plants. However the ratio between the plants inoculated with 100 and 1000 nematodes was not significant. The plants inoculated with 10000 nematodes had a ratio of 11.34 and were 2.5 times more, compared to the ratio of 3.078 of check plants. This marked difference in the ratio could be due to heavy root damage caused by high nematode inoculum. In case of plants inoculated with 100 and 1000 nematodes the ratios were 5.081 and 5.793 which were 1.6 and 1.9 times more respectively compared to the ratio of the check plants. The results indicated that with the increase in nematode inoculum in soil the normal growth of the plants were adversely affected.

Reproduction rate and final population

The final population of nematodes in soil, roots, total population and percentage increase are summarised in Table XVI

TABLE XV

Pathogenecity of R.similis to black pepper. Shoot weight/root weight ratio of plants at 150 days after nematode inoculation

(Mean of eight replicates)

	Check	Nematodes per pot			
		10	100	1000	10000
Shoot weight/ root weight ratio	3.078 (0.4954)	3.923 (0.5888)	5.081 (0.7040)	5.793 (0.7404)	11.134** (1.0449)
S.Em ±		0.03049		0.05378	0.03916
C D at 1% level		0.08539		NS	0.10966

Values in parentheses are log transformed means

* Mean of six replicates. Mean of five replicates.

$ Between the check and 100 nematodes inoculated pots.

& -do- 100 " 1000 -do- -do-

£ -do- 1000 " 10000 -do- -do-

TABLE XVI

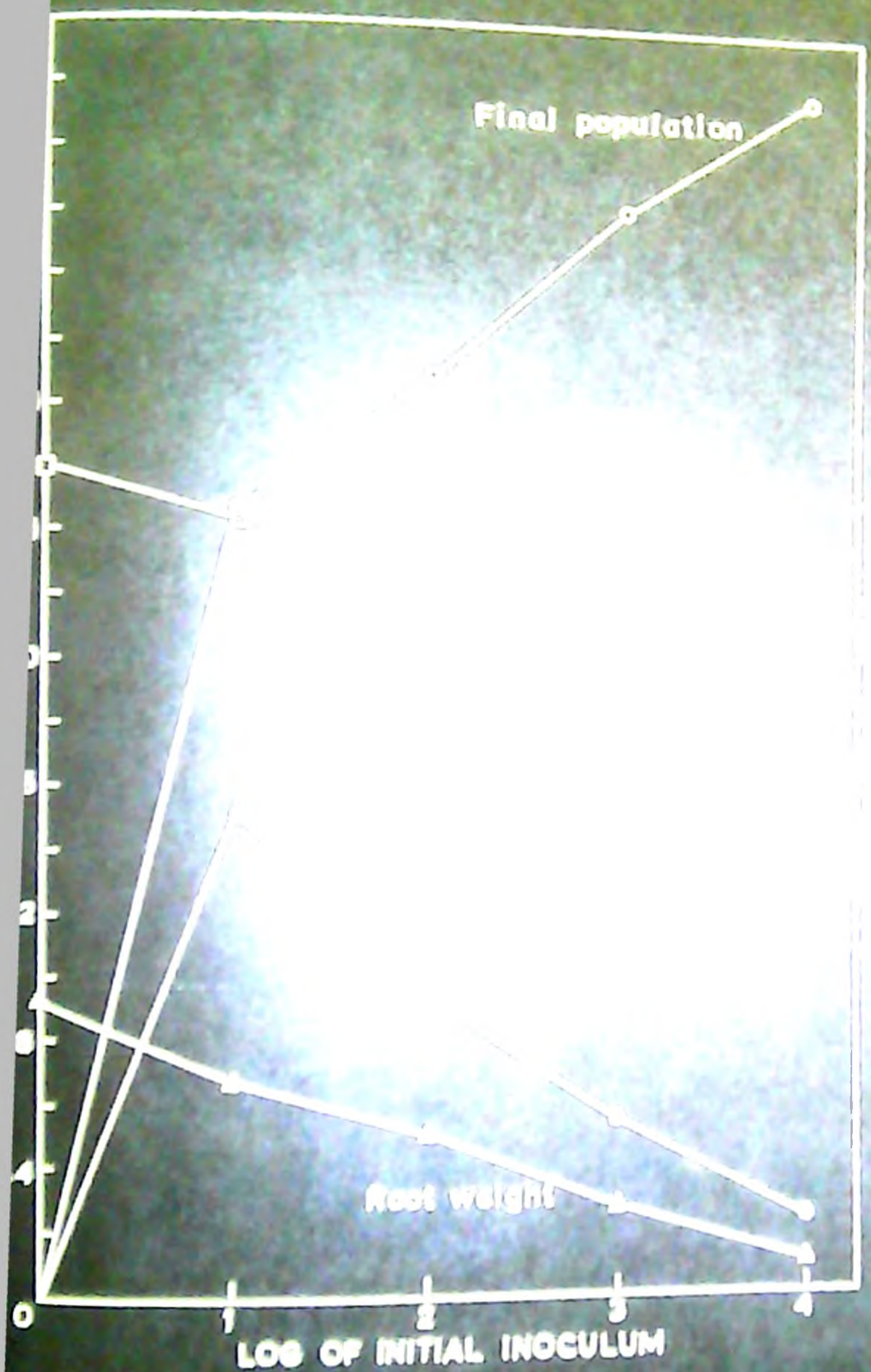
Pathogenecity of R.similis to black pepper. Final population in various inoculum levels at 150 days after nematode inoculation

(Mean of eight replicates)

Inoculum level	Mean soil population per pot	Mean root population		Mean total population per pot	Per cent increase/ decrease
		Per plant	Per g		
0	-	-	-	-	-
10	139	186	48	325	32.5
100	377	455	199	832	8.32
1000*	665	2060	2300	2725	2.73
10000**	6056	1221	2964	7277	0.73

* Mean of six replicates

** Mean of five replicates

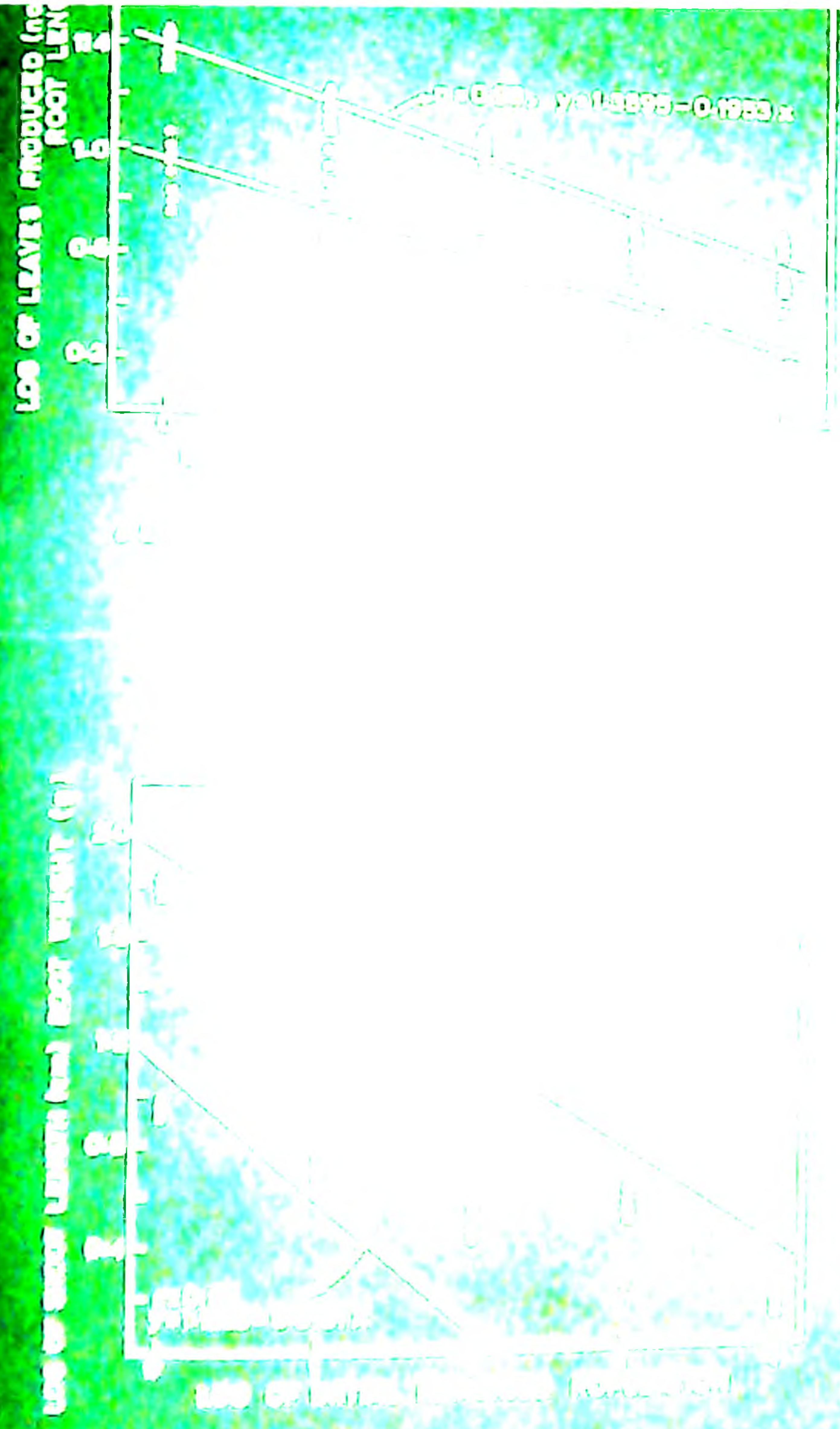


PATHOGENECITY OF R. SIMILIS TO BLACK PEPPER. EFFECT ON ROOT LENGTH AND WEIGHT REPRODUCTION RATE AND FINAL POPULATION AT 150 DAYS AFTER NEMATODE INOCULATION

(Fig.25). It was observed that the rate of increase in final nematode population was maximum by 32.5 per cent in plants inoculated with 10 nematodes. The rates of increase of the total population in plants inoculated with 100 and 1000 nematodes were 8.32 and 2.73 per cent respectively. The final nematode population declined to 0.73 per cent in plants inoculated with 10000 nematodes. The observations also revealed that more nematodes were present in the roots than in soil, in plants inoculated with 10, 100 and 1000 nematodes and vice - versa in plants which were given an initial inoculum of 10000 nematodes. The highest rate of multiplication(the factor on final population increase over initial inoculum, reproduction rate) of R.similis in plants inoculated with lowest number of nematodes showed that the test plant was a suitable host.

Relationship of initial inoculum level to plant growth characters

Various plant growth characters were studied with initial inoculum levels to know whether any relationship existed between these two factors. Regression analysis revealed correlation coefficient of negative values of over 0.90 in all cases. The results suggested that these two factors follow a negative correlation (Figs. 26 and 27), further supporting the evidence, that the increased inoculum level was related to plant damage. Thus this nematode is capable of effecting plant damage at a time when there is optimal nematode population level in the soil.


y=1.3895-0.1953x

Host range of pepper isolate of R.similis

This experiment was carried out to know the infectivity of pepper isolate of R.similis, to citrus, coffee, banana and pepper and the data on nematodes extracted from the root and soil are presented in Table XVII. The data revealed that none of the three citrus species and coffee plants were infected when inoculated with the pepper isolate, whereas banana and pepper (Piper nigrum) were readily infected. Roots of these host (citrus, coffee) plants were well developed with plenty of feeder roots and when washed and examined they were white creamy in colour and did not reveal presence of any lesions. The soil samples washed from the pots of these hosts did reveal the presence of R.similis at 160 - 285 nematodes per pot of 500 ml soil, still surviving in free soil. In banana and pepper the roots showed lesions and decay as normally observed in R.similis infected plants. The nematodes extracted from roots of these two hosts contained large number of larvae at different stages of development. The population recovered from roots showed that the nematodes had increased 2.0-2.5 times more than the original inoculum in banana and pepper respectively. These results indicated that these two plants were the favoured hosts for this nematode. The overall increase in population in banana also indicated that the pepper isolate of this nematode favoured banana more as a suitable host.

TABLE XVII

Infectivity of pepper isolate of R.similis to citrus, coffee, and banana. Number of nematodes extracted from roots and soil at 95 days after nematode inoculation

(Mean of five replicates)

Hosts inoculated	Number of nematodes extracted		Total final population
	From roots per plant	From 100 ml soil	
Citrus sinensis	-	42	210
Citrus reticulata	-	39	195
Citrus aurantifolia	-	57	285
Coffea arabica	-	32	160
Banana	1232	129	1877
Pepper	1055	144	1775

Original inoculum level 500 nematodes, per pot of 500 ml soil.

* Mean of 3 replicates

Cross infectivity behaviour of pepper isolate towards banana, coconut and arecanut

On depotting the seedlings from each set of pots, it was observed that the root system of both the plants were entangled and came into close contact with each other. They were separated carefully without losing root materials of either of the plants. The details on the number of nematodes extracted from roots of the plants and soil from the same pot are summarised in Table XVIII. No lesions could be found on the roots of coconut or arecanut seedlings. Eventhough, the roots were kept for over 10 days for extraction of nematodes, none could be recovered. In case of banana and pepper roots, patchy discolouration and rotting of roots could be observed. *R.similis* in all stages of development were readily obtained from the roots of banana and pepper. Eventhough no nematodes were obtained from roots of coconut and arecanut, the soil in the pots contained the nematodes. These results indicated that the Kannivur pepper isolate of *R.similis* could infect only banana but not coconut and arecanut (Fig.28).

Cross infectivity behaviour of banana isolate to pepper, coconut and arecanut

In this case also the root system of both host plants in each pot was observed to be mingled with each other. On washing the roots of these plants and carefully separating them, small dark spots could be located on the roots of pepper, coconut and

TABLE XVIII

Cross infectivity of "pepper isolate " of R.similis to banana, coconut and arecanut by "double plant" method. Root weight and number of nematodes extracted from roots and soil at 140 days after planting of the host plants.

Host plants	Root weight (g)	Number of nematodes extracted	
		Roots per plant	Soil per 100 ml
Pepper ..	8.84	2574	63
Banana ..	77.73	2344	
Pepper ..	6.36	2651	54
Coconut ..	17.81	Nil	
Pepper ..	7.17	2287	48
Arecanut ..	12.57	nil	

* From the same pot

arecanut plants, on close observation. These small spots were found to be lesions and they were separated and teased out on a glass slide in few drops of water. In the roots of coconut, pepper and arecanut, adult females, males and larvae could be seen from such lesions. The details on the number of nematodes recovered from the roots of susceptible hosts are presented in the Table XIX. The number of nematodes recovered from arecanut roots was comparatively less than the number collected from roots of banana, pepper and coconut. The results suggested that the banana isolate could infect all the three hosts (Fig.28).

Cross infectivity behaviour of coconut isolate to pepper, banana and arecanut

On removal of the seedlings from each pot, it was observed that the root system of susceptible hosts were entangled with roots of coconut seedlings. Some roots of the susceptible hosts were observed to have even penetrated into the husk portion of coconut seedlings. The details on the recovery of nematodes from the roots of original host, susceptible hosts and from soil in each of the pots are presented in Table XX. Examination of the roots revealed the presence of lesions only on roots of pepper and banana plants. The roots of these two hosts yielded R.similis. Lesions from pepper and banana roots contained nematodes of all stages of development, confirming the infection and reproduction in the hosts. Though the soil revealed the presence of nematodes on termination of the experiment in the

TABLE XIX

Cross infectivity of "banana isolate" of R.similis to pepper, coconut and arecanut by "double plant" method. Root weight and number of nematodes extracted from roots and soil at 140 days after planting the host plants.

Host plants	Root weight (g)	Number of nematodes extracted	
		Roots per plant	Soil per 100 ml*
Banana	43.35	3155	51
Pepper	9.47	1680	
Banana	69.27	2377	45
Coconut	41.12	1156	
Banana	49.83	2840	57
Arecanut	14.78	829	

* From the same pot

TABLE XX

Cross infectivity of "coconut isolate" of R.similis to pepper, banana and arecanut by "double plant"method. Root weight and number of nematodes extracted from roots and soil at 200 days after planting the host plants.

Host plants	Root weight (g)	No.of nematodes extracted	
		Roots per plant	Soil per 100 ml
Coconut	31.70	1072	47
Pepper	9.92	339	
Coconut	33.80	865	59
Banana	21.41	1520	
Coconut	29.26	784	36
Arecanut	16.85	nil	

from the same pot

pots planted with both hosts, no nematodes could be extracted from roots of arecanut. This indicated that the coconut population did not infect arecanut (Fig.28). Large number of nematodes recovered from banana roots suggested that it was more favoured than the other hosts.

Cross infectivity behaviour of arecanut isolate towards pepper, banana and coconut

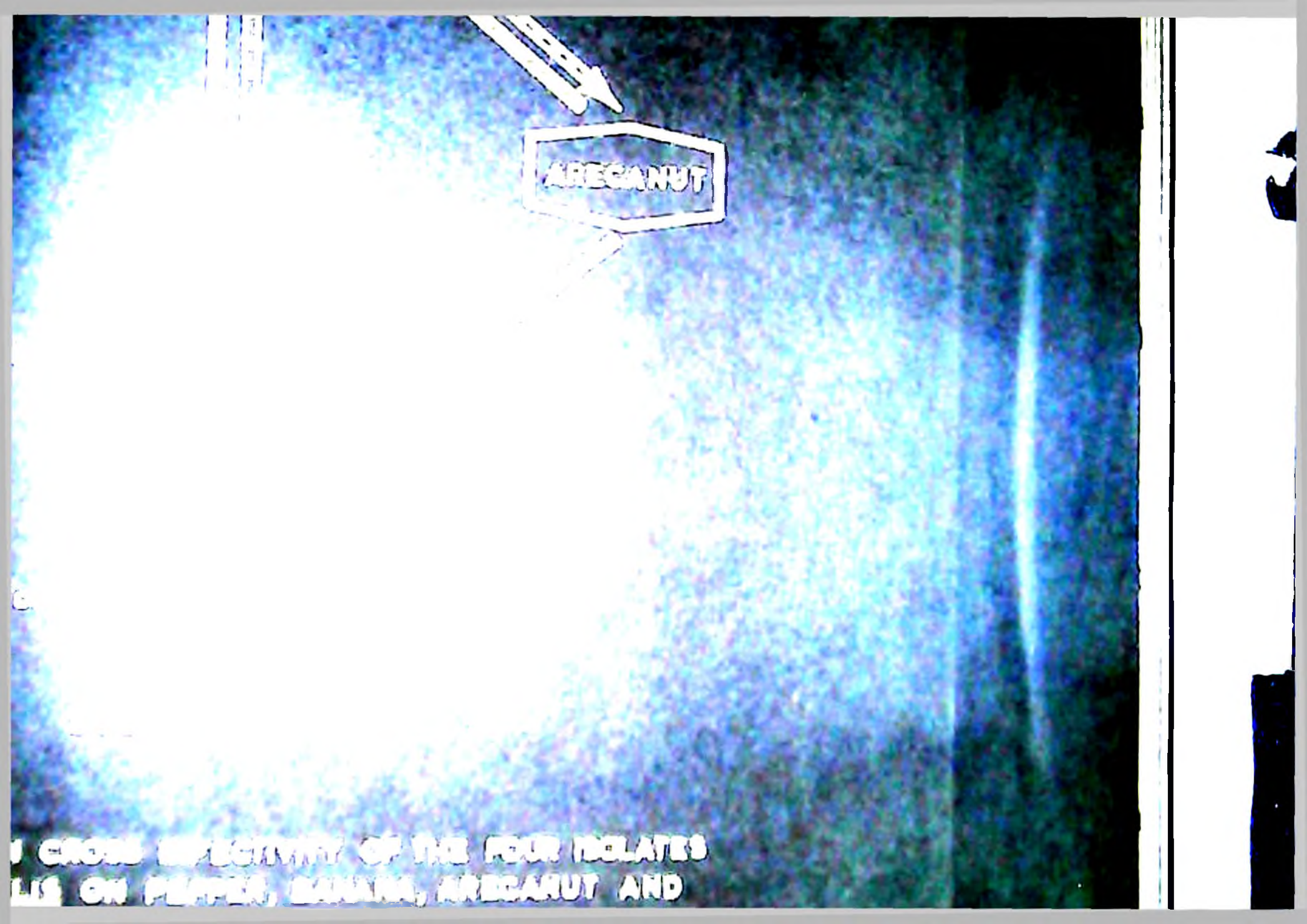
Examination of depotted plants revealed intermingling and entanglement of root system of both the host plants in each pots. The number of nematodes recovered from the roots of original and susceptible hosts and from soil are presented in the Table XYI. Root examination showed well developed lesions, partial dark discolouration and rotting on roots of banana and pepper, but not on roots of coconut. Lesions teased on a glass slide in few drops of water and examined showed nematodes of all stages of development. Eventhough, the pot was planted with both hosts, R.similis was recovered from roots of banana, pepper and arecanut but not from coconut. Thus the observations indicated that the arecanut isolate was capable of infecting banana and pepper only (Fig.28). More number of nematodes recovered from banana again suggested that this host was the more favoured one for the nematode than the other two hosts.

TABLE XXI

Cross infectivity of "arecanut isolate" of R.similis to banana, coconut and pepper by "double plant"method. Root weight and number of nematodes extracted from roots and soil at 140 days after planting the host plants.

Host plants	Root weight (g)	Number of nematodes extracted	
		Roots per plant	Soil per 100 ml*
Arecanut	22.86	887	41
Banana	34.57	2065	
Arecanut	27.29	928	38
Coconut	37.20	nil	
Arecanut	18.40	689	33
Pepper	7.74	487	

* From the same pot


ARECA NUT

Morphological characters and body measurements of the four isolates of R.similis

The details of measurements of females and males of the four isolates of R.similis population are presented in Tables XXII and XXIII respectively. Measurements on all criteria used for this nematode genus were recorded. The mean body measurements of different isolates of R.similis showed slight variations which were not significant. The range in the measur ments recorded were within the morphologic limits of R.similis. Critical examination of body organs did not reveal any special features or deviations from the already recorded characters.

Screening of black pepper cultivars and wild species against R.similis

Nine cultivars each, from Kerala and Karnataka, two cultivated species, two wild species and five wild collections of Piper were screened for their resistance against R.similis. Observations were recorded on root weights of plants and nematodes recovered from soil and roots. These are summarised in Table XXIV. It was observed that none of the plant types showed immunity or resistance to R.similis.

The root weight reduction and nematode multiplication rate (the factor on final population increase over initial inoculum) for each of the plant types are given in Table XXV (Fig.29). Root weight reduction of over 60 per cent due to R.similis was observed in 23 types whereas in four types

LE XXII

...s of R.similis from Pepper, Banana, Coconut and Arecanut

	♂	H (μ)	CF (μ)	BW (μ)	○	Stylet (μ)	Vulva %			
		3.51	10	3.75	13	20.8	17.7	58.61	3.61	49.2	1.37
...1		3.5-4.2	9-12	3-4	12-14	19-14	17-19	55.7-65.7	3.1-4.1	47.0-55.5	1.21-1
		3.2	12.7	4.7	15.3	18	18.1	56.07	3.54	47.36	1.17
...0		2.9-3.7	9-16	4-5	15-16	13-24	17-20	53.4-60.2	3.2-4.1	44.4-51.0	1.1-1.
		3.9	11.6	4.7	14	22	19.5	55.3	3.47	52.3	1.25
...5		3.4-4.4	9-18	4-5	13-16	19-25	17-20	53.2-59.3	3.1-3.7	47-55.5	1.2-1.3
		4.3	10	4.7	15.8	19.4	17.8	56.4	3.5	51.6	1.12
...5		3.5-4.8	9-14	4-5	15-17	16-24	17-19	51.4-60.8	3.1-3.8	50-55.5	1.06-1.

...x 100 ÷ total stylet length

Table

Body measurements (in water) of adult fema

Populations	L (μ)	a	b	b'	c
Pepper					
(Mean of 12 ♀♀)	592	27.0	7.0	4.2	9.2
(Range)	481-692	25.2-31.6	6.4-7.7	3.9-4.6	8.1-1
Banana					
(Mean of 12 ♀♀)	674	26.7	8.0	5.5	10.6
(Range)	539-783	25-29	5.8-9.9	4.2-6.5	9.1-1
Coconut					
(Mean of 10 ♀♀)	642	26.7	8.1	4.4	9.5
(Range)	522-721	24.7-29.5	7.5-8.9	4.1-4.8	8.1-1(
Arecanut					
(Mean of 10 ♀♀)	625	26.2	6.0	4.5	9.7
(Range)	540-716	23.2-28.6	5.2-7.0	3.8-5.3	8.5-1(

1. H = Hyaline area length
2. CP = Cephalic frame
3. BW = Body width at spear base
4. at = Tail length ÷ stylet length
5. M = Length of needle (Anterior part) of style
6. S = Stylet length + body width at stylet ba

TABLE XXIII

Body measurements in water o[illegible] adult males of R.similis from Pepper, Banana, [illegible]

Populations	L (µ)	a	b	b'	c	c'	[illegible] µ	[illegible] µ	[illegible] µ	Spicules (µ)	[illegible]
Pepper											
(Mean of 11 ♂♂)	[illegible]	[illegible]	[illegible]	[illegible]	5.5	4.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(Range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]–5.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Banana											
(Mean of [illegible] ♂♂)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(Range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Coconut											
(Mean of [illegible] ♂♂)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(Range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Arecanut											
(Mean of [illegible] ♂♂)	[illegible]	[illegible]	[illegible]	4.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(Range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE XXIV

Screening of black pepper cultivars and wild species, against P.similis. Root weight (g) of plants and nematode population extracted from soil and roots.

(Mean of two replicates)

Cultivar/ Wild species	Root weight of check plant	Inoculated plants				
		Root weight	% root weight to check	Nematodes		Total nematodes
				Soil	Root	
Karimunda	1.345	0.450	33.96	38	572	610
Kalluvally	1.610	0.150	9.29	17	236	253
Othirankotta	1.825	0.705	38.35	17	57	74
Panniyur-I	1.790	0.155	8.64	256	510	766
Kottanadan	1.250	0.230	18.40	37	195	232
Balankotta	1.470	0.420	21.45	39	198	237
Arakulam munda	1.970	0.710	35.55	69	466	535
Cheriyakaniya-kadan	1.620	0.255	15.70	120	370	490
Mundi-odi	1.570	0.505	32.20	63	297	360
Vakkal kunja	1.450	0.400	27.40	14	98	112
Singarem[illegible]	1.275	0.270	21.15	33	193	226
Doddiga	1.[illegible]35	0.550	29.45	26	135	161
Mortiga	1.[illegible]50	0.[illegible]50	26.65	35	214	249
Motakare	1.360	0.385	28.20	36	166	202
Karemenain ai	1.[illegible]00	0.425	27.03	25	262	287
Uddakare	1.490	0.350	23.50	58	254	312
Karemellaippanam	1.[illegible]00	0.400	23.80	57	284	341
Belemellaippanam kara	1.390	0.260	18.70	48	329	377
Piper longum	2.120	1.800	84.82	34	436	470
Piper betle	[illegible].600	1.00[illegible]	27.78	8	77	85
Piper attenuatum	1.870	1.335	69.10	39	81	120
Piper hymenophyllum	1.540	1.210	78.50	40	74	114
Wild collection (Vittal) No.430	1.100	0.915	81.70	44	31	75
-do- No.341	1.750	0.430	24.50	74	841	915
-do- No.348	1.560	0.395	25.30	62	255	317
-do- (Tamil Nadu) No.3	1.[illegible]00	0.235	21.50	48	398	446
No.7	1.200	0.450	37.50	69	239	308

TABLE XXV

Screening of black pepper cultivars and wild species, against R.similis. Effect on per cent of root reduction and nematode multiplication rate.

(Mean of two replicates)

Cultivar/wild species	Root reduction	Multiplication rate
Karimunda ..	66.04	6.10
Kalluvally ..	90.71	2.53
Othirankotta ..	61.65	0.74
Panniyur-I ..	91.36	7.66
Kottanadan ..	81.60	2.32
Balankotta ..	78.55	2.37
Arakulam munda ..	64.15	5.35
Cheriyakaniyakadan ..	84.30	4.90
Mundikodi ..	67.80	3.60
Vakkal gunja ..	72.60	1.12
Singaremane ..	78.85	2.26
Doddigya ..	70.55	1.61
Mortiga ..	73.35	2.49
Matakare ..	71.80	2.02
Karemensinkai ..	72.97	2.87
Uddakare ..	76.50	3.12
Karamellemenasa ..	76.20	3.41
Belemellemenasa ..	81.30	3.77
Piper longum ..	15.15	4.70
Piper betle ..	72.22	0.85
Piper attenuatum ..	30.90	1.20
Piper hymenophyllum ..	21.50	1.14
Wild collection (Vittal) No.430	18.30	0.75
-do- " No.341	75.45	9.15
-do- " No.348	74.70	3.17
-do- (Tamil Nadu) No. 3	78.50	4.46
-do- -do- No. 7	62.50	3.08
S.Em ±	15.283	0.7016
C.D at 1% level	42.487	1.9506

Fig. 29. Screening of pepper cultivars and wild species against R.similis per cent root reduction and nematode reproduction rate at 90 days after nematode inoculation

Cultivars/wild species

1. Karimunda
2. Kalluvally
3. Othirankotta
4. Panniyur-I
5. Kottanadan
6. Balankotta
7. Arakulam munda
8. Cheriyakeniyakaran
9. Mundikodi
10. Vakkal gunja
11. Singaremane
12. Doddigya
13. Mortiga
14. [illegible]
15. [illegible]
16. [illegible]
17. [illegible]
18. [illegible]
19. Piper longum
20. Piper betle
21. Piper attenuatum
22. Piper hymenophyllum
23. Wild collection (Vittal) No.430
24. " No.341
25. " No.348
26. " (Tamilnadu) No. 3
27. " No. 7

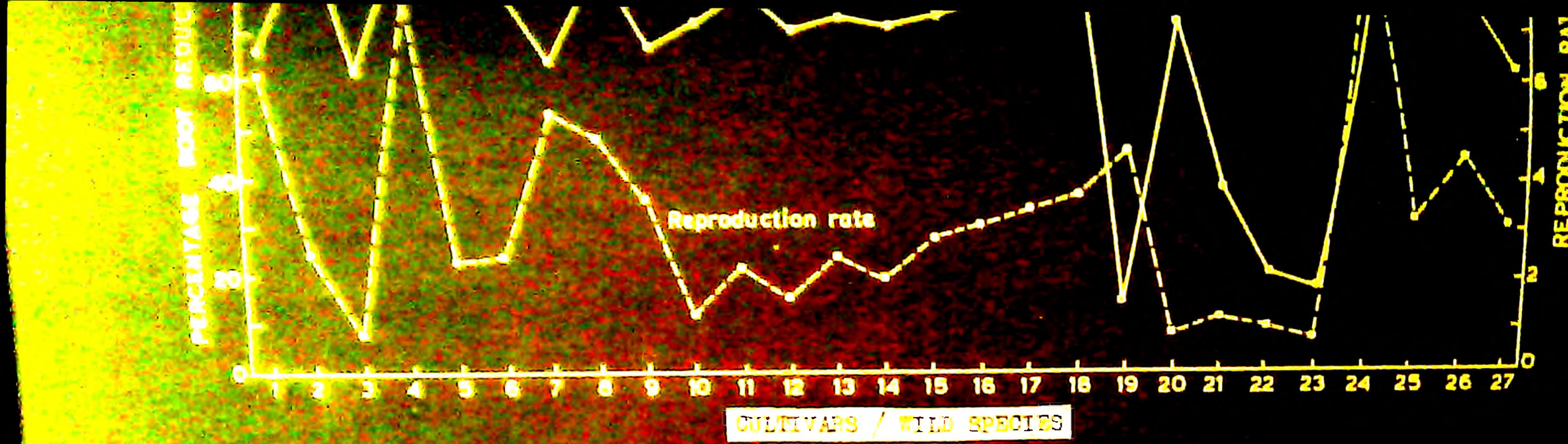


FIG. 29. SCREENING OF PEPPER CULTIVARS AND WILD SPECIES AGAINST R. SIMILIS. PER CENT ROOT REDUCTIO AND NEMATODE REPRODUCTION RATE AT 90 DAYS AFTER NEMATODE INOCULATION

namely P.longum, P.hymenophyllum, P.attenuatum and wild collection (Vittal) No.430 the per cent reduction of root weights were 15.15, 21.50, 18.30 and 30.90 respectively. The maximum multiplication rate of 7.66 times and root weight reduction of 91.36 per cent were recorded in Panniyur - I. In case of Cheriyakaniyakadan, Bellemellegessara and wild collection (Vittal) No.341, the root reduction varied from 75.45 to 84.3 per cent and the multiplication rate from 3.47 to 4.9 times. Though the nematode multiplication rate was from 3.6 to 6.1 times in Arakulammunda, Karimunda and Mundikodi the root reduction ranged from 64.15 to 67.8 per cent. In Kalluvelly the multiplication rate was observed to be 2.53 times, but the root reduction was as high as 90.7 times. Balankotta and Kottanadan though recorded, root reduction of 78.55 and 81.66 per cent respectively the multiplication rate in them was only 2.3 times. Othirankotta recorded a decline in final population of 0.74 per cent but showed root reduction of 61.65 per cent.

Though the multiplication rates in Karnataka cultivars ranged from 1.12 to 3.77 times, the root weight reduction recorded in them ranged between 70.5 to 81.3 per cent indicating that they were very susceptible. P.betle also recorded a decline in the final population, however it showed root weight reduction of 72.22 per cent. The plant types screened were

classified mainly into four groups based on their susceptibility reaction towards R.similis. The details on the groups are presented in Table XXVI.

Chemical control of R.similis on black pepper

The aim of this experiment was to know the reaction of pepper plants to various treatments of neem cake and three nematicides and their efficacy in controlling R.similis on pepper. The growth characters like shoot length, fresh shoot weight, leaf production, root length, fresh root weight and final nematode population were recorded with respect to the treatments adopted. A list of treatments are presented in Table XXVII.

Effect on shoot length

The shoot length of plants under various treatments at 90 and 150 days after nematicidal application are summarised in Table XXVIII and XXIX. Statistical analysis revealed significant differences between check and various treatments, between different nematicides, their dosages and methods of application. The increase in shoot length of check plant at 90 days was only 4.6 cm compared to 11.56 cm in plants under T 12 followed by T 24, T 21 and T 11 treatments. This increase in shoot length by these treatments was over 2.25 times compared to the increase of 1.1 - 2.1 times in rest of the treatments. The shoot length at 150 days after nematicidal

TABLE XXVI

Screening of black pepper cultivars and wild species, against R.similis- Reaction of the types tested

Highly susceptible	Susceptible		Less susceptible
High MR* > 3.0 High RR** > 75%	High MR 1.5-3.0 or Low RR 33-75%	High RR 33-75% Low MR 1.5-3.0	Low MR < 1.5 Low RR < 33%
Panniyur-I	Karimunda	Kalluvally	Wild collection (Vittal) 430
Cheriyakanialadan	Aralulammunda	Balankotta	P.hymenophyllum
Belemellegassara	Mundiyodi	Kottenadan	P.Attennuatum
Aaremellegassara	Wild collection (Vittal) 348	Valkalgunja	
Uddakkare	V.C.(T.Nadu) 7 (Wild collection)	Singaremane	
Wild collection(T.Nadu) 3	P. longum	Doddigyya	
Wild collection(Vittal)341		Kotalaro	
		Karemeneinkai	
		Othirankotta	
		P. betle	

* MR = Multiplication rate

** RR = Root Reduction

TABLE XXVII

Chemical control of R.similis on black pepper.
List of treatments

Code No.	Pre-inoculation treatments		Code No.	Post-inoculation treatments	
T 1	Neem cake	@ 1250 kg/ha	T13	Neem cake	@ 1250 kg/ha
T 2	-do-	@ 2500 kg/ha	T14	-do-	@ 2500 kg/ha
T 3	-do-	@ 5000 kg/ha	T15	-do-	@ 5000 kg/ha
T 4	D B C P	@ 22 l/ha	T16	D B C P	@ 22 l/ha
T 5	-do-	@ 33 l/ha	T17	-do-	@ 33 l/ha
T 6	-do-	@ 44 l/ha	T18	-do-	@ 44 l/ha
T 7	Fensulfothion	@ 2 kg/ha	T19	Fensulfothion	@ 2 kg/ha
T 8	-do-	@ 4 kg/ha	T20	-do-	@ 4 kg/ha
T 9	-do-	@ 8 kg/ha	T21	-do-	@ 8 kg/ha
T10	Aldicarbsulfone	@ 2 kg/ha	T22	Aldicarbsulfone	@ 2 kg/ha
T11	-do-	@ 4 kg/ha	T23	-do-	@ 4 kg/ha
T12	-do-	@ 8 kg/ha	T24	-do-	@ 8 kg/ha
			T25	Check	

Note: 1) T1, T13, T4, T16, T7, T19, T10, T22 are considered low dose level

2) T2, T14, T5, T17, T8, T20, T11, T23 are considered medium dose level

3) T3, T15, T6, T18, T9, T21, T12, T24 are considered high dose level

TABLE XXVIII

Chemical control of R.similis on black pepper. Effect on shoot length (cm) at 90 days after nematicidal application (Mean of three replicates)

Ts*	Sl*	Ts	Sl	Ts	Sl	Ts	Sl
T1	5.26	T7	6.63	T13	5.1	T19	8.43
T2	6.73	T8	7.73	T14	6.0	T20	9.6
T3	6.93	T9	8.26	T15	5.76	T21	11.03
T4	3.93	T10	9.7	T16	8.53	T22	8.63
T5	5.86	T11	10.3	T17	6.63	T23	9.43
T6	6.13	T12	11.56	T18	7.4	T24	11.53
						T25	4.6

*Ts=Treatments

*Sl=Shoot length

Nematicides x dosages

Nematicides	Low	Dosage levels Medium	High	Mean
Neem cake	31.1	38.3	38.1	5.9
D B C P	37.4	37.5	40.6	6.1
Fensulfothion	45.2	52.0	57.0	8.6
Aldicarbsulfone	55.0	60.2	69.3	10.2
Mean	7.0	7.8	8.5	

Nematicides x methods of application

Nematicides	Application method: Pre-inoculation	Post inoculation
Neem cake	56.9	50.6
D B C P	47.8	67.7
Fensulfothion	67.9	87.2
Aldicarbsulfone	95.7	88.8
Mean	7.4	8.1

TABLE XXVIII - (Contd.)

Dosages x method of application

Dosages	Application method		Mean
	Pre inoculation	Post inoculation	
Low ..	76.6	92.1	7.0
Medium ..	93.0	95.0	7.8
High ..	98.7	107.2	8.5
Mean ..	7.4	8.1	

Treatments	C.D at 5% level	C.D at 1% level
Between Nematicides	0.3712	0.9948
Dosages	0.3214	0.8614
Methods	0.2625	0.7035
Nematicides x dosages	NS	
Nematicides x methods	0.2756	0.7386
Dosages x methods	NS	
Check vs treatments	0.9093	2.4369

TABLE XXIX

Chemical control of *R.similis* on black pepper. Effect on shoot length (cm) at 150 days after nematicidal application.

(Mean of three replicates)

Ts*	Sl*	Ts	Sl	Ts	Sl	Ts	Sl
T1	8.4	T 7	21.6	T13	8.4	T19	13.9
T2	8.9	T 8	22.8	T14	8.6	T20	16.3
T3	9.1	T 9	24.9	T15	9.7	T21	25.0
T4	13.1	T10	21.8	T16	12.8	T22	23.2
T5	15.5	T11	23.6	T17	12.7	T23	23.9
T6	18.2	T12	27.8	T18	16.5	T24	29.4
						T25	8.1

*Ts=Treatmen

*Sl= Shoot length

Nematicides x dosages

Nematicides x methods of application

Nemati-cides	Dosage level			Mean	Method of application	
	Low	Medium	High		Pre inoculation	Post inoculation
Neem cake	50.6	52.7	56.5	8.8	79.4	80.4
D B C P	78.0	84.9	104.3	14.7	140.8	126.4
Fensulfothion	106.9	117.7	150.0	20.8	208.4	166.2
Aldicarb-sulfone	135.4	142.7	171.7	24.9	219.8	230.0
Mean	15.4	16.5	20.1		18.0	16.7

TABLE XXIX - (Contd.)

Dosages x methods of application

Dosages	Methods of application		Mean
	Pre inoculation	Post inoculation	
Low	195.4	175.5	15.4
Medium	212.9	185.1	16.5
High	240.1	242.4	20.1
Mean	16.0	16.7	

Treatments	S.Ed ±	C D at 1% level
Between		
Nematicides	0.4163	1.1157
Dosages	0.3606	0.9664
Methods	0.2944	0.7890
Nematicides x dosages	0.7211	1.9325
Nematicides x methods	0.3467	0.9292
Dosages x methods	0.5099	1.3665
Check vs treatments	1.0198	2.7331

application in check plants was 8.1 cm compared to the maximum shoot length of 29.4 cm in plants under T 24 followed by T 12, T 21 and T 9 with 27.8 cm, 25.0 cm and 24.9 cm respectively (Fig.30). Thus increase in shoot length recorded at 150 days after nematicidal application was 3.64 times higher in T 24 treatment and varied between 1.5 - 3.43 times in other treatments compared to check plants. But in case of all treatments with neem cake the increase over the check was only between 1.04 -1.20 times which was very negligible. The results indicated that at 90 days, fensulfothion and D B C P were more effective by post - inoculation application, in increasing shoot length, whereas aldicarb sulfone was more effective when applied before nematode inoculation. At 150 days the pre-inoculation application of chemicals in general, were effective in increasing the shoot length, whereas aldicar sulfone was found more effective as post-inoculation application. Neem cake was least effective. Among chemicals tried, aldicarb sulfone and fensulfothion at the rate of 8 kg per hectare irrespective of method of application were found more effective in inducing shoot length.

Effect on fresh shoot weight

The data on the fresh shoot weight of plants at 150 days after application of nematicides are presented in Table XXX (Fig.31). Statistical analysis revealed significant differences between check and all other treatments,

TABLE XXX

Chemical control of R.similis on black pepper. Effect on fresh weight of shoot(g) at 150 days after nematicidal application

(Mean of three replicates)

Ts	WS	Ts	WS	Ts	WS	Ts	WS
T1	10.4	T7	16.5	T13	11.6	T19	12.6
T2	12.4	T8	21.5	T14	12.6	T20	18.9
T3	13.2	T9	26.5	T15	14.3	T21	30.7
T4	8.3	T10	23.5	T16	11.3	T22	18.5
T5	12.1	T11	24.6	T17	15.9	T23	27.4
T6	14.7	T12	31.0	T18	18.6	T24	35.4
						T25	5.4

Ts = Treatments WS = Fresh weight of shoot per plant

Nematicides x dosage

Nematicides	Dosage levels			Mean
	Low	Medium	High	
Neem-cake	66.3	75.[illegible]	[illegible].6	[illegible].1
D B C P	59.0	83.9	1[illegible].1	13.[illegible]
Fensul-fothion	87.5	121.3	177.[illegible]	21.4
Aldicarb-sulfone	126.4	156.3	199.5	26.8
Mean	14.1	18.2	23.4	

Nematicides x methods of application

Methods of application		
	Pre-inoculation	Post-inoculation
Neem-cake	1[illegible].3	115.6
D B C P	105.[illegible]	137.5
Fensul-fothion	199.8	186.8
Aldicarb-sulfone	237.7	244.5
Mean	18.1	19.0

TABLE XXX - (Contd.)

Dosages x methods of application

Dosages	Methods of application		Mean
	Pre-inoculation	Post inoculation	
Low ..	176.9	162.3	14.1
Medium ..	211.9	224.6	18.2
High ..	262.5	297.5	23.4
Mean..	18.1	19.0	

Treatments	S.Em. ±	C D at 1% level
Between		
Nematicides	0.4359	1.1682
Dosages	0.3775	1.0111
Methods	0.3082	0.8260
Nematicides x dosages	0.7549	2.0231
Nematicides x methods	0.6164	1.6520
Dosages x methods	0.5339	1.4309
Check vs treatments	1.0677	2.8614

BLACK PEPPER. EFFECT ON SHOOT PRODUCTION (nos) AT 180 DAYS

between nematicides, different dosages and methods of application. Significant differences were also observed between the interaction effects due to main factors, Among nematicides tried, aldicarb sulfone was most effective by both methods of application in increasing shoot weight of plants, which was 6 - 7 times more over check plants. This chemical was also superior to all other chemicals in their respective dosages in effecting increased shoot weight. Post-inoculation of chemicals was in general superior to pre-inoculation application. Aldicarb sulfone at low dosage or fensulfothion at medium dosage were found more effective than D B C P at high dosage. Post-inoculation application of neem cake, D B C P and aldicarb sulfone resulted in better shoot weight whereas pre-inoculation of fensulfothion was more effective.

Effect on leaf production

The number of leaves produced by plants under various treatments at 150 days after nematicidal application are summarised in Table XXXI (Fig.32). Statistical analysis of data showed significant differences between check and various treatments, between nematicides and their three dosages. The two methods of application did not show significant difference on leaf production. Plants under T 24 produced maximum number of 7.6 leaves per plant as compared to 3.3 leaves produced by the check plants, which showed a two fold increase over check plants. Aldicarb sulfone was most effective for leaf producti

TABLE XXXI

Chemical control of R.similis on black pepper. Effect on leaf production at 150 days after nematicidal application

(Mean of three replicates)

Ts	Lp	Ts	Lp	Ts	Lp	Ts	Lp
T1	3.3 (1.821)	T7	4.0 (1.989)	T13	3.6 (1.911)	T19	4.0 (1.989)
T2	4.3 (2.079)	T8	4.3 (2.079)	T14	4.0 (1.989)	T20	4.3 (2.079)
T3	4.3 (2.079)	T9	4.3 (2.079)	T15	4.3 (2.079)	T21	5.3 (2.307)
T4	3.0 (1.732)	T10	5.3 (2.307)	T16	3.6 (1.911)	T22	4.3 (2.079)
T5	3.6 (1.911)	T11	5.6 (2.379)	T17	4.0 (1.989)	T23	5.0 (2.229)
T6	4.0 (1.989)	T12	7.0 (2.641)	T18	4.3 (2.079)	T24	7.6 (2.759)
						T25	3.3 (1.821)

Values in parentheses are $x + 1$ log transformed mean

Ts = Treatments

Lp = Number of leaves produced per plant

TABLE XXXI - (Contd.)

Nematicides x dosages

Nematicides x methods of application

	Dosage levels			Mean	Methods of application	
	Low	Medium	High		Pre-inoculation	Post-inoculation
Neem-cake	11.196	12.204	12.472	1.993	17.936	17.936
B B C P	10.928	11.700	12.204	1.934	16.896	17.936
Fensulfothion	11.936	12.472	13.158	2.087	18.440	19.126
Aldicarb sulfone	13.158	13.822	16.202	2.379	21.982	21.200
Mean	1.55	2.091	2.251			

Dosages x methods of application

Dosages	Methods of application	
	Pre-inoculation	Post-inoculation
Low	23.550	23.668
Medium	25.340	24.858
High	26.364	27.672

Treatments	S.Em ±	C D 1% level
Between		
Nematicides	0.0616	0.1651
Dosages	0.0529	0.1418
Methods	NS	
Nematicides x dosages	NS	
Nematicides x methods	NS	
Dosages x methods	NS	
Check Vs treatments	0.1507	0.4039

followed by fensulfothion. The effect on leaf production by fensulfothion at high dosage was equal to low or medium dosage on aldicarb sulfone. Neem cake and D B C P showed equal effect on leaf production at all the three dosages.

Effect on root length

Root length of plants under various treatments at 150 days of nematicidal application are presented in Table XXXII (Fig.33). Statistical analysis revealed significant differences, between nematicides, their dosages and between check and all the other treatments. The maximum root length was observed in plants under T 24, with 273.4 cm followed by plants in T 12, T 21, T 11, T 23 and T 9. The check plants recorded only 30.6 cm root length, which is 8.9 times less than the maximum recorded root length. Among the chemicals tested, aldicarb sulfone at high dosage resulted in maximum root length, followed by fensulfothion, D B C P, and neem cake. Aldicarb sulfone at medium dosage showed equal effect as fensulfothion at high dosage, in root length production of plants. fensulfothion at low dosage, D B C P at medium dosage or neem cake at high dosage showed equal effect. Similarly aldicarb sulfone at low dosage was more effective than D B C P or neem cake at their high dosage. Thus aldicarb sulfone was found superior over other chemicals tested.

TABLE XXXII

Chemical control of R.similis on black pepper. Effect on root length (cm) at 150 days after nematicidal application.

(Mean of three replicates)

Ts	Rl	Ts	Rl	Ts	Rl	Ts	Rl
T1	34.6 (5.88)	T7	119.0 (10.91)	T13	36.9 (6.10)	T19	118.1 (10.87)
T2	61.8 (7.76)	T8	185.5 (13.62)	T14	69.1 (8.33)	T20	176.4 (13.28)
T3	119.4 (10.93)	T9	247.9 (15.76)	T15	123.6 (11.13)	T21	255.7 (16.00)
T4	72.4 (8.81)	T10	210.9 (14.51)	T16	91.3 (9.57)	T22	213.9 (14.62)
T5	119.5 (10.93)	T11	254.2 (15.93)	T17	116.8 (10.81)	T23	246.1 (15.69)
T6	144.9 (12.04)	T12	270.3 (16.44)	T18	156.4 (12.43)	T24	273.4 (16.53)
						T25	30.6 (5.56)

Ts = Treatments

Rl = Root length per plant

Values in parentheses given are square root transformation

TABLE XXXII

Chemical control of R.similis on black pepper. Effect on root length (cm) at 150 days after nematicidal application.

(Mean of three replicates)

Ts	Rl	Ts	Rl	Ts	Rl	Ts	Rl
T1	34.6 (5.88)	T7	119.0 (10.91)	T13	36.9 (6.10)	T19	118.1 (10.87)
T2	61.8 (7.86)	T8	185.5 (13.62)	T14	69.1 (8.33)	T20	176.4 (13.28)
T3	119.4 (10.93)	T9	247.9 (15.76)	T15	123.6 (11.13)	T21	255.7 (16.00)
T4	72.4 (8.81)	T10	210.8 (14.51)	T16	91.3 (9.57)	T22	213.9 (14.62)
T5	119.5 (10.93)	T11	255.2 (15.98)	T17	116.8 (10.81)	T23	246.1 (15.69)
T6	144.9 (12.04)	T12	270.3 (16.44)	T18	156.4 (12.43)	T24	273.4 (16.53)
						T25	30.6 (5.56)

Ts = Treatments Rl = Root length per plant

Values in parentheses given are square root transformation.

TABLE XXXII - (Contd.)

Nematicides x dosages

Nematicides x methods of application

Nematicides	Dosage levels			Mean	Methods of application	
	Low	Medium	High		Pre-inoculation	Post inoculation
Neem cake	35.94	49.13	66.20	8.91	74.67	76.60
D B C P	55.13	65.24	73.43	10.76	95.33	98.45
Fensulfothion	65.35	80.69	95.27	13.91	120.86	120.45
Aldicarb-sulfone	87.38	95.00	98.91	15.63	140.79	140.50
Mean	10.16	12.08	13.91			

Dosages x methods of application

Dosages	Methods of application	
	Pre-inoculation	Post inoculation
Low ..	120.32	123.48
Medium ..	145.84	144.22
High ..	165.51	168.30

	Treatments	S.Em ±	C D at 1 level
Between			
	Nematicides ..	0.4583	1.2282
	Dosages ..	0.3969	1.0637
	Methods ..	NS	
	Nematicides x dosages	NS	
	Nematicides x methods	NS	
	Dosages x methods	NS	
	Check x treatments	1.1225	3.0083

Effect on fresh root weight

The data collected on fresh root weight of plants under various treatments are summarised in Table XXXIII (Fig.33). Statistical analysis showed significant differences between the check and various treatments. Significant differences were also observed between the nematicides, three dosages, and the two methods of application . The maximum root weight was recorded by plants in treatment T 24 with 12.90 g as compared to 2.07 g by plants in treatment T 25, and the differences were over six folds. Among the nematicides, aldicarb sulfone was found superior at the three dosages compared to all the other nematicides. However better results were obtained with other nematicides under treatments T 21, T 12, T 23, and T 9 which recorded an increase in root weight of 5.4, 5.0, 4.8, and 4.7 times more respectively over the check. In general post-inoculation application of chemicals was found superior to pre-inoculation application.

Effect on the nematode population in roots and soil

The data on nematode counts recorded in all treatments from roots and soil are summarised in Table XXXIV. The details on final nematode population counts per plant at 150 days after nematicides application are presented in Table XXXV. The statistical analysis revealed high level of significance between the check and treatments, nematicides, their dosage and methods of application. Maximum reduction in total population was observed in T 24 followed by T 12, T 21, T 23

TABLE XXXIII

Chemical control of R.similis on black pepper. Effect on fresh root weight (g) at 150 days after nematicidal application
(Mean of three replicates)

Ts	Rw	Ts	Rw	Ts	Rw	Ts	Rw
T1	4.25	T7	6.26	T13	4.08	T19	4.81
T2	4.45	T8	7.35	T14	4.30	T20	7.70
T3	5.09	T9	9.74	T15	5.10	T21	11.30
T4	2.47	T10	8.07	T16	4.10	T22	7.90
T5	4.65	T11	8.54	T17	6.07	T23	9.90
T6	5.99	T12	11.27	T18	7.51	T24	12.90
						T25	2.07

Ts=Treatments
Rw=Fresh root weigh per plant

Nematicides x dosages

Nematicides	Dosage level			Mean
	Low	Medium	High	
Neem cake	25.00	26.27	30.61	4.55
D B C P	19.72	32.19	40.61	5.14
Fensulfothion	33.23	45.17	62.32	7.82
Aldicarb-sulfone	47.93	55.44	72.64	9.78
Mean	5.28	6.62	8.59	

Nematicides x methods of application

Nematicides	Method of application	
	Pre-inoculation	Post inoculation
Neem cake	41.41	40.47
D B C P	39.33	53.18
Fensulfothion	70.07	70.65
Aldicarb-sulfone	83.69	92.32
Mean	6.51	7.13

TABLE XXXIII - (Contd.)

Dosages vs methods of application

Dosages	Methods of application		
	Pre inoculation	Post inoculation	Mean
Low	63.18	62.70	5.28
Medium	75.01	84.05	6.62
High	93.31	109.87	8.59
Mean	6.51	7.13	

Treatments	S.Em ±	C D at 1% level
Between		
Nematicides	0.1414	0.3790
Dosages	0.1225	0.3283
Methods	0.1000	0.2680
Nematicides x dosages	0.2449	0.6563
Nematicides x methods	0.2000	0.5360
Dosages x methods	0.1732	0.4642
Check vs treatments	0.3464	0.9284

TABLE XXXIV

Chemical control of R.similis on black pepper. Effect on the number of nematodes recovered from roots and soil at 150 days after nematicides application

(Mean of three replicates)

Treatments	Number of nematodes recovered		
	In roots/ plant	In soil/ pot	Total
T1	724	905	1629
T2	666	765	1431
T3	522	685	1207
T4	582	580	1162
T5	529	390	919
T6	566	340	906
T7	311	335	646
T8	199	150	349
T9	87	95	182
T10	116	230	346
T11	28	85	113
T12	-	65	65
T13	833	898	1731
T14	665	738	1403
T15	496	713	1209
T16	553	625	1178
T17	501	435	936
T18	378	320	698
T19	287	190	477
T20	68	133	201
T21	28	70	98
T22	96	325	421
T23	69	35	104
T24	-	15	15
T25	2634	925	3559

TABLE XXXV

Chemical control of R.similis on black pepper. Effect on final nematode population per plant/pot at 150 days after nematicide application

(Mean of three replicates)

Ts	Np	Ts	Np	Ts	Np	Ts	Np
T1	1629 (40.36)	T7	646 (25.42)	T13	1731 (41.59)	T19	477 (21.83)
T2	1431 (37.78)	T8	340 (18.71)	T14	1403 (37.46)	T20	201 (14.18)
T3	1207 (34.75)	T9	182 (13.51)	T15	1209 (34.77)	T21	98 (9.86)
T4	1162 (34.10)	T10	346 (18.61)	T16	1178 (34.33)	T22	421 (20.49)
T5	919 (30.84)	T11	113 (10.61)	T17	936 (30.59)	T23	104 (10.22)
T6	906 (30.10)	T12	65 (8.67)	T18	698 (26.42)	T24	15 (3.52)
						T25	3559 (59.64)

Ts = Treatments

Np = Nematodes per plant/pot

Values in parentheses are $\sqrt{x+1}$ transformation

TABLE XXXV - (Contd.)

Nematicides x dosages

Nematicides x methods of application

Nematicides	Dosage levels			Mean	Methods of applicatio	
	Low	Medium	High		Pre-inoculation	Post inoculation
Neem-cake	245.887	225.783	208.587	37.792	338.731	341.526
D B C P	205.311	184.340	169.560	31.067	285.144	274.067
Fensulfothion	141.799	98.692	70.151	17.258	172.977	137.665
Aldicarb sulfone	117.335	62.512	34.784	11.924	111.893	102.738
Mean	29.597	23.805	20.128		25.243	23.777

TABLE XXXV - (Contd.)

Dosages x methods of application

Dosages	Methods of application		Mean
	Pre-inoculation	Post inoculation	
Low ..	355.523	354.809	29.597
Medium ..	293.909	277.418	23.805
High ..	259.313	223.769	20.128
Mean	25.243	23.777	

Treatments	S.Em ±	C D 1% level
Between		
Nematicides ..	0.4088	1.0956
Dosages ..	0.3539	0.9485
Methods ..	0.2891	0.7748
Nematicides x dosages ..	0.7080	1.8974
Nematicides x methods ..	0.5781	1.5493
Dosages x methods ..	0.5007	1.3419
Check vs treatments ..	1.0013	2.6835

Control of R.similis on black pepper

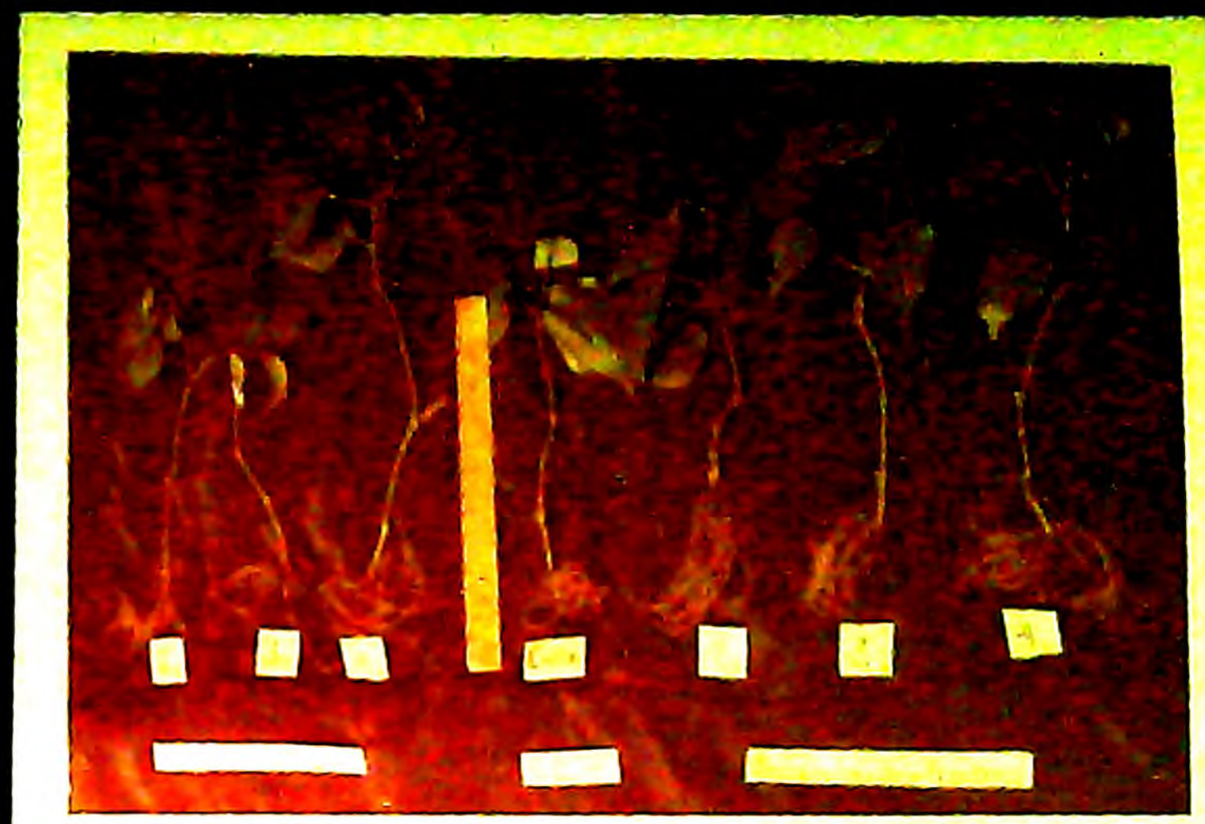
Fig. 35. Effect of Neem cake at three dosage levels, under two methods of applications at 150 days after nematicidal application on growth of vines and root development

Fig. 36. Effect of [illegible] at three dosage levels, under two methods of applications at 150 days after nematicidal application on growth of vines and root development

Fig. 37. Effect of Vensulfothion at three dosage levels, under two methods of applications at 150 days after nematicidal application on growth of vines and root development

Fig. 38. Effect of Aldicarb sulfone at three dosage levels, under two methods of applications at 150 days after nematicidal application on growth of vines and root development

Fig. 36



and T 11. In the above five treatments, the per cent reduction in original inoculum populations were 97, 87, 80.4, 79.2 and 77.4 respectively. Whereas the final nematode population in T 25 (check) increased to 3559 which was 7.1 times more than the original inoculum (Fig.34). Among the chemicals tried, aldicarb sulfone was found to be most effective, followed by fensulfothion, D b C P and neem cake (Figs.35, 36, 37 and 38). Aldicarb sulfone at high dosage prevented the roots from nematode infection. No nematodes were recovered from the roots of plants treated with this chemical. In general, population counts were much less in plants treated with aldicarb sulfone or fensulfothion. Fensulfothion at low dosage was not as effective as aldicarb sulfone, but at high dosage it was as effective as aldicarb sulfone at medium dosage. The post-inoculation treatments in general, were more effective in bringing down the nematode population, whereas in case of neem cake pre-inoculation treatment was found to be more effective.

DISCUSSION

V. DISCUSSION

The black pepper contributes a lion's share of foreign exchange earnings among the spice crops in India. Present reports indicate the prevalence of the "slow wilt" disease which is destroying the vines in the pepper growing tracts. Hence, it was considered imperative that a study be initiated to understand the nature of the problem, especially the role of the burrowing nematode, Radopholus similis in "slow wilt" disease.

This is the first time that such a study has been made in India. Though black pepper was recorded as a host of R.similis by Tom Goodey (1936), a preliminary study of the damage caused by this nematode was conducted only fourteen years later (van der Vecht, 1950). Christie, (1957) based on his tour conducted in the Island of Bangka, confirmed the findings of van der Vecht. Thorne (1961) considered this nematode as ranking high among the most important plant parasitic nematodes in the tropics and as the major economically important species in those regions. D'Souza et al. (1970) reported the occurrence of R.similis on pepper for the first time in South Western India. They did not investigate the problem further. Venkitesan (1972) reported the presence of this nematode on pepper in the northern parts of Kerala and suspected its association with "slow wilt" disease.

The field symptoms of "slow wilt" observed in the present study are similar to those reported by van der Vecht (1950) and Christie (1957) (Figs.2, 3 and 4). This is basically a root disease. Root rot and decay symptoms were observed in the case of affected vines. Above ground symptoms are manifested by occasional or partial to complete yellowing of leaves (Figs.2 and 3). Cessation of growth of vines was followed by gradual leaf drop and final death of vines (Fig.4). Examination of root system of affected vines showed extensive necrosis and decay of roots. Feeder roots were completely destroyed. In such cases the vines could be pulled out easily. From the time of infestation to death of the vines, a period of two to three years may elapse. The final stages of leaf drop to death of vines however occur in the course of three to four months. The disease comes to the notice of the farmer only at this final stage by which time the vines are irretrievably lost.

The present survey revealed the presence of R.similis in 25 out of 41 locations, in and around the disease suspected vines. This is 60.9 per cent of the total area surveyed. The nematode population was higher in the roots of diseased vines and the soil around the roots. The samples collected from healthy looking vines revealed the presence of the nematode in 18 out of 41 samples in the corresponding locations. Soil around healthy looking vines contained the nematode, but the roots of these vines contained practically no nematode. Wide

difference in nematode population density has been observed. This could probably be due to the time of sampling, effect of intercropping or different soil types in the diseased area (Tables VII and VIII).

The other plant parasitic forms encountered in the samples were Meloidogyne spp., Rotylenchulus spp., and Helicotylenchus spp. (Table 6). Their population levels did not vary markedly between the healthy and slow wilt affected vines and areas. R.similis was associated with several popular cultivars in the disease affected plantations (Table IX). Similar observations have been made by several workers who studied the nematode incited root diseases. Mountain and Boyce (1958), also reported similar findings investigating the peach replant problem in Canada who found that only 50 per cent of the orchards surveyed with a history of replant problem contained the suspected nematode pathogen Pratylenchus penetrans. They also observed a higher population in the samples in replant problem areas than in the healthy areas. Suit and DuCharme (1953) also observed a similar situation in respect of the spreading decline of citrus in Florida. They reported that frequent sampling may be necessary before the nematode was found. Failure to detect R.similis in all the samples collected during the survey is not therefore unusual and does not necessarily indicate that the disease is not associated with the nematode. Its wide

occurrence in the diseased areas and the presence of root lesions in all cases of diseased plants strongly point to R.similis being the incitent of slow wilt disease of pepper. Further the locations in which the nematode was not detected were not contiguous in the disease tract. These are scattered along with other locations from which the nematode was detected in the samples. Thus the survey data could be considered adequate to postulate the close association of the nematode with the slow wilt disease of pepper. The results of pathogenecity of R.similis on pepper reported in this thesis strengthen this conclusion.

The invasion of the roots by the nematodes leading to formation of lesions was studied. This was done in the laboratory, simulating as far as possible conditions prevailing in nature (fig.13). The invasion and establishment of the pathogen are closely similar to those reported by DuCharme (1959),in case of R.similis on citrus roots. Hand sections of the infected root showed that the nematode also feeds on the root parenchyma cells and multiplies in root tissues (Figs.18 and 19) These findings conform with the observations reported by van der Vecht (1950) in pepper, DuCharme (1959) in citrus and Vilsoni et al. (1976) in case of ginger infected by R.similis.

The inoculation experiments proved the pathogenicity of R.similis on pepper roots. Pathogenicity experiments were conducted as per the techniques reported by Mountain (1960) for

evaluating the plant nematode relationship. These experiments were conducted on whole plants (rooted cuttings) in pots with fumigated soil and with non-sterile monospecific inoculum. The enhancement of intensity of symptoms with increasing dosages of the pathogen was very striking (Figs.20, 21 and 23). The above ground symptoms observed on the plants in this experiment closely resembled the slow wilt symptoms in the field except for the lack of pronounced chlorosis in experimental plants. It is considered that these results conclusively establish the pathogenicity of R.similis on pepper and as the incitant of slow wilt disease. This the first report in this regard. Though van der Vecht (1950) inoculated pepper seedlings with R.similis, he did not conduct experiments with differential inoculum levels. He could not therefore arrive at the same threshold level of inoculum for proving pathogenic effect. The present results have shown that the threshold level of inoculum to induce visual symptoms on 55 days old rooted cuttings of pepper at the end of 150 days is 1000 nematodes per 1500 ml of soil or attaining a nema population level of about 2300 per gram of root. Likewise under field conditions whenever the population level increases to this stage, it may bring visible level of damage leading to the diseased condition of the vines. Results of the survey showed that the soil population around diseased vines ranged between 3-61 (average 21) nematodes per 100 ml soil. However this population level may vary depending upon the age and growth of vines and

soil factors. Van der Vecht (1950) could not also reproduce the yellowing of leaves in inoculated plants. However, symptoms of decline of above ground parts, necrosis and decay of roots and destruction of feeder roots were well reproduced and closely resembled field symptoms.

The role of other plant parasitic nematodes encountered in the soil samples in "slow wilt" disease is questionable. The conspicuous reduction in growth and prounced root rot in plants inoculated with R.similis at threshold level as above, strongly indicate that this nematode alone could cause all the symptoms of slow wilt. Further into (1972) reported that, M.incognita and M.javanica caused reduction in plant weight of pepper seedlings by 37.4 and 10.2 per cent respectively to those of controls. This he observed 10 months after of inoculation with these nematodes. Lumar et al. (1971) reported that P.nigrum is a poor host for Rotylenchulus reniformis and that its multiplication rate was less than two times. These observations indicated that the other nematodes found in pepper field soil do not have any major role in slow wilt disease. It is also probable that these nematodes are pathogens of the plants used as standards of pepper vines.

The rapid multiplication and high population build up in inoculated plants at low inoculum levels clearly indicated that pepper is a favourable host of R.similis. However there was a decline in population level at the highest inoculum level

of 10000. Similar results have been recorded with Praty-
lenchus thornei on wheat (Anon., 1971a). This phenomenon
can occur only when the reproductive potential of the pathogen
is dependent on and related to the availability of nutrition
and substratum. This was evident from the specimens of the
nematode with sluggish movement and empty body cavities re-
covered from soil washings of the above treatment. Suit and
DuCharme (1953) observed that H.similis invade feeder roots
of 1/16 inch diameter. The pepper plants used for pathogeni-
city test had fewer feeder roots. When few roots are inocu-
lated with such large numbers of nematodes as 10000, the
population per unit area of available root is so high, that
the feeder roots are quickly damaged. Fresh root production
was too slow to replace the roots lost. The high population
pressure and severe competition for food would have lead to
a high mortality which could not be compensated by the rate
of multiplication. Such a situation had been reported by
DuCharme and Price (1966). They postulated that such a pheno-
menon could also occur under natural conditions. They observed
that after 55 days, the actual population began to fall due to
population pressure.

The H.similis population isolated from pepper, when inocu-
lated to citrus, coffee, banana and pepper could infect, re-
produce and multiply only in pepper and banana indicating that

the pepper isolate belonged to a different race (Fig.28). Coffee was not infected, thus supporting the findings of D'Souza *et al.* (1970) who could not observe any infection of *R.similis* on coffee roots during their survey.

Cross infectivity behaviour of the four populations (isolated from banana, pepper, coconut and arecanut) indicated the presence of four "host types" (Fig.28). All populations infected banana and multiplied best on banana. The infectivity behaviour of the four populations was as follows. The pepper isolate did not infect coconut and arecanut, but infected banana. The banana isolate infected pepper, coconut and arecanut. The coconut isolate infected banana and pepper and not arecanut. The arecanut isolate infected banana and pepper but not coconut. It is difficult to say whether the four isolates encountered here represent four "races" of the nematode. For the present, therefore it may be advisable to designate them as "host types" as has been observed by Martin *et al.* (1969), Keetch (1972) and Milne and Keetch (1976). It is clear however that when pepper is interplanted with banana, arecanut or coconut, it is liable to be infected by "host types" from these plants and by its own "host type". Arecanut and coconut are also unsuitable standards for pepper since they encourage multiplication of their "host types" which pass on to pepper. Van der Vecht (1950) has pointed out that the probability and spread of infection to pepper could be from

infected banana plants. Edwards and Wehunt (1971) found the banana population of R.similis from Panama and Honduras had differential infectivity indicating additional races. In Kerala (Koshy, 1975) the coconut population was found infecting banana, pepper and arecanut. But the present studies indicated that the coconut population infect only pepper and banana and not arecanut. This indicates the possibility of a mixture of "host types" in Kerala coconut population, which might infect other hosts. Fischler (1901) and Steiner (1925) postulated a hypothesis of evolution of strains or host types or races with host selectivity. Van Heerdt (1957) also found that population of R.similis isolated from different hosts had inconsistent infectivity behaviour. Sturhan (1971) reported that every local population of R.similis had a considerable amount of genetic variability, regarding host specificity. This author also observed that he obtained isolates of R.similis only from pepper vine roots and not from arecanut roots used as standards for the pepper vine. A similar infectivity behaviour was observed by Das Gupta and Seshadri (1971) in case of Rotylenchulus reniformis with two populations, collected from Andhra Pradesh.

The four isolates did not differ significantly in measurements or morphology (Tables XXII and XXIII). The variation in body measurements were similar to those reported by Godfry (1931), DeCharme and Birchfield (1956), and Sher (1968) in the populations

from different hosts. Van Weerdt (1958) found that body measurements of progenies from a single female isolated from different hosts varied, but he was unable to separate them into different specific groups. Thorne (1961) suggested that variation of ten per cent or even more could be expected in any population. He further stated that specimens from favourable and unfavourable hosts may exhibit marked differences.

The pepper cultivars, wild collections and other Piper species screened did not reveal any immunity or resistence to R.similis. However, the materials were grouped into four categories, (Table XVI). This is based on their reaction to R.similis related to the per cent root reduction and multiplication rate of the pathogen in the host plant (Fig.29). The criteria used for such a classification are those of Rohde (1965). According to him, resistance is to be measured in terms of ability of the parasite to survive and multiply and not always directly related to plant growth. In their report (Anon, 1966a) the committee on nematode control also recommended that the rate of reproduction be determined while assessing resistance. Feder et al. (1959), screened several citrus plants against R.similis first by selective screening, subjecting the candidate seedlings for infection in soil tanks. Those showing less damage were later tested for the ability of nematodes to penetrate into roots, lay eggs and maintain

populations. The screening in the present studies was done using rooted cuttings and not seedlings. Genetic uniformity was therefore maintained. Day and Serr (1951) used an index-ing method based on the number and size of lesions on walnut roots against Pratylenchus vulnus. Such a method was impracti-cable in the present studies. This is due to the fact that pepper roots infected with R.similis changed to dark brown and turned black in colour. In such condition the lesions could not be distinguished clearly. Since the home of pepper is India (Abraham, 1959) and several wild species of Piper are known to occur in the hill region of the sub continent (Anon, 1959b) further screening has to be done to find some suitable resistant material. Moreover information on the degree of in-fection and damage likely to be caused by populations from other hosts are to be observed and studied.

The experimental studies on the control of R.similis indicated that aldicarb sulfone and fensulfothion at high dosage levels were effective in complete elimination of root infection and improvement of plant growth (Figs.33 and 35 to 38). None of the materials namely, aldicarb sulfone, fensulfothion, D B C P and neem cake were phytotoxic even at high doses tried to the pepper cuttings. The results obtained with aldicarb sulfone and fensulfothion were consistent with the three dosage levels tried as compared to neem cake and D B C P. Similar results were obtained using aldicarb on Calathea makoyana in-

fected with R.similis (Heungens, 1971) and on cabbage infested with Pratylenchus spp. (Davide and Comedis, 1972). Aldicarb sulfone is a sulfone derivative of aldicarb and this was the most promising nematicide among the four tried. Though D B C P is being recommended for control of R.similis in banana by various authors (Loos and Loos, 1960; Vilardebo and Robin, 1969; Keetch, 1973) the present results were not encouraging. D B C P penetration was insufficient for complete kill of this nematode in banana (Blake, 1961). It failed to control Pratylenchus penetrans in antirrhium and chrysanthemum and in reducing root and soil populations (Krumberg, 1971). Neem cake was practically ineffective.

The post-inoculation treatments (Fig.35 to 37) of chemicals was found superior to the pre-inoculation treatments. This effect was consistently significant in influencing the improvement of plant growth. Only in the case of shoot length of the plants, the pre-inoculation treatments (Fig.34) were found superior. This shows that the chemicals were more effective as therapeuants, inactivating and killing the invaded nematodes and also possibly preventing further infection. Reddy and Seshadri (1971) reported the same phenomenon in case of aldicarb treatment against M.incognita on tomato. The chemical aldicarb sulfone is a non-fumigant systemic nematicide and only intake into plant system and can act upon the nematodes while feeding by inactivating or killing them. Nelmes (1970) observed that aldicarb in water solution inhibited

locomotion in case of larvae of Heterodera rostochiensis. post-plant treatment given in citrus to control R.similis failed (Cohn, 1972) apparently because of poor soil penetration and diffusion of fumigants through sub-soil and the deep rooted nature of citrus trees, which aided in survival of nematodes in root-lets in deeper layers of soil. But R.similis was effectively controlled in citrus seedlings in closed containers when soil was treated with aqueous solutions of D B C P, fensulfothion (dasanit), and aldicarb (temik) (Suit, 1969). fensulfothion (dasanit) was used in granular form which might have accounted for its low efficacy, when compared to aldicarb sulfone which was used in aqueous solution and drenched around the plants.

The investigations carried out here revealed that R.similis had widespread occurrence and close association with the slow wilt disease of pepper. The pathogenecity experimental results proved that R.similis was the incitant of the slow wilt disease. The cross infectivity studies of the four isolates from pepper, banana, coconut and arecanut indicated the presence of four 'host types', and banana was a more favoured host for these four isolates. These findings have significant relevance to cropping pattern where banana is planted as a subsidiary crop in the initial phase of raising pure plantations of pepper, coconut or arecanut. Moreover, pepper could be infected by all the isolates. Hence caution has to be exercised while introducing pepper as mixed crop along with coconut or arecanut, which

are already infected with R.similis.

Cultivars and wild species of pepper screened for their resistance against R.similis showed that a few have less susceptibility. Screening all available germ plasm is therefore necessary which could serve as a source of resistance or tolerance for evolving suitable types. The chemical control results have shown that aldicarb sulfone, followed by fensulfothion (Dasanit) at eight kg a.i. per hectare prevented infection of R.similis on pepper. These nematicides could now be tried under field conditions to explore the possibilities of controlling this nematode and check the spread of the disease.

Thus the present investigations have opened a new thinking on the slow wilt disease problem and more lacunae exist in our knowledge on various aspects of this nematode and its host plant the pepper vine. It is not known whether all the common standard used for trailing pepper vines are also infected by this nematode. Studies on these aspects would be valuable to advocate effective control practices. Further research on the biology of the nematode, biochemical mechanisms involved in the host-parasite relationships and the ecological factors responsible for the occurrence, spread and severity, would be quite rewarding to control this nematode problem on black pepper.

SUMMARY

VI. SUMMARY

Investigations were carried out on the role of the burrowing nematode, Radopholus similis (Cobb), in the "slow wilt" disease of black pepper Piper nigrum L., prevalent in Karnataka and Kerala. The salient findings of these investigations are summarised below.

A survey was conducted in Karanataka and Kerala in the pepper growing areas where slow wilt disease is prevalent. A total of 28.5 and 29.3 hectares were covered by the survey in Karanataka and Kerala respectively. The percentages of disease incidence in the surveyed areas were 12.50 and 11.51 in Karnataka and Kerala respectively.

Samples from 41 locations collected from both the states, revealed the presence of R.similis in 18 healthy and in 25 disease suspected vines.

The population of R.similis ranged from 3 to 49 in healthy and 3 to 61 in diseased per 100 ml of soil around the vines. The population per gram root varied from 7 to 42 in healthy compared to 4 to 326 in diseased vines.

Meloidogyne spp., Rotylenchulus spp., and Helicotylenchus spp. were frequently encountered in the soil samples in the surveyed locations. Occasionally Criconemoides spp., Tylenchorhynchus spp. and Hoplolaimus spp. were also recorded. Pratylenchus spp. was observed both in soil and root samples only from Siddapur (Karnataka).

infected pepper and banana but not arecanut. Population of R.similis isolated from arecanut infected pepper and banana but not coconut. These findings indicated that the four isolates are different "host types".

The four populations isolated from pepper, banana, coconut and arecanut did not differ significantly in their morphologic dimensions and proportions or anatomical features.

Eighteen cultivars, four Piper species, and five wild collections of Piper, screened against R.similis did not reveal any immunity or resistance. But wild collection (Vittal) No.430, P.hymenophyllum, and P.attenuatum recorded less than 33 per cent root reduction and 1.5 times of nematodo reproduction, showing less susceptibility.

Among the three nematicides namely aldicarb sulfone, fensulfothion and D B C P and neem cake tested for control of R.similis on pepper, aldicarb sulfone was found to be the best at a dosage of 8 g a.i. per hectare preventing root infection and resulting in improvement of plant growth, (leaf production, shoot and root growth). Fensulfothion was next best in this respect followed by D B C P. Neem cake was ineffective.

Between the two methods tested, post-inoculation application of the chemicals was found to be superior over the pre-inoculation method of application.

The survey revealed that red lateritic soil types contained more R.similis This nematode was not observed in lateritic clay or clay loamy soils.

R.similis was found frequently in areas where pepper was intercropped with arecanut, followed by coconut and banana. But this nematode was not encountered when pepper was intercropped with coffee or ginger.

Most of the common cultivars of pepper grown in Karnataka and Kerala were observed to be infected with R.similis in the plantations.

R.similis invaded pepper feeder roots prefering the root tip region, above the elongation zone. Lesions were formed within 72 hours of nematode inoculation to the roots.

The nematodes were observed to feed cortical parenchyma cells. The cells at infection site were turned brown and finally to black in colour. Stelar portion of root was not affected.

R.similis effected 93.8 and 79.2 and 94.97 and 81.6 per cent reductions in shoot length and weight in 55 days old pepper rooted cuttings when inoculated with 1000 and 10000 nematodes respectively, at the end of 150 days compared to the check plants.

When check plants produced an average of 7.5 number of leaves per plant, the plants inoculated with 1000 and 10000 nematodes per pot containing 1500 ml soil produced only 1.5 and 1.4 leaves respectively.

REFERENCES

VII. REFERENCES

Abraham, P., 1959, Pepper cultivation in India. Farm Bull.no.55, Farm information unit, Directorate of Extension, Ministry of Food and Agric. New Delhi. 80 pp.

*Addoh, P.G., 1971, The distribution and economic importance of plant parasitic nematodes in Ghana. Ghana J.Agric.Sci. 4: 21-32.

Anonymous, 1968a, Control of plant parasitic nematodes, vol. 4. Principles of plant and animal pest control. Publication no.1696. Nat. Acd.Sci. Wash. 172 pp.

* _____ 1968 b, Surinam agricultural research report, 1968. Neded Landbroefsn, Suriname no. 44. 156 pp (Plant nematodes 139-153 pp).

* _____ 1969a, Report of the secretary for agriculture, 1967-68. Salisbury. Government printer. 83 pp (Plant nematodes 34 p).

_____ 1969b, Health of India, Raw materials. Vol. III Publication and information directorate, Council of Scientific and Industrial Research, New Delhi. 83-118 pp.

_____ 1971a, Research on plant parasitic nematodes (1966-1971). Division of Nematology, Indian Agric.Res.Inst.New Delhi. (Mimiographed) 81 pp.

* _____ 1971b, West Indies University. Report of the faculty of Agriculture 1969-70. St.Augustine, Trinidad. 142 pp. (Plant Nematology, 19-20 pp).

* _____ 1972, Annual report for the year 1971-72. Plant Pathology services, Bvumbeve Research Station, Limbe. (Plant Nematology, 5,6, 13 p).

Anonymous, 1974a, Annual report for 1973. Central Plantation Crops Research Institute, Kasargode, Kerala, India (Nematology 62 p).

* ———— 1974b, Annual report for 1972. East African Community, East African Agriculture and Forestry Research Organisation. Record of Research, Nairobi, Kenya,182 pp. (Plant Nematology, 122-126 pp).

Arnon, D.I. and Hoagland, D.R., 1940, Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Science. 50: 463-484.

Ayala, A. and Roman, J., 1963, Distribution and host range of the burrowing nematode in Puerto Rican soils. J.Agric.Univ.Puerto Rico. 47: 28-37.

Bally, ?. and Reydon, G.A., 1931, Detegenwoordige Stand van het Vraagstuk van de worteelaaltjes in die koffiecultuur. Arch.Koffiecult. Nederl. Indie.5: 23-216.

*Beccari, F. and Scavazzon, R., 1966, I resultati di trattamenti nematocidi eseguiti in Somalia su materiale moltiplicato del Banano prima dell' impianto. Riv.Agric.subtrop. trop. 60: 123-140.

*Beugnon, M. and Villardebo, A., 1973, The nematodes of the Banana tree in Madagascar. Aspects of the problem and its economic importance. Fruits. 28: 607-612.

*Birchfield, W.,1954, The hot water treatment of nematode infested nursery stock. Proc. Fla. St. hort. Soc. 67: 94-96.

Blake, C.D., 1961, Root rot of bananas caused by Radopholus similis (Cobb)and its control in New South Wales. Nematologica 6: 295-310.

————, 1963, Root and corm diseases of banana. Agric. Gaz. N.S.W. 74: 526-531, 523.

Blake, C.D., 1966, The histological changes in banana roots caused by Radopholus similis and Helicotylenchus multicintus. Nematologica 12: 129-137.

_____, 1969, Nematode parasites of banana and their control. In Peachy, J.E. (ed). Nematodes of tropical crops. Tech. Commun.Commonw. Bur. Helminth. No.40, 109-132 pp.

_____, 1972, Nematode disease of banana plantations. In Webster, J.M. (ed). Economic nematology, London, Academic Press, 245-267 pp.

*Brooks, T.L. and Perry, V.G., 1963, Apparent parthenogenic reproduction of the burrowing nematode, Radopholus similis (Cobb) Thorne. Proc. Soil and Crop Sci.Soc. Fla. 22nd annul meeting (1962). 160-162 pp.

*Casamayor, ..,Seidel,T. and Decker, H., 1966, Tratamiento con agua caliente contra nematodos parasitos en platano. Boln. Cent. Investnes Agropec. Univ. Cent, Las villas. No.1.6 pp.

*Cassidy, G., 1930, Nematodes associated with sugarcane in Hawaii. Hawaii Plant. Rec. 34: 379-387.

Christie, J.R., 1957, The yellows disease of pepper (Piper) and spreading decline of citrus. Fl.Dis.Contr. 41: 267-268.

*Coates, P.L., 1972, Response of an established banana planting to four nematicides. Aust Arti. and Lews Summ. 18: 165-170.

*Cobb, N.A., 1893, Nematodes, mostly Australian and Fijian. Macleay Mem. vol. Linn. Soc. N.S.W. 252-308.

*_____, 1909, Nematodes (In Fungus maladies of the sugarcane). Bull. Div.Path. Physiol. Hawaiian Sug.Plrs. Assn.Exp.Stn. 6: 51-74.

_____, 1915, Tylenchus similis, the cause of a root disease of sugarcane and banana, J. agric.Res. 4: 561-568.

Cohn, E., 1972, Nematode diseases of citrus. In Webster, J.M. (ed), Economic nematology. London, Academic Press. 215-244 pp.

*Colbran, R.C., 1964, Effects of treatments of banana' bits' for nematode control on emergence and yield. Qd.J.agric. Sci. 21: 237-238.

_____, 1970, Studies of plant and soil nematodes. 15. Eleven new species of Radopholus Thorne and a new species of Radopholoides De Gurian (Nematoda: Tylenchoidea) from Australia. Qd.J.agric. and anim. Sci. 27: 437-460.

*Collins, R.J. and Feldman, A.W., 1965, Attempt to improve the growth of Radopholus similis infected citrus with under tree drenches of zinophos. Fl.Dis.Reptr. 49: 865-867.

*_____, 1966, Studies on the penetration of D D, E D B and D B C P for the control of Radopholus similis and for the destruction of live citrus roots. Proc.Fla. St.hort. Soc. 78: 63-69.

Das Gupta, D.R. and Seshadri, A.R., 1971, Races of the reniform nematode, Rotylenchulus reniformis Linford and Oliveira, 1940. Indian J.Nematol. 1: 21-24.

*Davide, R.G. and Comedia, A., 1972, Chemical control of nematodes on cabbage at Atok Benguet. Phillippine Agriclt. 55: 282-288.

*Day, L.H. and Serr, E.F., 1951, Comparative resistance of root stock of fruit and nut trees to attack by a root lesion or meadow nematode, Proc. Amm. Soc. Hort. Sci. 57: 150-154.

*Decker, H., Casamayor, G.R., and Rodrigues, F.M.L., 1971, New investigations on the hot water and nematicide treatment of banana rhizomes, for nematode control. Revista Agropecuaria. 3: 27-35.

D'Souza, D.I., Kumar, A.C., Kasiviswanathan, P.R. and Shamanna, H.V., 1970, Relative distribution and prevalence of plant parasitic nematodes in the coffee tracts of South western India. Indian Coff. 34: 329-342.

DuCharme, E.P., 1954, Cause and nature of spreading decline of citrus. Citrus Ind. 35: 6-7.

_____, 1959, Morphogenesis and histopathology of lesions induced on citrus roots by Radopholus similis. Phytopathology, 49: 388-395.

_____, 1969, Temperature in relation to Radopholus similis (Nematoda) spreading edcline of citrus. Proc. First Intern. Citrus Symp. Riverside, 1968. Vol.2: 979-983.

_____,1972, Kallstroemia maxima a new host of Radopholus similis. Pl.Dis. Reptr. 56: 85.

_____ and Birchfield, ., 1956, Physiologic races of the burrowing nematode. Phytopathology, 46: 615-616.

_____ and Price, .C., 1966, Dynamics of multiplication of Radopholus similis. Nematologica 12: 113-121.

Edwards, D.I. and Lehunt, W.J., 1971, Host range of Radopholus similis from banana areas of Central America with indication of additional races. Pl.Dis.Reptr. 55: 415-418.

*Everisto, F.N., 1969, Contribuicno para O reconhecimento nematologico das bananeiras em Mocambique. Agronomia Mocamb. 3: 169-178.

Feder, W.A., Ford, H.W., Feldmesser, J., Gardner, F.E., Suit, R.F., Pieringer, A. and Hutchins, P.C., 1958, Citrus varieties, species and relations susceptible to attack and damage by the burrowing nematode, Radopholus similis. Pl.Dis.Reptr. 42: 934-937.

Feldman, A.W., DuCharme, E.P. and Suit, R.F., 1963, Attempts to control spreading decline of citrus with high rates of nematicides applied by sprinkler irrigation. Ibid. 47: 927-931.

George, C.K., 1976, Pepper production programmes in five year plans. Paper presented at the Intern. Seminar on pepper, Cochin, India, 7-10 pp.

Godfrey, G.H., 1931, The host plants of the burrowing nematode, Tylenchus similis. Phytopathology, 21: 315-322.

Goodey, T., 1933, Plant parasitic nematodes and the diseases they cause. London, Methuen & Co., Ltd. 306 pp.

_____, 1936, On Anguillulina oryzae (v. Breda de Haan,1902) Goodey, 1932, a nematode parasite on the roots of rice, Oryza sativa L. J.Helminth. 14: 107-112.

Gowen, S.R., 1973, The effect of hydrogen peroxide, temperature and detergent on the recovery of nematodes from macerated banana roots. Second Intern.Cong.Pl.Path. Minneapolis, Minnesota, Sept. 5-12 (Abstr.)

*Guercut, ..., 197., Study of 3 new nematicides in a banana plantation. Fruits, 25: 769-77.

*Heungens, A., 1971, Nematode control in Calathea culture. Med.van de Facult. Landbouw. wetn, Schappen, Rijks universiteit. Gent. 36: 1309-1317.

*Huang, C.S.,1972, Plant parasitic nematodes in Taiwan. Monograph series, Inst. of Botany, Academia Swice Taipei.

*Hutton, D.G. and Chung, D.C., 1973, Effect of post planting application of the nematicide D B C P to plantain. Nematropica, 3: 46-50.

Keetch, D.P., 1972, Some host plants of the burrowing eelworm, Radopholus similis (Cobb) in Natal. Phytophylactica, 4: 51-58.

Keetch, D.P., 1973, Damage caused by the burrowing eelworm, Radopholus similis on banana in Natal. Banana series No.J 15. Dept.Agric. Tech. Service, Pretoria, 6 pp.

Koshy, P.K., 1975, Annual report for 1974. Central Plantation Crops Res. Inst. Kasargode, Kerala India (Nematology 61-63 pp).

Krusberg, L.R., 1971, Control of Pratylenchus penetrans in heavy green house soil. Fl.Dis. Reptr. 55: 276-279.

*Kuhne, F.A. and Milne, D.B., 1969, Burrowing nematode in South Africa 'First report'. S.Afr.Citrus J. No.423, 3-5 pp.

Kumar, A.C., Kasiviswanathan, P.K. and D'Souza, D.I., 1971, A study on the plant parasitic nematodes of certain commercial crops in coffee tracts of South India. Indian Coff. 35: 222-224.

*Larter, A.H.H. and Allen, B.F., 1953, Notes on current investigations; October to December, 1953. Malayan agric. J. 36: 36-43.

*Leach, R., 1956, Blackhead toppling disease of bananas (Correspondence), Nature, London, 171: 204-205.

Loos, C.A., 1961, Eradication of the burrowing nematode, Radopholus similis, from bananas. Fl.Dis.Reptr. 45: 457-461.

_____, 1962, Studies on the life history and habits of the burrowing nematode, Radopholus similis, the cause of black head disease of banana. Ir c. Helminth.Soc. Wash. 29: 43-52.

_____ and Loos, S.B., 1960, The blackhead disease of banana (Musa accuminata) Ibid. 27: 189-193.

*Luc, M. and Vilardebo, A., 1961, Les nematodes associes aux bananier cultives dans l'ouest African, especes parasites et dommages causes. Fruits 16: 205-219.

Martin, G.C., James, G.L., Bissett, J.L. and Way, J.I., 1969, Trials with field crops and Radopholus similis with observations on Pratylenchus sp. Meloidogyne sp. and other plant parasitic nematodes. Rhod.J.agric. Res. 7: 149-157.

Mass, P.W.I., 1969, Two important cases of nematode infestation in Surinam. In, Peachy, J.E (ed) Nematodes of Tropical Crops. Tech. Commun. Commonw. Bur. Helminth. No. 40, 149-154, pp.

Menon, K.K., 1949, The survey of pollu and root disease of pepper. Indian J.agric. Sci. 19: 89-136.

Milne, D.L., and Keetch, D.P., 1976, Some observations on the host plant relationships of Radopholus similis in Natal. Nematropica 6: 13-17.

Mountain, W.B., 1960, Theoretical considerations of plant-nematode relationships. In Sasser, J.N. and Jenkins, W.R.(eds), Nematology: Fundamentals and recent advances with emphasis on plant parasitic and soil forms. Chapel Hill, Univ. North Carolina Press. xv 480 pp.

_____ and Boyce, H.L., 1958, The peach replant problem in Ontar V. The relation of parasitic nematodes to regional differences in severity of peach replant failure. Can. J. Botany, 36: 125-136.

Nair, M.R.G.K., Rao, P.N. and Menon, M.K., 1966, On the occurrence of the burrowing nematode, Radopholus similis (Cobb, 1893 Thorne, 1949 on banana in Kerala, Indian J.Ent.28:553-55

Nambiar, K.K.N. and Sarma, Y.R., 1974, Annual report for 1973 Central Plantation Crops Research Institute, Kasargode, Kerala, India (Pathology, 149-151 pp).

Nambiar, M.C., 1976, Pepper research problems. Paper presented at the Intern. seminar on pepper, Cochin, India, 4-6 pp.

Helmes, D.P., 1970, Behavioural responses of Heterodera rostochiensis larvae to aldicarb and its sulfoxide and sulfone, J. Nematol. 2: 223-227.

*Ngundo, B.W. and Taylor, D.P., 1973, The burrowing nematode, Radopholus similis fromTanzania and Kenya. East Afr. Agric. Forestry J. 38: 405-406.

O'Bannon, J.H., 1973, Momordica charantia a new host of Radopholus similis. Fl.Dis.Reptr. 58: 561.

_____ and Taylor, A.L., 1967, Control of nematodes on citrus seedlings by chemical bare root dips. Ibid. 51: 995-998.

_____ and Tomerlin, A.T., 1971, Control of nematodes on citrus seedlings by chemical dips. Ibid. 55:154-157.

*Peachy, J.E. and Hooper, D.J., 1963, Chemical treatment of quarantined banana stocks infested with plant parasitic nematodes. Fl.Nath. London, 12: 117-120.

*Pereira, A.F., Figueiredo, D.M., Jr. and Hussni, J., 1960, Nematode cavernicole nos bananais do litoral de Sao Paulo. Biologico Sao Paulo 26: 27-31.

Poucher, C., Ford, H.W.,Suit, R.F. and DuCharme, E.P., 1967, Burrowing nematode in citrus. Fla. Dept.Agric.,Div. Plant Ind. Bull. No. 7, 63 pp.

*Raemaekers, R.H. and Patel, B.K., 1973, Burrowing nematode on banana. Pl.Prot. Bull. F.A.O. 21: 67.

Reddy, P.D.R. and Seshadri, A.R., 1971, Studies on some systemic nematicides. I. Evaluation of systemic and contact action against the root-knot nematode, Meloidogyne incognita. Indian J.Nematol. 1: 199-208.

Reis, L.G., 1974, Nematology in Mocambique. Nematol. News Letter. 20: 2.

*Renninger, G., Coffey, J. and Sokolof, B., 1958, Effect of hydrogenated fish oil on citrus tree destroying nematodes. Fl.Dis. Reptr. 42: 1057-1065.

Rohde, R.A., 1965, The nature of resistance in plants to nematodes. Phytopathology 55: 1159-1162.

*Scotto La Massase, C., 1967, De CouVerte' d'um nematode phytophag non encore signale en Europe. Phytoma 19: 29-33.

Senanayake, A.., 1969, Root and rhizome necrosis of blackhead disease of banana. Trop. agric. Ceylon. 125: 119-121.

Sher, S.A., 1962, Revision of the genus Radopholus Thorne, 1949 (Nematoda : Tylenchoidea). Proc. Helminth. Soc.Wash. 35: 219-237.

Sivapalan, P., 1966, Association of Radopholus similis with decline in young tea fields. Pl. Dis. Reptr. 52:528.

Southey, J. F., 1970, Laboratory methods for work with plant and soil nematodes, London, Her Majesty's Stationery Office. Tech. Bull. No.2, 148 pp.

*Spears, J.F., 1955, Progress report of burrowing nematode contro research for calendar year 1955. Ieaville New York. USDA agric. Res. Service Pl. Pest control Br.IV 38 pp.

Steiner, G., 1925, The problem of host selection and host specialisation of certain plant infesting nemas and its application in the study of nemic pests. Phytopathology 15: 499-534.

*Steiner, G. and Buhrer, E.M., 1933, The nematode Tylenchus similis Cobb, as a parasite of the tea plant (Thea sinensis, Linn.), its sexual dimorphism, and its nemic associates in the same host. Zeitsch. Parasitenk. 5: 412-420.

Strover, R.H., 1972, Banana, plantain and abaca diseases. Kew England: Commonw. Mycol. Inst. xii-316 pp.

Strover, R.H. and Fielding, M.J., 1958, Nematodes associated with root injury of Musa spp in Honduran banana soils. Fl.Dis.Reptr. 42: 938-940.

*Sturhan, D., 1970, Radopholus similis ein fur Deutschland newer pflanzen nematodes. Nachr. Bl.dt.Pflschutz dienst, Stuttg. 22: 1-2.

_____, 1971, Biological races. InZuckerman, B.M., Mai,W.F. and Rohde, R.A.(Eds.), Plant parasitic nematodes. Vol.II. New York, Academic Press. 51-71 pp.

*Suit, R.F., 1961, A comparison of pre-plant soil treatment chemicals for control of burrowing nematode in citrus groves. Fl.Dis.Reptr. 45: 454-456.

*_____, 1969, Treatment of citrus trees for burrowing nematode contrdl. Proc. First Intern.Citrus Symp. Riverside, Calif. 2: 961-968.

_____ and Cohoon, R.E., 1953, The burrowing nematode and other plant parasitic nematodes in relation to spreading decline of citrus. Fl.Dis. Reptr. 37: 379-383.

_____, _____ and Feldman, A.W., 1961, Effectiveness of D B C P and fumicides for the control of Radopholus similis on citrus trees. Ibid. 45: 62-66

_____ and _____, 1961, Treatment of citrus trees with cynem for control of Radopholus similis. Ibid, 45: 782-786.

_____ and _____, 1964, Further test with D B C P for control of spreading decline of citrus trees caused by the burrowing nematode, Radopholus similis. Ibid. 48: 544-547.

*Tabondo, J. and Caballero, D.J., 1968, Research on chemical control of Radopholus similis on banana plants. An.Inst.Biol.Univ.Mex.Ser Zool. 39: 29-33.

*Tarjan, A.C., 1960a, A comparison of polythene plastic bags and glass jars as incubation chambers for obtaining nematodes from roots. Pl.Dis.Reptr. 44: 474-477.

_____,1960b, Some effects of African Marigold on the citrus burrowing nematode, Radopholus similis(Abstr.) Phytopathology 50: 577.

*_____,1962, Attempts at controlling citrus burrowing nematodes using nematode-trapping fungi. Proc.Soil Crop Sci. Soc. Fla. (1961) 17-36 pp.

_____,1967, Influence of temperature and hydrogen-peroxide on the extraction of burrowing nematodes from citrus roots. Pl.Dis.Reptr. 51: 1024-1029.

*_____,1971, Some interesting associations of parasitic nematodes with cacao and coffee in Costa Rica (Abstr.) Nematropica 1: 5.

_____ and Couto, A.., 1965, Stimulative and nematicidal effects of ethion on citrus seedlings parasitized by the burrowing nematode. Proc. Fla.St.Hort.Soc. 77: 60-66.

Taylor, A.., 1960, Control of the banana root nematode in Fiji. Pl.Prot. Bull. F.A.O. 17: 97-103.

Taylor, A.L. and O'Bannon, J.H., 1968, Experiments with an apparatus for sub-surface application of nematicides to nursery plants in containers. Pl.Dis.Reptr. 52: 218-222.

Taylor, J., 1948, Errors of treatment comparisons when observations are missing. Nature, London 162: 262-263.

Thorne, G., 1949, On the classification of Tylenchida, new order (Nematoda, Phasmidia). Proc.Helminth.Soc.Wash.16: 37-73.

_____, 1961, Principles of Nematology. New York, McGraw Hill Co. 553 pp.

Ting, W.P., 1975, Plant Pathology in peninsular Malaysia. Rev.Pl.Path. 54: 297-305.

*Tischler, G., 1901, Über Heteroderagallen an den wurzeln von Circaea lutetiana L. Ber.Deut.Bot.Gesell. 19: 95-107.

Van Weerdt, L.G., 1957, Studies on the biology of Radopholus similis (Cobb, 1893) Thorne 1949. Part I. Pl.Dis.Reptr. 41: 832-835.

_____, 1958, Studies on the biology of Radopholus similis (Cobb, 1893) Thorne 1949. Part II. Morphological variations within and between progenies of single females. Nematologica 3: 184-196.

_____, 1960, Studies on the biology of Radopholus similis (Cobb, 1893) Thorne 1949. Part III. Embryology and post embryonic development. Ibid. 5: 43-52.

*_____, Birchfield, W. and Esser, R.P., 1960, Observations on some sub-tropical plant parasitic nematodes in Florida. Proc. Soil Crop Sci. Soc.Fla. 19th Annual meeting (1959): 443-451.

*Vecht, J.v.d., 1950, De planten parasiterende aaltjes. In L.G.Seinhorst (ed) 'De planten van de cultuurgewassen in Indonesie'. 's-Gravenhage: W.van Hoeve, 16-41 pp.

Venkitesan, K., 1972, On the occurrence of four parasitic nematodes associated with different crops in Cannanore district, Kerala. Agric.Res.J.Kerala. 10: 179-180.

Vilardebo, A. and Robin, J., 1969, Nematicidal treatment of banana planting material. In Peachy, J.E (Ed), Nematodes of Tropical Crops. Tech.Commun. Commonw.Bur. Helmith. No.40, 133-141 pp.

Vilsoni, F., Michael, A.M. and Butler, L.D., 1976, Occurrence, host range and histopathology of Radopholus similis in ginger (Zingiber officinale) Pl.Dis.Reptr. 60: 417-420.

*Wehunt, E.J. and Edwards, D.I., 1968, Radopholus similis and other nematode species on banana. In Smart, G.C. Jr. and Perry, V.G (Eds) Tropical nematology. Univ. Fla. Press Gainsville, 1-19 pp.

Weicher, B., 1967, Plant parasitic nematodes. Report to Govt. of India. U N D P ., F.A. O., Rome, Report No.PL/TA/52.TA. 2332.

West, J.[illegible]., 1957, Recommended changes in recovery techniques for burrowing nematode. Pl.Dis.Reptr. 41: 600-602

*Winto, R.[illegible]., 1972, Effect of Meloidogyne sp. on the growth of Piper nigrum. Malaysian agric. Res. 1: 86-90.

Yates, F., 1933, Analysis of replicated experiments when the field results are incomplete. Emp. J. Exp.agric. 1: 129-142.

Young, T.[illegible]., 1954, An incubation method for collecting migratory endoparasitic nematodes. Pl.Dis.Reptr. 38: 794-795.

*Zimmerman, A.[illegible]., 1898, De nematoden der koffiewortels. Meded.Pl.tuin.Batavia, 27: 16-41.

* Original not seen - only abstracts consulted.

APPENDICES

APPENDIX I

SURVEY OF DECLINE (SLOW WILT) OF PEPPER
DATA FORM/SHEET

(Please fill in this form from the Field/Spot when you take Soil/Root samples)

Name of Officer supplying sample:

Village :

NES Block :

Taluk :

District :

Name of Cultivator:

Total area under Pepper .. :

% of Area under disease .. :

Varieties grown :

From where samples taken

Soil Type

1. Name of Locality :

2. Age of vines :

3. Variety .. :

4. Standard used for support :

Visual symptoms on Vines in the Garden

Yellowing of leaves (tick / appropriate one)

a) Whether occasionally seen : Yes/No

b) Whether all leaves are yellowish : Yes/No

c) Whether Die back observed : Yes/No

d) Normal flowering and fruit Set : Yes/No

Root System (While taking samples)

a) Main Root and feeder Roots Intact & Healthy: Yes/No

b) Main Root devoid of feeder Roots .. : Yes/No

c) Commencement of Decay & Death of Main Root : Yes/No

Distribution of Diseased Looking Vines — General/Localised

Inter cropping Done with ——— Banana/Arecanut/Coconut

-do- ——— Within/on Border Rows

APPENDIX II

SURVEY OF SLOW DECLINE (SLOW WILT) OF PEPPER
INFORMATION SHEET ON COLLECTION/SENDING OF SOIL/ROOT SAMPLES

The 'Slow wilt' of pepper (*Piper nigrum*) also known as "pepper yellows" is suspected to be caused by the burrowing nematode (*Radopholus similis*). The object of this survey is to gather information on the extent of distribution and prevalence of this nematode in the pepper growing areas in our state and its role on the cause of the disease. Plant nematodes are primarily soil organisms infecting roots, hence soil and roots samples are required for laboratory examination.

The following points may please be strictly adhered to while taking and sending the soil and root samples.

1. Do not take samples from spots of completely died out vines.
2. Remove 3-5 cm surface soil, collect soil upto 55 cm depth round feeder root zones of pepper vines.
3. Pool 3-4 such samples and take a representative sample of 250 g.
4. Take 20-30 g of feeder roots from same vines (Plants).
5. Immediately put the soil and root samples together in polythene bags, put one lable inside with given code number and tie the bag with a rubber band to make air tight and make another lable outside. Polythene bags & labels are provided along with this for that purpose.
6. Take two such samples from (1) Healthy vines, (2) Diseased vines where disease spread is suspected; in the same localition.
7. Fill in the details in the data sheet on the spot of sampling.
8. Do not expose the sample bags to sun or in hot places as drying of soil and roots will kill the nematodes.
9. Do not allow the bags to get holes etc. as it will result in moisture escape and drying of soil and roots.
10. Arrange to send the samples, the same day as far as possible or at least next day itself of taking sample without fail.
11. Send the samples by Post, well packed in cloth bags or card board boxes by Unregistered Post Parcel, to the address given under item (12).
12. Sri T.S.Venkitesan, Ph.D. Scholar, Department of Plant Pathology, Agricultural College, U.A.S. Hebbal, Bangalore-560024