STUDIES ON
THE BURROWING NEMATODE OF ARECANUT

Thesis
submitted to
The University of Kerala
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for the degree of
Doctor of Philosophy
in Botany (Nematology)

by
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INDIA

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DECLARATION

I, P. SUNDARARAJU, hereby declare that the materials contained in the Thesis entitled "STUDIES ON THE BURROWING NEMATODE OF ARECANUT", has not been previously formed the basis for the award of any degree or diploma of any University to the best of my knowledge and belief.

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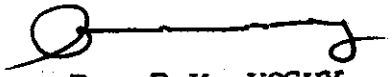
CERTIFICATE

THIS IS TO CERTIFY THAT

the Thesis entitled "STUDIES ON THE BURROWING NEMATODE OF ARECANUT" submitted for the degree of 'DOCTOR OF PHILOSOPHY' of the UNIVERSITY OF KERALA is a bona fide research work carried out by Shri. P. Sundararaju, M.Sc. under my supervision and no part of the Thesis has been submitted for any other degree or diploma.



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Introduction

INTRODUCTION

The arecanut palm Areca catechu Linnaeus (Family: Arecaceae) is one of the important cash crops of India. The nuts of this palm popularly known as arecanut, betelnut or supari are extensively used all over the country for chewing with the leaves of Piper betle. They form also an essential requisite for several religious and social ceremonies. Arecanut chewing habit is prevalent throughout Asia and the crop is grown in Sri Lanka, Indonesia, Malaysia, Bangaladesh, Fiji, Philippines, Solomon Islands, Singapore, China, Papua New Guinea etc. Though the crop is cultivated in many of the Asian countries, research on various aspects of this palm is being carried out only in India. In India, the total area under arecanut during 1980-81 was 1,84,500 ha of which 60,900 ha were in Kerala, 54,300 ha in Karnataka, 50,800 ha in Assam, 6,500 ha in Meghalaya, 4,300 ha in Tamil Nadu, 3,100 ha in West Bengal; the remainder lies in the States of Andhra Pradesh, Maharashtra, Goa and Tripura. The large scale cultivation of arecanut is done in Kerala, Karnataka and Assam, the three states accounting for nearly 90 per cent of the area under the crop contributing about 95 per cent of the total production in the country. India is the largest arecanut producing country in the world with a total production of 1,91,400 tonnes, valued at Rs.2,500 million (Mohan Rao, 1982).

The production as well as area under arecanut cultivation in Kerala showed a decreasing trend in recent years. During 1966-'67 the area was 71,200 ha, which increased steadily to 93,000 ha by 1974-'75. Then onwards it was on the decline and came down to 60,900 ha by 1980-81. The main reason for decline was the incidence of a number of diseases that takes a heavy toll of palms annually besides reducing the yield and quality of nuts (Velappan and George, 1982). About 20 diseases, causing varying degrees of damage to the palm have been recorded in India. They are associated with 40 pathogenic and non-pathogenic forms of fungi and one bacterium. Based on the extent of damage and nature of disease, yellow leaf disease, mahali, anabe, inflorescence die-back and button shedding are considered to be the major diseases (Koti Reddy et al., 1978).

The yellow leaf disease (YLD) remains today as the most serious malady affecting the crop. This disease known in Kerala State as "kattuveezhcha" was reported from Muvattupuzha, Meenachil and Chalakudi areas of Kerala about a century ago (Nambiar and Srinivasan, 1951). In earlier years, it was felt that YLD was more or less similar to the leaf and root disease of the coconut palm (Nambiar, 1949). In the Malnad areas of Karnataka State the disease is known as "Chandiroga" (Dastagir, 1963, 1965).

The prevalence of the disease has also been reported from the central regions of Maharashtra and Tamil Nadu (Anonymous, 1963). The malady does not kill the palm outright, but is only debilitating in nature. The major symptoms of the disease are yellowing of leaves and shedding of both mature and immature nuts. The yellowing starts from the tips of the outer leaves gradually extending to the middle of laminae. Tips of the chlorotic leaves eventually dry up. In the advanced stage, leaves are reduced in size, become stiff and pointed, closely bunched and abnormally puckered. Ultimately, the crown falls off leaving a bare trunk (Plate 1a). The endosperm of the diseased nut has a blackish appearance (Plate 1b) and is soft to touch, which renders it unsuitable for consumption and fetches only very low price in the market.

Though a number of biotic agents have been implicated, the exact cause of the disease is not yet known. Water-logging, acidity and nutritional disorders have been attributed as factors favouring disease incidence (Anonymous, 1961; Dastagir, 1963; Velappan, 1969 and Rawther, 1976). Fungi isolated from leaves (Menon, 1959 and Menon and Kalyanikutty, 1961) and roots (Anonymous, 1969); bacteria (Srivastava et al., 1970); virus particles (Menon, 1963, Reddy et al., 1982) and mycoplasma like organisms (Nayar, 1971, 1976 and Nayar and

Plate 1

a. Different intensities of yellow leaf disease of arecanut

- A. A healthy arecanut palm
- B. Palm in the early stage of disease
- C. Palm in the middle stage of disease
- D. Palm in the advanced stage of disease
- E &F. Palms succumbed to the disease

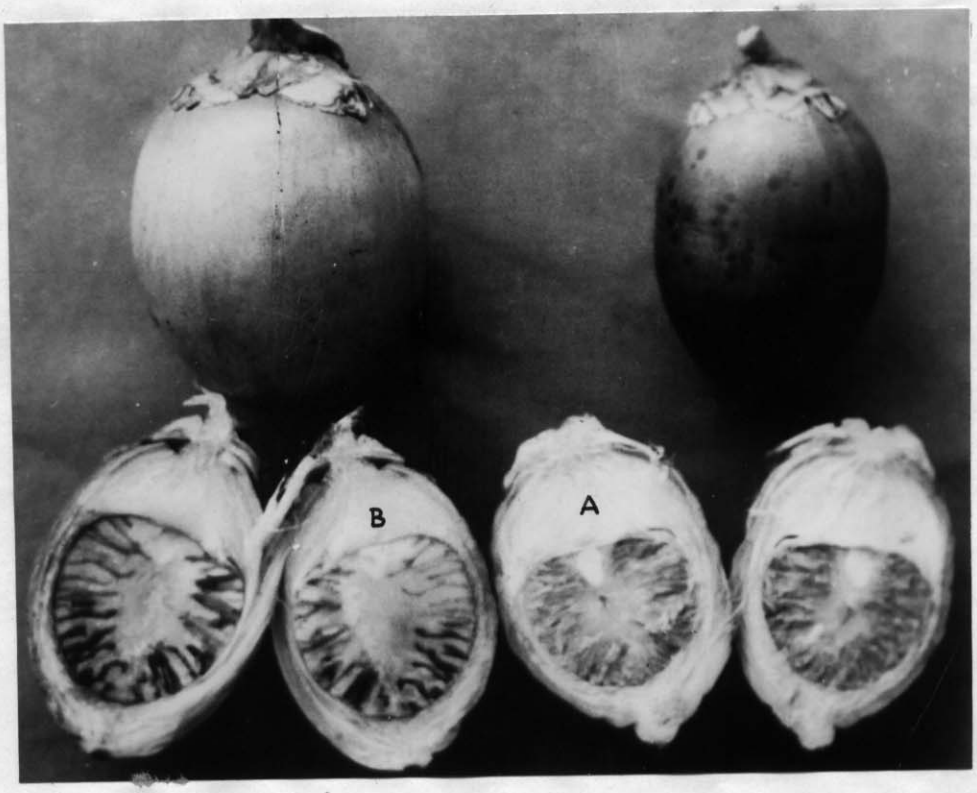
b. Split nuts of yellow leaf disease affected arecanut palms showing the discolouration of the kernel in comparison to nuts from healthy palms.

- A. Healthy
- B. Diseased

Plate.1

a

b



Seliskar, 1978) obtained from the root region, when inoculated, have not so far reproduced the symptoms.

The burrowing nematode, Radopholus similis (Cobb, 1893) Thorne, 1949 was first reported in India from roots of coffee in Coorg, Karnataka by Mayne and Subramhanyam in 1933. Kumar et al. (1971) recorded the burrowing nematode from soil around roots of arecanut palm for the first time in Mysore, India. Later, Koshy et al. (1975, 1976) recorded lesions, rotting and black tip of roots of arecanut by R. similis. They recorded 22 genera of plant parasitic nematodes from the root zone of areca palms and isolated R. similis from more than 50 per cent of the root samples. Radopholus similis was the only endoparasite encountered and its population was also comparatively high in samples collected from the YLD affected tracts. The burrowing nematode is known to be the causal agent of spreading decline of citrus in Florida (Suit and DuCharme, 1953), pepper yellows in Bangka Island of Indonesia (van der Vecht, 1950) and root and rhizome rot of banana in all the banana growing tracts of the world, except in Jordan valley, Israel (Minz et al., 1960) and Taiwan (Huang, 1972). Hence, the following investigations were carried out at the Central Plantation Crops Research Institute (CPCRI), Research Centre, Palode, Trivandrum and CPCRI Regional Station, Kayangulam, Kerala State to ascertain the role of

the burrowing nematode on arecanut and in the incidence of yellow leaf disease, if any.

1) Survey of different YLD prevalent and non-prevalent arecanut growing tracts in Kerala, Karnataka and Tamil Nadu for R. similis with a view to map out the infested locations.
2) Pathogenicity of R. similis on arecanut.
3) Histopathology of R. similis infested arecanut roots.
4) Axenic culturing of R. similis.
5) Isolation and identification of fungal organism associated with lesions of R. similis on arecanut roots.
6) Studies on population fluctuations of R. similis on arecanut.
7) Control of R. similis on arecanut in pots and under field conditions.
8) Screening of Areca germplasm for resistance to R. similis.

Plate 1

c. A healthy arecanut garden

d. A flowering arecanut palm

c

d

Review of literature

REVIEW OF LITERATURE

Sveshnikova (1940) was the first to record the incidence of root-knot nematode, Heterodera marioni, on Areca rubra from the Soviet Union. Subsequently, Aphelenchus sp. was recorded from Mysore, India on Areca sp. by Thirumalachar in 1946. The association of nematodes with the arecanut palm, Areca catechu L., was first reported in India by Nair (1964). He observed Meloidogyne javanica, Helicotylenchus sp. and Tylenchorhynchus sp. from the root zone of yellow leaf disease affected arecanut palms at Palode, Trivandrum district, Kerala. Subsequently Weischer (1967) recorded seven genera viz. Meloidogyne, Rotylenchulus, Hoplolaimus, Pratylenchus, Helicotylenchus, Tylenchorhynchus and Xiphinema from a few soil samples of healthy and yellow leaf disease affected palms. But he did not correlate the population of any of these with the incidence of the disease because of the low number of samples he observed. Swamy and Reddy (1969) reported the occurrence of Aphelenchus sp. and Helicotylenchus sp. from arecanut rhizosphere in Mysore. Meloidogyne incognita was noticed on arecanut palms in the Philippines by Pizzarro in 1969. Kumar et al. (1971) reported the occurrence of Radopholus similis for the first time in soil samples collected from the root zone of arecanut in the coffee tracts of South India.

Other plant parasitic nematodes encountered by them in the root zone of arecanut were Hemicriconemoides sp., Pratylenchus coffeae and Tylenchorhynchus spp. Later Koshy et al. (1975, 1976) had recorded 22 genera of plant parasitic nematodes viz. Aphelenchoides, Aphelenchus, Caloosia, Criconemoides, Ditylenchus, Dolichodorus, Helicotylenchus, Hemicriconemoides, Hoplolaimus, Longidorus, Meloidogyne, Paralongidorus, Paratylenchus, Pratylenchus, Psilenchus, Radopholus similis, Rotylenchulus reniformis, Scutellonema, Trichodorus, Tylenchorhynchus, Tylenchus and Xiphinema from the root zone of arecanut during their survey. Among them, R. similis was the only endoparasite they have come across in more than 50 per cent of the root samples collected and its population was also high in samples collected from the YLD-affected tracts. In addition to the above, a new species Brachydorus swarupi (Nematoda : Dolichodorinae), was described from soil around the roots of arecanut palms in Kerala State, India (Koshy et al., 1981). Rotylenchulus spp., Tylenchus spp. and Xiphinema insigne were reported on arecanut from Thailand by Reddy (1978). Phukan and Sanwal (1981) reported Hadronchus karengenesis from the rhizosphere of Areca catechu in Assam, India.

The available literature on nematodes associated with arecanut has been dealt with in the preceding pages and the review of literature on R. similis pertaining to the present work is grouped under different captions for the sake of convenience.

Systematic position

Cobb (1893) first described the male specimens of a parasitic nematode as Tylenchus similis from the necrotic root lesions of Musa sapientum in Fiji. In the same year, he described the female specimens as Tylenchus granulosus obtained from banana roots. Zimmermann (1898) described both the sexes of this nematode, which he called Tylenchus acutocaudatus, found in the roots of diseased coffee plants grown in Java. In 1906, Cobb found both males and females of a species infesting the roots of sugarcane, Saccharum officinarum, in the Hawaiian Islands, which was described as T. biformis. Several years later he received diseased rhizomes of the Gros Michel variety of banana from Jamaica which was also infested with nematodes. While studying this situation, he concluded that the nematodes observed first in Fiji, later in the Hawaiian Islands and finally from Jamaica were identical and belong to the same species. Therefore, he retained Tylenchus similis as the type species in the detailed redescription published in 1915. In 1949,

Thorne erected the genus Radopholus and placed the burrowing nematode as the type species and it became Radopholus similis (Cobb, 1893) Thorne, 1949. This was further confirmed by Sher (1968) and he suggested that it was indigenous to Australia and New Zealand. Colbran (1971) provided a key to Radopholus species.

Phylum	: Nematoda (Diesing, 1861) Potts, 1932
Class	: Secernentea (von Linstow, 1905) Dougherty, 1958
Sub-class	: Phasmidia Chitwood and Chitwood, 1933.
Order	: Tylenchida Thorne, 1949
Sub order	: Tylenchina (Orley, 1880) Geraert, 1966
Super family	: Tylenchoidea (Orley, 1880) Chitwood and Chitwood, 1937
Family	: Pratylenchidae (Thorne, 1949) Siddiqi, 1963
Sub family	: Radopholinae Allen and Sher, 1967
Genus	: Radopholus Thorne, 1949
Type species	: Radopholus similis (Cobb, 1893) Thorne, 1949

Economic importance

The burrowing nematode occupies the second position only to the root-knot nematode among the economically important plant parasitic nematodes in the tropical and sub-tropical regions (O'Bannon, 1977). Parasitization by R. similis causes gross reduction in the quality and

quantity of yield. It had wiped out over 22 million black pepper vines in the Bangka Island, Indonesia over a short period of two decades (Christie, 1959). Spreading decline of citrus caused by R. similis reduces yield from 50 to 80 per cent for grape fruit and from 40 to 70 per cent for oranges (DuCharme, 1954 and Suit et al., 1954). Suit and DuCharme, 1957 reported the spreading decline of citrus to an extent of nearly 6000 acres in Florida. It was observed by Poucher et al. (1967) that healthy citrus groves yielded 535 boxes of fruits/acre/year compared to only 25 boxes/acre/year by the infested groves. In banana, the disease caused by R. similis is known throughout the world by different names, the most common are Radopholus root rot, blackhead, blackhead toppling disease and decline (Blake, 1969). Vilardebo (1957, 1959) reported that in R. similis infested banana plots in French Guinea, production increased from 22.5 to 40 metric tons/ha after application of DD at planting time. Yields in plots treated with EDB increased from 26.7 to 37.15 metric tons/ha. Wehunt and Edwards (1968) observed an increase of 17,000 lbs of fruit production/acre/year in the nematode-free banana plots compared to the nematode-infested plots in Central America. Similar observations made by Maas (1969) at Surinam had also shown that 100 per cent infestation of banana reduced the yield to 30 tons/ha/year, whereas plantations with

three per cent infestation had yielded 73 tons/ha/year. Radopholus similis probably causes greater worldwide losses in banana yields than any other banana pathogen (with the exception of Sigatoka disease). The yield losses of 12.5 tons/ha had been reported as the result of R. similis infection alone. Other reports indicated that fruit yields on infested plants may be suppressed by as much as 50 per cent within 3 to 4 years after planting, and the number of uprooted or blown-down plants may be increased by 60 per cent (O'Bannon, 1977). Yield increases of 30 to 60 per cent were obtained following the control of R. similis on banana (Blake, 1972).

Recently R. similis had been recorded on banana from Kerala, India (Nair et al., 1966) and in association with diseases such as slow wilt of pepper (Venkitesan, 1976), root (wilt) of coconut (Weischer, 1967; Mathen, 1969; Mathen et al., 1970 and Koshy et al., 1975, 1978b) and yellow leaf disease of arecanut (Koshy et al., 1976). However, data on crop losses are yet to be available.

Distribution and spread

The burrowing nematode, R. similis has a wide geographical distribution and probably occurs in most areas of the world where bananas are produced commercially with a notable exception of the Jordan Valley, Israel

(Minz et al., 1960) and Taiwan (Huang, 1972). The geographic distribution of R. similis reported in association with different crops is furnished in Table 1. Some of the reported geographical localities are too cold to support field infestations of the burrowing nematode and thus are represented by greenhouse infestations. The most important means by which R. similis is introduced into new geographical areas are through the infested planting materials (O'Bannon, 1977). Mechanical equipment for working citrus grove soil might occasionally be the means for spreading R. similis to new locations within the same grove or to other groves (Tarjan, 1956, 1957). Within the plantations Suit and DuCharme (1953) reported that R. similis had spread at a rate of 6 to 60 m/year in citrus groves. The rate of spread of nematode through Lakeland fine sand type of citrus soils was found to be enhanced by the flow of water in the soil and in infested groves it was faster downhill than uphill (DuCharme, 1955). In greenhouse tests on citrus, Feldmesser et al. (1960) found that the nematode moved at about 15.2 to 21.6 cm per month. The movement of R. similis was studied by O'Bannon and Tomerlin (1969) on a weed host, Solanum nigrum and their results had revealed that it was capable of migrating to a distance of 216 cm in 44 weeks with an average of 21.2 cm/month. Studies conducted in Honduras

Table 1. Geographic distribution of *Radopholus similis* on different crops in the world

Country	Host	Reference
Australia	Banana	Colbran (1955)
	Sugarcane	Williams (1969)
Bangka Island, Indonesia	Black pepper	van der Vecht (1950)
Brazil-Bahia	Banana	Lordello (1973)
Brazil, Sao Paulo	Banana	Carvalho (1959)
Belgium*	*Calathea makoyana*	Coolen *et al.*(1971)
Cameroon	Banana	Price (1960)
Ceylon	Tea	Sivapalan (1968)
	Banana	Senanayake (1969)
Colombia	Banana	Cardenosa-Barriga (1948)
Congo	Banana	Luc *et al.* (1964)
Costa Rica	Banana	Taylor and Loegering (1953)
	Coffee	Tarjan (1971)
Cuba	Banana	Decker *et al.* (1966)
	Coffee	" " "
	Sugarcane	" " "
Denmark*	*Maranta tricolor*	Jacobsen (1972)

Contd...

Table 1 (Contd...)

Country	Host	Reference
Dominican Republic	Banana	Wehunt and Holdeman (1959)
Ecuador	Banana	" " "
El. Salvador	Banana	Holdeman (1960)
England*	Banana	Peachey and Hooper (1963)
France*	Ornamentals	Scotto la Massese (1967)
French Guinea	Banana	Mallamaire (1939)
French Polynesia	Taros and avocados	Scotto la Massese (1967) in Reboul (1978)
Germany*	Calathea makoyana	Sturhan (1970)
Ghana	Banana	Addoh (1971)
Guatemala	Banana	Wehunt and Holdeman (1959)
Honduras	Banana	Stover and Fielding (1958)
India		
1) Karnataka	Coffee	Mayne and Subramhanyam (1933)
	Cardamom	D'Souza et al. (1970)
	Arecanut	Kumar et al. (1971)
2) Kerala	Banana	Nair et al. (1966)
	Coconut	Weischer (1967)
	Black pepper	Venkitesan (1972)

Contd...

Table 1 (Contd...)

Country	Host	Reference
3) Gujarat	Banana	Sethi et al. (1981)
4) Maharashtra	Banana	Darekar et al. (1981)
Ivory Coast	Banana	Vilardebo (1957)
Jamaica	Banana	Cobb (1915)
	Coconut	van Weerdt et al. (1959)
Java, Indonesia	Tea	Zimmermann (1899)
	Sugarcane	Williams (1969)
Kenya	Banana	Anonymous (1972a)
Kent	Calathea zebrina	Southey (1978)
Madagascar	Banana	Beugnon and Vilardebo (1973)
Malawi	Banana	Siddiqi (1973)
Malaysia	Banana	Larter and Allen (1953)
	Rubber	Anonymous (1975b)
Mexico	Banana	Holdeman (1960)
Mozambique	Banana	Evaristo (1969)
Natal, South Africa	Banana	Kuhne and Milne (1969)
	Sugarcane	Anonymous (1971)
	Coffee	Milne and Keetch (1975)

Contd...

Table 1 (Contd...)

Country	Host	Reference
Netherlands*	*Anthurium andreanum*	Anonymous (1975a)
New Guinea	Banana	Fisher and Shaw (1971)
New Zealand	Banana	Sher (1968)
Nicaragua	Banana	Holdeman (1960)
Nigeria	Banana	Anonymous (1973a)
Pakistan	Rice	Timm and Ameen (1960)
	Pinus excelsa	Siddiqi (1963)
Panama	Banana	Newhall (1958)
Pacific Islands		
1) Fiji	Banana	Cobb (1893)
	Ginger	Vilsoni (1974)
2) Niue	Banana	Orton Williams (1980)
3) Western Samoa	Banana	Anonymous (1976)
	Coconut	Orton Williams (1980)
4) Tonga	Banana	Taylor (1968)
	Orange	Orton Williams (1980)
Peru	Banana	Sasser *et al.* (1962)
Philippines	Sugarcane	Williams (1969)
	Banana	Boncato and Davide (1980)
	Black pepper	" "
	Coffee	" "

Contd...

Table 1 (Contd..)

Country	Host	Reference
Puerto Rico	Banana	Ayala and Roman (1963)
Rhodesia	Banana, rice, Sugarcane, wheat, tobacco, potato, soybean	Martin et al. (1969)
Surinam	Banana	Anonymous (1968)
Taiwan*	Anthurium	Huang (1972)
Tanzania	Banana	Ngundo and Taylor (1973)
Uganda	Banana	Anonymous (1972b)
United States		
1) Arizona*	Citrus	Lanier (1957)
2) California	Banana	Hart (1956)
3) Baton Rouge, Louisiana	Sugarcane	Rands (1930)
	Ornamental banana plants	Whitlock (1957)
4) Florida	Citrus	Suit and DuCharme (1953)
	Avocados	DuCharme and Suit (1953)
	Coconut	van Weerdt et al. (1959)
5) Hawaii Islands	Sugarcane	Cobb
6) Texas*	Ornamental nurseries	Rogers et al. (1977)
Venezuela	Banana	Yepez et al. (1972)
West Africa	Banana	Vilardebo and Guerout (1976)

Contd...

Table 1 (Contd...)

Country	Host	Reference
West Indies	Banana	Nowell (1923)
Windward Islands	Banana	Edmunds (1969)
Zambia	Banana	Raemaekers and Patel (1973)
Zululand	Banana	Anonymous (1973b)

*Greenhouse detection: area probably too cold or otherwise not suitable for field infestations, or intercepted before getting to the field.

on the rate of spread of R. similis on 'Valery' banana indicated a movement of approximately 2.5 m in one year (Wehunt, 1966).

Host range

The burrowing nematodes have been found to parasitize more than 250 species of plants throughout the tropical and sub tropical regions (O'Bannon, 1977). In India, the burrowing nematode was recorded as early as in 1933 by Mayne and Subramhanyam and followed by Pattabhiraman (1949) from roots of coffee plants. Other economically important plants infested by R. similis in India are coconut, arecanut, banana, black pepper and

cardamom as given in geographic distribution (Table 1). Fortyeight species of plants belonging to 44 genera of 17 families were recorded as hosts of coconut population of R. similis at Kayangulam. Twenty of them were new host records which included a number of economically important plants (Koshy and Sosamma, 1975; Sosamma and Koshy, 1977, 1981).

Biotypes

Bally and Reydon (1931) reported for the first time the possibility of biological races in R. similis when they were unable to infect Gigantochola apus with the populations obtained from Coffea robusta. Later DuCharme and Birchfield (1956) established the existence of two biotypes as "banana race" which parasitized banana roots but not citrus and "citrus race" which parasitized both citrus and banana. They reported the possibility of existence of a third race in the field infesting citrus alone. Adult females of both the biotypes were morphologically similar with no significant dimensional differences. This had been confirmed by van Weerdt (1958) and Sher (1968). Edwards and Wehunt (1971) tested thirtysix crops and sixtyfour weed species against two populations (Panama and Honduras) of R. similis infesting banana areas in Central America. They found that both the

populations, infested in common only Rabiza bean, Tephrosia candida, Sorghum bicolor, Calapogonium mucunoides and had differential infectivity to all other plant species tested by them, showing the different infective behaviour of the two populations. Studies conducted at Kayangulam, Kerala revealed that the populations of R. similis from coconut, arecanut and banana belong to banana race (Koshy and Sosamma, 1977). Pinochet (1979) found that populations from Panama, Coto, Costa Rica and Armuelles caused greater damage to the root system of banana than an isolate from Honduras. Population increase in the Honduran isolate was considerably less than that of the other three isolates suggesting it could be a different biotype. Tarte et al. (1981) studied the physiological differences and morphological variations of the banana race. In their observations on morphology of female tails differences in the frequency of pointed and rounded tails were noticed within 13 populations. The ratio of pointed and rounded tailed females ranged from 25 : 75 to 98.8 : 1.2 respectively. Latest observations made by Huettel and Dickson (1981) revealed differences in the developmental stages of oogenesis between the two races. They, were found to be distinct with respect to karyotype. There were four chromosomes in the banana race

and five in the citrus. Huettel et al. (1982) studied the sex attractants and pheromone mediated sexual and copulatory behaviour between and within the citrus and banana races of R. similis. They found that the males of citrus race did not respond to the females of banana race. The pheromone of citrus race females attracted banana race males but they did not mate.

Biology and life history

The burrowing nematode is a migratory endoparasite and is capable of spending its entire life in the roots. All larval stages and adults of R. similis are vermiform and infest only healthy, succulent feeder root tips.

The embryonic and post-embryonic development of R. similis were studied by van Weerdt (1960). He had illustrated the development of R. similis from the undivided egg through the four larval stages to the mature male and female. At the second stage, male and female larvae could be differentiated by the arrangement of cells in the genital primordia. During the third and fourth larval stages there was a general increase in size and further differentiation of the gonad cells. At the fourth moult males did not increase in length, the heavily sclerotized cephalic frame work and strong stylet were replaced by an offset unsclerotized head resulting in

degenerated forms. Females emerged with fully formed gonads and the anterior ovaries were usually longer than the posterior ones. Loos (1962) studied the life history of the nematode (banana race) on Tephrosia candida. Females laid 3.5 to 4.6 eggs per day for two weeks. The egg period lasted for 5 to 7 days in water and 7 to 8 days in the root. Larvae persisted for 10 to 13 days after hatching, but many of them matured to adults in 11 days. Adults began to lay eggs 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 to 25 days at 24 to 32°C. According to DuCharme and Price (1966) the duration of life cycle was 18 to 20 days at 24 to 27°C on citrus. The number of eggs laid ranged from one to six 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 had laid viable eggs and produced active colonies of males and females, although no males were introduced into the culture. Loos (1962) was not able to produce progeny from single larva of the banana race of R. similis. Brook and Perry (1963), however, obtained three generations of R. similis without the participation of males by using single egg on grapefruit

seedlings. Huettel and Dickson (1979) also studied the parthenogenesis in the two races of R. similis from Florida. Inoculations of single R. similis larva of the citrus and banana races into okra seedlings produced only female populations after 80 days and male and female populations after 180 days. All the female populations produced female progeny with viable eggs, but no spermatozoa were observed in the spermatheca. No intersexes were also observed.

Host parasite relationship

Leech (1958) found that roots and corms of banana are extensively invaded by R. similis and other secondary organisms. The nematodes were found in small lesions on young roots and at the junction of healthy and rotted regions of the roots. The penetration and histopathology of R. similis were studied on citrus (DuCharme, 1959) and banana (Blake, 1961, 1966). In both banana and citrus, R. similis burrowed in the cortex forming extensive cavities. On banana, the cavities coalesced and enlarged due to the activity of nematodes tunnelling laterally and towards the endodermis, producing the characteristic reddish brown lesions through out the cortex. In citrus, unlike banana, however, nematodes entered the stele via endodermal passage cells and accumulated in the phloem

and cambium which in the course of time might be completely destroyed leaving a nematode-filled cavity separating the remains of the stele from the cortex. Cell reaction included wound gum deposition in the cortex, hyperplasia and tumour formation in the pericycle. External cracks appeared over the lesions 3 to 4 weeks after infection of both citrus and banana roots. In a large rhizome, roots passed through 6 to 12 cm of cortex before emerging. R. similis migrated from an infected root into the rhizome cortex and caused diffuse black lesions (Loos and Loos, 1960a).

DuCharme and Price (1966) isolated up to 739 individuals from one lesion of citrus root and estimated that a 20-year-old tree supported 1,00,000 to 8,00,000 nematodes. Cassidy (1930) obtained 2632 specimens of R. similis from one linear inch of sugarcane root in Hawaii. Hubert (1957) reported that yellows disease of black pepper is characterized by the gradual abnormal yellowing of foliage, especially in young vines. Plant growth ceased soon after yellowing became apparent, and production of pepper berries rapidly declined. Severe die-back and eventual death of the plants followed. The root-lets of affected vines showed small lesions which enlarge gradually leading to disintegration ultimately. Vilsoni et al. (1976)

reported that the burrowing nematode entered the rhizomes of ginger and penetrated the tissue intercellularly. Heavy infestation resulted in destruction of tissues and formation of channels or galleries in the rhizome. Infested ginger plants exhibited stunting, chlorosis and sparse tillering.

Koshy et al. (1975) studied in detail the symptoms and population density of R. similis in coconut roots. The infested roots showed small reddish brown cortical lesions which later coalesced resulting in extensive rotting. They recorded maximum number of nematodes from the semi-hard orange coloured portion of the main roots. Peeling and slicing of roots followed by submersion in water at 4 to 14°C for 48 hours yielded maximum number of nematodes. Govindankutty and Vellaichamy (1976) studied the histopathology of R. similis infested coconut roots and found that sections of roots having lesions revealed the presence of nematodes and their eggs in cortical burrows which were encircled by deeply stained cells and abnormal sclerenchyma.

Studies made by DuCharme (1969) on root invasion and reproduction of R. similis on citrus had shown that female nematode usually entered a root in less than 24 hours. The optimum temperature for root invasion and reproduction

was 24°C, the minimum 12°C and the maximum 29.5 to 32.5°C. Venkitesan and Setty (1977) reported that the burrowing nematode penetrated the pepper roots within 24 hours and dark brown lesions appeared within 72 hours after inoculation. Similar studies carried out on coconut by Koshy and Sosamma (1982) had also shown the formation of typical elongate orange coloured lesions on coconut roots after 24 hours of inoculation.

The pathogenicity of R. similis was established in black pepper (Venkitesan and Setty, 1977), ginger (Sundararaju et al., 1979), turmeric (Sosamma et al., 1979) and coconut (Koshy and Sosamma, 1983). Attempts were made earlier by various workers for mass culturing R. similis. It was cultured on okra roots (Feder 1958), on root tissues developed from lucerne seed (Myers et al., 1965), on carrot discs (O'Bannon and Taylor, 1968; Koshy and Sosamma, 1980) and on roots developed on leaf petioles of several citrus species and cultivars (Inserra and O'Bannon, 1975).

Associations with fungal organism

Incidence of Panama wilt caused by Fusarium oxysporum f. cubense doubled in Gros Michel bananas when R. similis was added to the soil (Newhall, 1958) and wilt

symptoms appeared faster on R. similis-infested bananas (Loos, 1959). F. oxysporum alone was unable to invade intact banana root cells, but colonized when cortical parenchyma cells were wounded mechanically or by the nematode. In plants inoculated with both F. oxysporum and R. similis, the fungus was able to grow through the endodermis, causing necrosis of the stele and eventual atrophy of the whole root distal to the point of stelar invasion (Blake, 1966). Feder and Feldmesser (1961) studied the individual and combined effects of F. oxysporum, F. solani and R. similis on the growth of Duncan grapefruit seedlings under greenhouse condition. The combined infections of R. similis and Fusarium spp. were found to cause more severe damage to the seedlings than did the nematode alone. Similar results were obtained by Blake (1966) on banana with R. similis and F. oxysporum. Booth and Stover (1974) recorded the constant association of Cylindrocarpon musae with R. similis from all banana growing regions of the world. Sosamma and Koshy (1978) reported the occurrence of C. effusum and C. lucidum from the root lesions caused by R. similis on coconut. In 1980, Pinochet and Stover isolated fungi, Fusarium solani, F. moniliforme, C. musae and Acremonium stromaticum from banana root lesions caused by R. similis. When inserted into rhizome

wounds or placed in contact with aseptic banana roots, C. musae caused the most extensive necrosis, followed by the Fusarium spp. Acremonium caused little damage by itself, but was destructive to roots in the presence of R. similis. All of the fungi associated with R. similis were found as wound invaders and not parasitic in the field.

Population fluctuation and longevity of R. similis

Population levels of plant parasitic nematodes would vary with the type of host crop, density of the root system and other environmental factors. DuCharme and Suit (1967) recorded high populations of burrowing nematode in citrus from October to December, lowest from January to July and also disappearance of the population from known infested areas in spring. Similar population fluctuations were recorded by Vilardebo in 1976 at Cameroon in banana. DuCharme (1969) studied the effect of temperature on R. similis on citrus and found that the optimum temperature for nematode reproduction and root invasion was 24°C, the minimum 12°C and the maximum, 29.5 to 32.5°C. According to Jimenez (1972) the soil temperature at a depth of 30 cm did not influence the population size, but the rainfall appeared to be the most important factor in the seasonal fluctuation of R. similis in the banana

growing areas of Pococi, Costa Rica. Low populations occurred during or after heavy rains, whereas high peaks, or initial accelerated growth noticed during dry months. Jaramillo and Figueroa (1974) also studied the population density of R. similis in the banana area of Guapiles, Costa Rica and recorded the population peaks in April, May, August and September, when the host plants were actively growing. Minimum population densities occurred during January, February, June and July after periods of heavy rainfall. Population dynamics of R. similis on banana in Cuba was studied by Shafiee and Mendez (1975). They recorded the increase in nematode population during the first two to three months of the year and the reduction in population particularly during wet season (April to October). Soil temperature had no apparent effect on the population build up. But Marcelino et al. (1978) recorded the highest population of R. similis in 'Valery' banana of Panama during November, the month of maximum rainfall and a second peak in May, the beginning of the rainy season other peaks in April and August. Studies conducted at Kayangulam, Kerala, revealed that maximum populations of R. similis on coconut and arecanut occurred during October and November and the lowest or nil population during May to July. (Koshy and Sosamma, 1978).

Laboratory and field experiments showed that R. similis survived for only 66 days in water and could not be recovered alive from infested soil and roots after four months in the field or in temperature tanks held at 74 to 76°F (Birchfield, 1957). Tarjan (1961) also studied the longevity of R. similis in host-free sandy soil and found that the nematode did not survive after six months in infested soil stored in buried plastic bags. However, the results of Hannon (1963) showed that R. similis survived for fourteen months in the field after the citrus tree was removed. Similar studies carried out by Keetch et al. (1975) on banana had also revealed that R. similis was able to survive in soil for 13 months, even after the plants were killed or removed from the field.

Control

Chemical methods

Burrowing nematodes were effectively eradicated from soil and roots of citrus seedlings in closed containers by soil applications of aqueous solutions of DBCP and Zinophos; but they could not be eradicated from diseased groves when the chemicals were applied by soil injection or sprinkler irrigation (Suit et al., 1961; Suit and Feldman, 1961; Feldman and Hanks, 1962 and

Suit, 1964). DBCP was successful on citrus as a preplant treatment @ 10 U.S. gal/acre and infection was controlled over four years (Suit, 1961). Treatment with DD at the recommended 50 U.S. gal/acre failed to control R. similis in the lower depths of a citrus grove in Florida, U.S.A., but at 500 U.S. gal/acre killed all R. similis up to a depth of 4.57 m (Collins and Feldman, 1966). In case of spreading decline of citrus in Florida, due to limited occurrence, made it possible to devise more uniform control measures applicable to local conditions (Cohn, 1972) whereas its worldwide distribution with banana had 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.

Use of systemic nematicides of organophosphates and carbamates have been tested recently on citrus. These chemicals were tried as bare root dips for nursery seedlings, soil drench, sub-surface drench injections and granular forms. Some methods have given promising results. The results of various workers have been summarised in Table 2.

Loos and Loos (1960b) found that paring banana suckers viz. removal of all discoloured tissues and dipping in Bordeaux mixture-DBCP paste reduced the

Table 2. Chemical control of *Radopholus similis* on citrus

Chemical tried	Dosage	Duration and type of treatment	Effectiveness	Reference
Thionazin Fensulfothion Prophos	1000 ppm	30 to 60 minutes bare root dips	Eradicated *R. similis* in roots	O'Bannon and Taylor (1967)
Phenamiphos	250 to 600 ppm	30 minutes dip	"	"
Fensulfothion Thionazin Prophos	100, 200, 400 ppm	Sub-surface drench mixed with water in nursery container	Eradicated *R. similis* in rough lemon seedlings	Taylor and O'Bannon (1968)
DBCP	@ 30 kg ai/ha	Soil application thrice a year (March, May and April)	Oxamyl applied as a foliar spray alone and in combination with DBCP treatments reduced the soil populations of *R. similis*	O'Bannon and Tomerlin (1977)
Oxamyl	@ 11.4 kg ai/ha	Soil application in March, July and May and foliar sprays 6 times a year at 6 week intervals		
Phenamiphos	11.2 kg ai/ha	Split application	Reduced population significantly	O'Bannon and Tarjan (1979)

nematode infection from 89 per cent to one per cent after eight months. Similar techniques adopted at Kew (Peachey and Hooper, 1963) and in the Windward Islands (Edmunds, 1969) on banana. However, phytotoxicity was encountered, especially with pared sets (Vilardebo and Robin, 1969), or nematicide penetration proved insufficient for complete kill (Blake, 1961). Soil fumigation with DD, EDB and DBCP was also investigated by Luc and Vilardebo (1961). DBCP was most satisfactory, and recommended at 40 litres/ha at planting during May and June and 25 litres/ha in October and 15 litres/ha in March of the following year. This programme gave an excellent control of nematodes and had been widely adopted in the Ivory Coast. Price (1960) reported that the application of DBCP by injection or watering to root areas of established bananas in Southern Cameroon resulted in increased growth, leaf production and leaf retention. Wehunt and Edwards (1968) observed that the DBCP treatment on banana plants increased the yield from 14 to 86 per cent in Central America. The recommendations for chemical control of R. similis in banana, based on the results obtained by various workers are presented in Table 3.

Table 3. Chemical control of Radopholus similis on banana

Chemical tried	Dosage	Duration and type of treatment	Effectiveness	Locality	Reference
DBCP	1.0% solution	Immersion of sets for five minutes	Eliminated infection	Cuba	Casamayor et al. (1966)
"	0.5% emulsion	Immersion of sets for five minutes and kept moist for 24 hours	"	"	Decker et al. (1971)
"	550 ml + 40 l clay soil + 50 l water	Pralinage-consists of soaking the sets for a few seconds	No attack or spread or multiplication till 14 months	Madagascar	Vilardebo and Robin (1969)
"	39.9 l/ha (75% EC)	Injected 15 to 20 cm deep at 8 points, 50 cm away from pseudostem initially and 10 months later on 18 weeks old plants	Produced heavier bunches than untreated plants	Jamaica	Hutton and Chung (1973)
"	3 ml/plant	Applied twice a year in 8 injections around the base of the plant, 20 to 25cm away from the pseudostem	42% increase in crop growth and bunch weight	Madagascar	Beugnon and Vilardebo (1973)

Contd...

Table 3 (Contd...)

Chemical tried	Dosage	Duration and type of treatment	Effectiveness	Locality	Reference
Phorate Phenamiphos	8 to 11 ml/plant 50 ppm (v/v) (40% EC)	Immersion of rhizomes for 15 to 30 minutes	Eliminated infection	Mexico	Taboada and Caballero (1968)
Phenamiphos	100 ppm (40% emulsion)	Immersion of rhizomes for 5 minutes	Eliminated infection	Cuba	Decker et al. (1971)
"	2.5 g ai/m^2	Applied 4 monthly intervals (April, August and December)	Reduced population significantly	Queensland, Australia	Burnett et al. (1974)
"	2 to 3 g ai/plant	Applied at 4 monthly intervals	"	Windward Islands	Gowen (1978)
"	2 g ai/plant	Applied at planting and after 4 months	"	Trichur, Kerala, India	Nair (1979)
"	2500 ppm	30 minutes dip followed by a 30 minutes water rinse	Reduced infection from 71% to 14%	South Africa	Jones and Reynolds (1980)

Contd...

Table 3 (Contd...)

Chemical tried	Dosage	Duration and type of treatment	Effectiveness	Locality	Reference
Aldicarb	60 g ai/ single mat	Applied twice a year	Reduced population significantly	Machala, Ecuador	Hasing-Lama et al. (1976)
"	6 g ai/mat	Applied four times a year	"	Costa Rica	Figueroa (1980)
Aldicarb sulfone	50 g ai/ single mat	Applied twice a year	Reduced population significantly	Machala, Ecuador	Hassing-Lama et al. (1976)
"	9 g ai/mat	Applied 4 times a year	"	Costa Rica	Figueroa (1980)
Carbofuran	W.P.750 ppm ai	Ten minutes immersion of rhizomes	"	"	Canderon (1974)
Carbofuran Fensulfothion	42 g ai/ sucker 17 g ai/ sucker	Immersion of suckers in clay slurry, followed by sprinkling uniformly with nematicides and paring followed by pralinage	Reduced population significantly and increased bunch weight	Coimbatore, Tamil Nadu India	Rajagopalan et al. (1977)

Venkitesan and Setty (1979) observed that aldicarb sulfone or fensulfothion at 4 to 8 kg ai/ha completely eliminated R. similis on black pepper seedlings, when Venkitesan and Charles (1979) tested six chemicals for the control of R. similis in the slow wilt affected pepper garden and found that phorate and DBCP were very effective. Koshy and Nair (1979) reported significant reduction of burrowing nematode population in coconut nursery with fensulfothion @ 50 kg ai/ha. Koshy and Sosamma (1979) reported eradication of R. similis from infested coconut seedlings by dip treatment in DBCP 1000 ppm for 15 minutes. Later Koshy et al. (1983) recorded complete control of R. similis with phenamiphos and phorate @ 25 kg ai/ha when applied thrice at three months interval in coconut nursery.

Non-chemical methods

Among the physical methods of control, hot water treatment of citrus root-stocks and pared banana suckers was reported to be effective for the control of R. similis. Birchfield (1957) in a preliminary test found that at 122°F for 10 minutes all the burrowing nematodes in the citrus roots were killed. The roots were cooled in cold water for 10 minutes immediately after heat therapy and

they were cared for in the planted sites by frequent watering (Cohn, 1972). Various practices of hot water treatment adopted by different workers for banana have been summarised in Table 4.

Flooding and fallowing for 5 to 6 months eliminated R. similis from banana fields in Honduras and Panama (Loos, 1961). This method was practised in Surinam also for the control of burrowing nematode, but bare fallowing was unsuitable (Maas, 1969). Colbran (1964) recommended a two-year fallow period with a cover crop of Panicum maximum var. trichoglume in Queensland, Australia. Salas et al. (1976) reported that adoption of fallow would be practically possible only when bananas were part of a rotation system with non-host crops of R. similis.

Resistance

In citrus, more than 1,400 clones were evaluated, only 15 were found sufficiently promising for further testing. Three of them - 'Milam' lemon (Citrus sp.) 'Ridge pineapple' sweet orange (C. sinensis) and 'Estes' rough lemon (C. limon) were found to be effective biological barrier against R. similis (Ford and Feder, 1964). Similar results were obtained by O'Bannon and Ford (1976).

Table 4. Hot water treatment of banana sets for control of Radopholus similis

Temperature range	Duration of treatment	Type of treatment	Effectiveness	Locality	Reference
55°C	5 minutes	Immersion	Eliminated infection	Sao Paulo, Brazil	Pereira et al. (1960)
"	30 "	"	"	New South Wales, Australia	Blake (1961)
"	25 "	"	Controlled infection	"	Blake (1963)
"	20 "	"	Controlled spread and damage	Cuba	Casamayor et al. (1966)
"	20 "	"	Eliminated infection	"	Decker et al. (1970)
55°C or 60°C	15 minutes or 10 minutes	"	Controlled infection and eliminated crop loss	Ceylon	Senanayake (1969)
52°C to 53°C	20 minutes	"	Controlled infection	Fiji	Taylor (1969)
53°C to 55°C	20 "	"	"	North Queensland, Australia	Broadley (1979b)

Wehunt et al. (1978) screened 70 cultivars of banana (Musa acuminata) and reported four cultivar groups were found resistant to R. similis. Charles et al. (1983) screened 125 cultivars of banana maintained under the germplasm collections at Kannara, Kerala, against R. similis and none of them was found immune to the nematode infection. Venkitesan and Setty (1978) screened 18 cultivars of black pepper, four Piper species and five wild Piper collections against R. similis. All of them were susceptible. Similar studies carried out on 27 cultivars/hybrids of coconut by Sosamma et al. (1980) did not reveal any of the cultivars/hybrids to be resistant to R. similis.

Besides the literature cited above the author had studied the distribution of R. similis in South India (Koshy et al., 1978b), proved the burrowing nematode as a potential pathogen on arecanut (Koshy and Sundararaju, 1981) and evaluated different arecanut germplasm collections against the burrowing nematode (Koshy et al., 1978a; Sundararaju and Koshy, 1982). The contents of these publications also have been utilised while writing this thesis.

Materials and methods

MATERIALS AND METHODS

Survey of *Radopholus similis* in Kerala, Karnataka and Tamil Nadu

The purpose of the survey was to map out the occurrence and distribution of the burrowing nematode, *Radopholus similis* in relation to the incidence of yellow leaf disease of arecanut. Extensive surveys were conducted during 1975 to 1982 in the arecanut growing tracts of Kerala (60,900 ha), Karnataka (54,300 ha) and Tamil Nadu (4,300 ha). The route of survey was mostly along the main roads and samples were collected from nearby gardens after every 10 to 15 km. A proforma (Appendix-I) with relevant information was prepared and used in the survey. A total of 822 each of soil and root samples was collected from healthy and yellow leaf disease-affected arecanut gardens covering soil groups of sandy loam, laterite, alluvial, clayey and red loam. The survey covered Chirayinkeezh, Nedumangad, Neyyattinkara and Trivandrum taluks of Trivandrum district; Karunagappally, Kottarakara, Kunnathur and Quilon taluks of Quilon district; Karthigappally, Mavelikara and Shertalai taluks of Alleppey district; Tiruvalla, Pathanapuram and Pathanamthitta taluks of Pathanamthitta district; Changanachery, Meenachil, Vaikom and Kottayam taluks of

Kottayam district; Alwaye, Cochin, Muvattupuzha, Parur and Kanayannur taluks of Ernakulam district; Chowghat, Kodungallur, Mukundapuram, Talappilly and Trichur taluks of Trichur district; Alathur and Ottapalam taluks of Palghat district; Eranadu and Perinthalmanna taluks of Malappuram district in Kerala state; Agastiswaram, Kalkulam, Thovala and Vilavancode taluks of Kanyakumari district; Nanguneri, Shencottah and Tiruchendur taluks of Tirunelveli district; Avanashi and Coimbatore taluks of Coimbatore district; Sankagiri taluk of Salem district; Coonoor and Gudalur taluks of Nilgiris district in Tamil Nadu State; Chikmagalur, Kadur, Koppa, Mudigere, Narasimharajapura and Sringeri taluks of Chikmagalur district; Bantwal, Belthangady, Karkala, Puttur and Sullia taluks of Dakshina Kannada district; Mercara and Somavarpet taluks of Kodagu district and Thirthahalli taluk of Shimoga district in Karnataka State.

Collection of samples

Soil and root samples were collected 75 cm away from the bole of the palm at a depth of 10 to 50 cm with a 75 mm diameter soil auger. Three such samples were taken within the basin at 120° to each other, mixed well and an aliquot of 250 cm^3 was drawn. Samples of tender to semi-hard portions of the main and lateral

roots, colour ranging from creamy white to light orange were taken and to this, root bits available in the auger samples were also added for the purpose of analysis. Root samples were restricted to 30 to 50 g each.

Samples were taken to represent healthy and diseased areas. Samples collected from healthy palms from fields/sites without any incidence of YLD in taluks that were broadly classified as YLD prevalent zones were designated as apparently healthy, in grouping of sampling sites for analysis.

One sample each was collected from every site/field in healthy and apparently healthy groups whereas up to four samples were taken from each sampling site in the disease affected fields, depending upon the availability of palms in different stages of the disease. In diseased tract, palms were categorised as apparently healthy, early, middle and advanced on the basis of disease index suggested by Rawther (1976) and George et al. (1980). They were (i) Apparently healthy-not showing any of the disease symptoms (ii) Early-palms showing initial stages of disease with index 10 to 25 (iii) Middle-palms in the middle stages of disease with index 25 to 50 and (iv) Advanced-palms showing advanced stages of disease with an index of 50 and above.

The data on the occurrence of R. similis were analysed both by total sample basis and sampling site basis. However, the samples collected from apparently healthy palms from disease affected fields along with samples collected from healthy palms from healthy fields in taluks designated as disease affected were considered together for analysis as apparently healthy.

Alongwith arecanut, root samples were collected also from coconut, cardamom, banana and black pepper grown in arecanut gardens wherever it was available.

Extraction of nematodes from soil

Soil samples were processed by Cobb's sieving and sifting method using 840, 250 and 38 µ aperture sieves (Cobb, 1918). The sievings from 38 µ mesh were collected in a beaker and transferred on to a moulded sieve of aluminium wire gauze containing two layers of face tissue paper and placed in a petridish (10 cm) with sufficient water. After 48 hours the suspension containing nematodes was carefully transferred to a clean 100 ml beaker.

Killing and fixing

The nematode suspension in the beaker was allowed to settle for one hour. The supernatant was decanted and boiling water equal to the volume of nematode suspension

was added to kill the nematodes. This was again left undisturbed for one hour. The supernatant was then poured off and eight per cent formalin of equal volume was added to the suspension for fixing nematodes. After two hours the fixed suspension was decanted further to reduce the volume to 25 ml. The total population of R. similis and other plant parasitic nematodes was estimated.

Extraction of R. similis from roots

Root samples were cut into 2.5 cm bits sliced longitudinally and left in petridish (15 cm) containing 100 ml of tap water for 72 hours in a B.O.D. incubator at 15° ± 1°C (Koshy et al., 1975). After 72 hours the suspension containing nematodes was passed through a set of 840, 250 and 38 µ size sieves and the sievings of 38 µ mesh were collected in a beaker.

Nematode suspension extracted from roots or soil was poured into a counting dish and counts were made under a stereoscopic binocular dissecting microscope.

Preparation of temporary and permanent mounts

For general observations temporary and permanent mounts of larvae, males and females were prepared in five per cent formalin (Goodey, 1963). For permanent mounts

Seinhorst's (1959) glycerol-ethanol method was followed. The slides were sealed with glyceel and labelled. These mounts were examined under high magnification of a compound microscope for detailed morphological studies. De Man's formula (Southey, 1970) was used for recording the measurements of different larval stages and adult males and females.

Soil sterilization

Sandy loam soil (coarse sand-54.79%., fine sand-28.51%, silt-0.04% and clay-15.55%) was collected on the farm of CPCRI and filled in the cement concrete tanks. The soil was fumigated with methyl bromide @ 1 kg/2.7 m^3 under polythene cover for 72 hours and used after a period of 20 days. Before using, the soil was analysed for nematodes and absence of nematodes confirmed.

Pathogenicity of Radopholus similis on arecanut

Twentyfive cement concrete pots (75 x 75 cm) were inserted 60 cm deep into laterite soil after opening pits, at one metre intervals in the field at CPCRI (RC) Palode. Each pot was filled with R. similis infested coarse sieved laterite soil (80 kg) from arecanut garden and treated with DBCP @ 60 l ai/ha. Soil samples drawn and analysed after 30 days did not yield any plant parasitic nematodes. One

year old arecanut seedling, variety South Kanara, raised in sterile soil was transplanted into each pot in September, 1977 after a lapse of 60 days. A thick growth of cover crop Pueraria phaseoloides was provided in the interspaces to reduce heat and avoid soil splashings during rainy season. Pots were shaded and irrigated during summer months, to maintain sustained vigorous growth. R. similis infested arecanut roots were collected from YLD affected arecanut gardens (Plate 2a) at CPCRI (RC) Farm, Palode and nematodes extracted were used for inoculation. The nematode suspension collected on 400 mesh sieve was placed on double layer tissue paper contained in an aluminium wire gauze on a petridish containing water to obtain active nematodes. Each treatment had five replications and was randomised. A logarithmic series of inoculum such as 0, 10, 100, 1,000 and 10,000 was used for inoculating plants on or very near to the roots in December, 1977. For treatments using 10 and 100 nematodes, active females and larvae were hand picked. The suspension containing the required number of nematodes was poured into three holes drilled with a wooden peg at different depths (10, 15 and 25 cm) around the base of the seedlings. The holes were closed and watered.

Observations on plant growth parameters such as number of leaves, shoot length and girth at collar region

were recorded at six monthly intervals after inoculation. Seedlings were dusted with five per cent BHC as and when required to protect them from infestations of mite (Raoiella indica Hirst.) and spindle bug (Carvalhoia arecae Miller and China). The plants were maintained for three years after nematode inoculation. When the experiment was concluded the plants were depotted carefully and plant growth parameters recorded. The lesions and rotting of roots (Plate 2b) were graded on a 0 to 5 scale as indicated below:

0 = no infection

1 = small, elongate lesions on white roots with necrotic root tips,

2 = prominent lesions of dark brown to black with necrotic root tips,

3 = coalescing lesions with necrotic root tips,

4 = partial decay of roots with necrotic root tips

5 = severe decay of roots with necrotic root tips.

Roots were cut into small pieces after indexing, mixed thoroughly and three aliquots of 10 g each were collected from each seedling, stained in boiling acid fuchsin lactophenol (Franklin and Goodey, 1949) for three minutes, cleared and churned for 40 seconds using a Waring blender for population assessment. Three aliquots

Plate 2

a. Yellow leaf disease affected arecanut seedlings in field

b. Lesions and rotting on young white to light orange coloured arecanut roots on infestation by *Radopholus similis*

Plate. 2

a

b

of five ml each of this suspension were drawn in a counting dish and the nematode population in each sample was assessed. The total root population was computed based on the average of these counts. The soil population of the nematodes was assessed by mixing thoroughly the available soil in the pots and drawing a sample of 250 cm^3 from each pot.

Histopathology of Radopholus similis infested arecanut roots

Twentyfive arecanut seednuts of variety South Kanara were sown in sterilised sand contained in earthen pots (50 cm). On germination the nut was removed carefully from the pot and a red silken thread was tied loosely on to the main root above 2.5 cm away from the root tip. Individual seedling was transferred to a small earthen pot (10 cm) and placed horizontally in moist sand filled to a depth of 8 cm. More dry sand was then added in such a manner as to cover the roots except the marked portion. A small quantity of sand was sprinkled on to the marked portion and few drops of water was added to fix this portion on sand surface and remained partially uncovered and visible. Suspension containing about 500 active R. similis from axenic culture was pipetted very close to the region between red silken

thread knot and root tip. After inoculation, the pots were then fully filled with sand and sprinkled with water immediately. Seven plants that served as control received only sterile water. Root sample was taken after 24, 48 and 72 hours, 5th, 10th, 15th and 30th day from different inoculated plants. Roots from the control plants were also collected. Two and a half centimetre portion of the root from the thread knot to the root tip was cut with the help of a sharp blade, fixed in F.A.A, dehydrated in TBA (tertiary butyl alcohol) series and embedded in paraffin. Longitudinal and transverse sections of 10 to 15 µ thickness were prepared and stained with safranin and fast green as described by Johansen in Plant microtechnique (1940).

Maintenance of Radopholus similis culture

Tender, main and lateral arecanut roots with the characteristic elongate, reddish brown cortical lesions of burrowing nematode were collected from yellow leaf disease-affected palms at the Central Plantation Crops Research Institute, (CPCRI) Research Centre, Palode, Trivandrum district, Kerala. Radopholus similis was extracted from the roots and inoculated on to healthy arecanut seedlings grown in sterile soil in cement tubs (75 x 75 cm) and earthen pots (35 x 35 cm) at CPCRI, Regional Station,

Kayangulam. On examination after two months, roots of these plants exhibited typical orange coloured, elongate cortical lesions separated from one another on young, white fleshy roots which yielded R. similis population. The nematode population from roots of plants in earthen pots inside greenhouse and in cement tubs outside greenhouse served as sources of inoculum for all further experiments.

Axenic culturing of Radopholus similis

The axenic carrot culture technique adopted is essentially based on the method of O'Bannon and Taylor (1968) modified by Koshy and Sosamma (1980). Fresh carrot tubers were thoroughly washed in water and dipped in 95 per cent ethyl alcohol. These were then flamed, pared and sliced into discs of 8-10 mm thickness. One such disc each was placed on one per cent sterile water agar (40 ml) in 100 ml Erlenmeyer conical flasks. These flasks were kept under observation in the laboratory for 48 to 72 hours to observe for contamination, if any, and also for initiation of callus growth.

The nematodes extracted from arecanut roots from culture pots were hand picked individually to sterile water contained in a Syracuse watch glass under a stereoscopic binocular microscope to avoid contamination. The

nematode suspension was then pipetted into sterile centrifuge tubes and run at 3000 rpm for one minute. The supernatant was decanted leaving about 0.5 ml suspension at the bottom of the tubes. Mercuric chloride (0.1%) was then added and the centrifuge was run again for one minute followed by removal of supernatant and rinsing the nematode suspension twice with sterile water, with 15 seconds centrifuguation each time. Then the population was washed thoroughly with 0.1 per cent streptomycin sulphate. About 0.5 ml of this treated population was then drawn out with a sterile syringe (needle No. 20) and released on or to the sides of carrot disc. The whole process was done on a laminar flow clean air bench. The inoculated flasks were then stored in dark at 20 to 25°C.

Discolouration of carrot discs was taken as a sign of multiplication of the nematode. Such culture could be kept up to two months. Subculturing was also done after 45 days of first inoculation. Nematodes obtained from the axenic cultures were used for different experiments.

Isolation and identification of fungal organism associated with lesions of Radopholus similis on arecanut roots

To study the association of other microorganisms with the lesions caused by burrowing nematode, roots showing typical lesions were collected from arecanut palms

at CPCRI (RC) Farm, Palode. Newly formed lesions on the white portion of the main roots were chosen for isolation. Three different methods were adopted for making the isolation viz. (1) transverse sections (1 mm) of roots having lesions were cut and surface sterilised with 0.1 per cent mercuric chloride for one minute and washed thrice in sterile water and transferred to potato dextrose agar (PDA) medium, (2) small lesions with surrounding root tissues were scooped after surface sterilisation with the help of a sterile sharp blade and transferred to PDA and (3) stele of the infested roots after surface sterilisation was separated and transferred to PDA.

Population fluctuation of Radopholus similis on arecanut

Ten to fifteen year old healthy and YLD affected arecanut palms with the early stage of disease were selected at CPCRI (RC) Palode, and the population fluctuation of R. similis was studied in two palms of each category. Tender, main and lateral roots of white to creamy white in colour with reddish brown cortical lesions were collected at fortnightly intervals during May, 1977 to April 1980. Care was also taken to sample only the above mentioned type of roots known to harbour maximum number of R. similis. Root samples of 25 to 30 g each were collected, processed and counts made as per the method described earlier.

Control of R. similis on arecanut in pots and under field conditions

Three experiments were carried out to evaluate the effect of nematicides and neem oil cake on the control of R. similis under pot and field conditions.

Effect of various nematicides in the control of Radopholus similis on arecanut seedlings in pots under field conditions

Twenty five cement pots (75 x 75 cm) were inserted 60 cm deep into laterite soil after opening pits, leaving the top 15 cm of the pot above the soil surface at a distance of one metre in the field at CPCRI (RC) Palode. These pots were filled with coarse sieved laterite soil (80 kg) from an infested field. One year old arecanut seedlings var. South Kanara raised in sterile soil were transplanted singly in the pots in September, 1977. Pueraria phaseoloides was grown in the interspace as a cover crop. Plants were shaded and irrigated, during summer months. The treatments consisted of four nematicides viz. aldicarb, aldicarb sulfone, carbofuran and fensulfothion @ 1 g ai/plant and control. The technical names, formulation of nematicides and sources of supply are given in Table 5. The treatments were completely randomised and replicated five times.

The required quantity of nematicides was applied on or very near to root system in the pot after removing the top 3 to 5 cm soil carefully. The soil was placed back after the application. Nematicides were applied three times a year during September, January and May. The first application was done immediately after planting. The plants in pots were irrigated immediately after every application of nematicides.

Seedlings were protected from mites and spindle bugs by applying five per cent BHC dust as and when required. Observations on plant growth parameters viz. number of leaves produced, shoot length and girth at collar region were recorded at six monthly intervals.

After three years, plants were removed carefully and data on shoot and root length, shoot and root weight, girth at collar region and number of leaves were recorded in October, 1980. Visual observations on the intensity of lesions and rotting in the roots were made and graded. Soil and root populations were also estimated following the same procedures described earlier under "Extraction of nematodes from soil and roots".

Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in YLD affected arecanut palms

Fifty bearing arecanut palms of uniform age exhibiting yellow leaf disease symptoms were selected at

CPCRI (RC), Palode. They were grouped into five lots on the basis of their disease intensity, pre-treatment soil and root samples were drawn for initial evaluation of R. similis and other parasitic nematode population. Treatments were randomised. There were five treatments comprised of fensulfothion @ 50 g ai/palm, aldicarb @ 10 g ai/palm, DBCP @ 10 ml ai/palm and neem oil cake @ 1.5 kg/palm and control. The treatments were randomised and replicated five times. The chemical nomenclature of the nematicides, method of application and sources of nematicides are given in Table 5. All the dosages of nematicides are expressed in terms of active ingredient per palm. Only common names of the chemicals have been used in the text.

The required quantity of nematicides and neem oil cake were applied very near to the root system after opening a basin during May/June, September/October and December/January depending upon the availability of soil moisture. In the case of DBCP, the calculated quantity of nematicides was mixed with water and then added to the arecanut basin as soil drench. The basins were covered with soil after the applications of nematicides and oil cake and irrigated immediately. Fertilizer application and other cultural practices were adopted as per standard schedules in vogue.

Pre-treatment soil and root population of nematodes were assessed every year during September/October. The severity of the disease was estimated as per the indexing method suggested by Rawther (1976) and modified by George et al. (1980). Yield data of individual palms was also recorded every year.

Effect of application of various nematicides on population build up of Radopholus similis and incidence of YLD symptoms on arecanut seedlings in field

A field infested with fairly high population of R. similis was selected and 64 pits (1 x 1 x 1 m) were prepared with an interspace of 2 x 2m. The pits were then filled with loose laterite soil up to 25 cm height. All pits other than those of control were treated with DBCP @ 60 l ai/ha. Pre-treatment soil samples were drawn and nematode populations assessed as per the method described earlier. After one month, one year old areca seedlings, variety South Kanara, raised in fumigated soil were transplanted in the pits. Treatments were aldicarb, phorate, phenamiphos, carbofuran, @ 3 g ai/ plant and DBCP @ 3 ml ai/plant, inoculations with R. similis extracted from infested arecanut roots, exenic population reared on carrot discs and control. The inoculum used per seedling were 10,000 nematodes. The

technical names, formulation and dosages of nematicides are shown in Table 5. The treatments were replicated eight times and laid out in a completely randomised block design.

The required quantity of nematicides was applied near the root system after opening a basin around the plant in the pit. In the case of DBCP and Nemacur E.C., the calculated quantity of nematicide was mixed with five litres of water and then added after opening a basin as soil drench. The root system was covered with the same soil after the application of nematicides. Plants were irfigated immediately after every application of nematicides. Nematicides were applied three times a year during May, September and January.

Pre-treatment soil and root population of the nematode were assessed every year during September/ October. The severity of the disease was estimated as per the indexing method mentioned earlier under the "control experiment No. 1."

Screening of Areca germplasm for resistance to Radopholus similis

All the Areca germplasm collections available at CPCRI, Regional Station, Vittal, Karnataka were screened

Table 5. Chemical composition, formulation, method of application and sources of supply of the materials used for control of Radopholus similis on arecanut seedlings/palms.

	Common name	Trade name	Chemical composition	Formulation	Method of application	Source
1)	Aldicarb	Temik	2-methyl-2(methylthio) propionaldehyde O-(methyl carbamoyl) oxime	10 G*	Broadcasted within basins and raked in to soil.	Union Carbide India Limited
2)	Aldicarb sulfone	Sulfocarb UC 21865	2 methyl-2-(methyl sulfomyl) propionaldehyde O-(methyl carbamyl) oxime	75% W.P+	"	"
3)	Carbofuran	Furadan	2,3-dihydro-2,2-dimethyl -7-benzofuranyl-N-methyl carbamate	3 G	"	Rallis India Limited
4)	Fensulfothion	Dasanit	(O-O-Diethyl-O-(4-methyl sulfinyophenyl)-monothio phosphate	5 G	"	Bayer (India) Limited
5)	Phorate	Thimet	O,O, Diethyl S-(ethylthio methyl) phosphorodithioate	10 G	"	Cyanamid India Limited
6)	Phenamiphos	Nemacur	ethyl 4-(methylthio)-m-totyl isopropylphosphoramidate	10 G or 40 EC**	Soil drenching in basin area	Bayer (India) Limited
7)	DBCP	Nemagon	1,2-dibromo 3-chloropane	60 EC W/W	"	Shell Chemical Company
8)	Neem cake	--	--	Cake powder	Soil application	Directorate of Non-edible Oils and soap industry, Khadi and Village Industries Commission Pune, Maharashtra

*G - Granules **EC - Emulsified Concentrate
+W.P.- Wettable powder

for locating resistance to the burrowing nematode. A total of 46 accessions, of which thirty one in 1977 and fifteen in 1981 was screened against R. similis. Twentyfive seednuts each of every accession were sown in 50 cm earthen pots containing 8 kg of fumigated sandy loam soil. Six months after sowing, ten seedlings of uniform growth in four leaf stage were selected. They were transplanted individually to small earthen pots (35 cm) filled with 5 kg of fumigated soil. In the first test, five seedlings each of all varieties were inoculated with 1,500 active (larvae and females) nematodes extracted from infested roots of seedlings maintained in culture pots at CPCRI (RS) Kayangulam. The remaining five seedlings were maintained as control and they received only the run off from the 400 mesh sieve used for collecting the nematode inoculum.

In 1981, seedlings of 15 cultivars were inoculated with axenic nematode population reared on carrot discs. Seedlings were maintained in a randomised manner in the greenhouse (Plate 3) where the ambient temperature ranged from 24° to 28°C for two years. Both the experiments were terminated in September, 1979, and September, 1983 respectively. When experiments were concluded data on length and weight of shoot and root, girth at collar region and

Plate 3

Potted arecanut seedlings in greenhouse for screening against *Radopholus similis*

Plate. 3

number of leaves were recorded. Final nematode populations in root and soil were also estimated. The extent of infection on the roots was also rated in the scale of 0 to 3 in the first experiment terminated in 1979 and 0 to 5 in the second experiment terminated in 1983 based on the formation of lesions.

Experimental results

EXPERIMENTAL RESULTS

Survey of *Radopholus similis* in Kerala, Karnataka and Tamil Nadu

The survey conducted in the major arecanut growing tracts of Kerala, Karnataka and Tamil Nadu recorded 28 genera of plant parasitic nematodes from the root zone of areca palms, when 822 samples of soil were analysed. *Radopholus similis* was the only endoparasitic nematode present in 264 root samples out of 822 samples analysed. Of these, one hundred and seventy samples were from Kerala, eightyseven from Karnataka and seven from Tamil Nadu. On a total sample basis, *R. similis* was observed in 32.1 per cent samples collected from yellow leaf disease-prevalent areas, closely followed by that of (31.9%) non-prevalent areas. Maximum population of 440 nematodes per g of root was recorded in YLD-affected palms, as against 48 from healthy palms of healthy tract. The distribution of *R. similis* in surveyed areas is presented in Tables 6 a, b, c and Figure 1.

The presence of *R. similis* in relation to soil groups was analysed based on the total number of samples and the results are presented in Table 7. The percentage occurrence was more in sandy loam soil (45.36%), closely followed by red loam (44.83%) and laterite (31.27%).

Table 6 (a). Occurrence of *Radopholus similis* in soil and root samples collected from YLD non-prevalent arecanut palms in Tamil Nadu

S. No.	District	Locations	Total samples collected	*R. similis* in	
				Soil	Root
I	Kanyakumari	Agastiswaram	2	-	-
		Kalkulam	8	4	2
		Thovala	3	1	2
		Vilavankode	6	1	-
II	Tirunelveli	Nanguneri	1	-	-
		Shengottah	4	-	-
		Tiruchendur	2	1	-
III	Coimbatore	Avanashi	5	3	2
		Coimbatore	2	1	-
IV	Salem	Sankagiri	1	-	-
V	Nilgiris	Coonoor	1	1	1
		Gudalur	1	-	-
		Total	36	12	7

- Not yielded *R. similis*

Table 6 (b). Occurrence of Radopholus similis in soil and root samples collected from YLD prevalent and non-prevalent areas in Kerala and Karnataka

S. No.	Locations District/Taluks	Healthy tract		Diseased tract				Total samples	Sampling sites
				Apparently healthy		Diseased			
		Soil	Root	Soil	Root	Soil	Root		
I	KERALA STATE Trivandrum								
	1. Chirayinkeezh	-	-	3/9*	3/9	6/10	5/10	9/19	5/7
	2. Nedumangad	-	-	12/32	14/32	46/199	54/199	68/231	21/25
	3. Neyyattinkara	-	-	6/24	7/24	6/24	10/24	17/48	18/19
	4. Trivandrum	-	-	0/9	3/9	3/3	1/3	6/12	2/5
II	Quilon								
	1. Karunagappally	-	-	2/3	3/3	2/2	2/2	5/5	3/3
	2. Kottarakara	-	-	3/12	4/12	1/11	2/11	6/23	5/9
	3. Kunnathur	-	-	10/20	8/20	3/9	0/9	13/29	12/14
	4. Quilon	-	-	2/6	0/6	0/3	1/3	3/9	1/5
III	Pathanamthitta								
	1. Pathanapuram	-	-	5/32	4/32	9/41	8/41	14/73	14/30

Contd...

Table 6 (b)(Contd...)

S. No.	Locations District/Taluks	Healthy tract		Diseased tract				Total samples	Sampling sites
				Apparently healthy		Diseased			
		Soil	Root	Soil	Root	Soil	Root		
	2. Pathanamthitta	-	-	0/1	0/1	0/1	0/1	0/2	0/1
	3. Thiruvalla	-	-	0/2	0/2	0/1	0/1	0/3	0/2
IV	Alleppey								
	1. Karthigappally	2/4	1/4	-	-	-	-	2/4	2/2
	2. Mavelikara	2/2	2/2	-	-	-	-	2/2	2/2
	3. Shertalai	1/2	0/2	-	-	-	-	1/2	1/2
V	Kottayam								
	1. Changanachery	-	-	-	-	0/1	0/1	0/1	0/1
	2. Kottayam	-	-	-	-	1/1	0/1	1/1	1/1
	3. Meenachil	-	-	1/1	0/1	-	-	1/1	1/1
	4. Vaikom	-	-	1/3	0/3	0/1	0/1	1/4	1/4
VI	Ernakulam								
	1. Alwaye	-	-	2/3	1/3	2/4	2/4	4/7	1/3

Contd...

Table 6 (b) (Contd...)

S. No.	Locations District/Taluks	Healthy tract		Diseased tract				Total samples	Sampling sites
				Apparently healthy		Diseased			
		Soil	Root	Soil	Root	Soil	Root		
	2. Cochin	-	-	1/2	0/2	1/2	1/2	2/4	2/2
	3. Kanayannur	-	-	0/1	0/1	-	-	0/1	0/1
	4. Muvattupuzha	-	-	0/1	0/1	-	-	0/1	0/1
	5. Parur	-	-	1/3	0/3	1/3	0/3	2/6	2/4
VII	Trichur								
	1. Chowghat	1/4	0/4	-	-	-	-	1/4	1/2
	2. Kodungallur	-	-	2/3	2/3	1/1	1/1	3/4	3/4
	3. Mukundapuram	-	-	8/29	8/29	20/44	18/44	28/73	23/28
	4. Talappilly	2/6	1/6	-	-	-	-	3/6	1/5
	5. Trichur	--	-	3/12	1/12	1/14	0/14	4/26	2/7
VIII	Palghat								
	1. Alathur	4/6	2/6	-	-	-	-	4/6	2/4
	2. Ottapalam	1/4	1/4	-	-	-	-	1/4	1/4

Contd...

Table 6 (b) (Contd...)

S. No.	Locations District/Taluks	Healthy tract		Diseased tract				Total samples	Sampling sites
				Apparently healthy		Diseased			
		Soil	Root	Soil	Root	Soil	Root		
IX	Malappuram								
	1. Eranadu	0/3	0/3	-	-	-	-	0/3	0/3
	2. Perinthalmanna	0/2	0/2	-	-	-	-	0/2	0/2
	Total in Kerala	13/33	7/33	62/208	58/208	103/375	105/375	201/616	127/203
	KARNATAKA STATE								
I	Chikmagalur								
	1. Chikmagalur	2/2	1/2	-	-	-	-	2/2	2/2
	2. Kadur	2/2	2/2	-	-	-	-	2/2	2/2
	3. Narasimharajpura	1/3	1/3	-	-	-	-	1/3	2/3
	4. Mudigere	2/5	4/5	-	-	-	-	4/5	3/3
	5. Koppa	-	-	6/12	9/12	6/15	11/15	20/27	8/10
	6. Sringeri	-	-	4/6	4/6	3/6	5/6	9/12	5/6

Contd...

Table 6 (b) (Contd...)

S. No.	Locations District/Taluks	Healthy tract		Diseased tract				Total samples	Sampling sites
				Apparently healthy		Diseased			
		Soil	Root	Soil	Root	Soil	Root		
II	Dakshina Kannada								
	1. Bantwal	17/20	17/20	-	-	-	-	17/20	14/16
	2. Belthangady	0/4	0/4	-	-	-	-	0/4	0/4
	3. Karkala	1/6	4/6	-	-	-	-	4/6	3/4
	4. Puttur	10/62	14/62	-	-	-	-	14/62	6/28
	5. Sullia	-	-	1/6	4/6	7/12	10/12	14/18	6/6
III	Kodagu								
	1. Mercara	0/3	0/3	-	-	-	-	0/3	0/3
	2. Somavarpet	1/1	0/1	-	-	-	-	1/1	1/1
IV	Shimoga								
	1. Thirthahalli	3/5	1/5	-	-	-	-	3/5	3/4
	Total in Karnataka	39/113	44/113	11/24	17/24	16/33	26/33	91/170	55/92

*Numerator is number of samples yielded R. similis and denominator is total number of samples collected.

- Location not sampled

Table 6(c). Details on the total number of samples collected and the number of soil and root samples that yielded Radopholus similis in Kerala, Karnataka and Tamil Nadu

S. No.	States	Healthy Tract			Diseased Tract						Grand Total		
					Apparently healthy palms			Diseased palms					
		Total	Soil	Root	Total	Soil	Root	Total	Soil	Root	Total	Soil	Root
1.	Kerala	33	13 (39.4)	7 (21.2)	208	62 (29.8)	58 (27.9)	375	103 (27.5)	105 (28.0)	616	178 (28.9)	170 (27.6)
2.	Karnataka	113	39 (34.5)	44 (38.9)	24	11 (45.8)	17 (70.8)	33	16 (48.5)	26 (78.8)	170	66 (38.8)	87 (51.2)
3.	Tamil Nadu	36	12 (33.3)	7 (19.4)	-	-	-	-	-	-	36	12 (33.3)	7 (19.4)
	Total	182	64 (35.2)	58 (31.9)	232	73 (31.5)	75 (32.3)	408	119 (29.2)	131 (32.1)	822	256 (31.1)	264 (32.1)

Figures in parentheses are the percentages

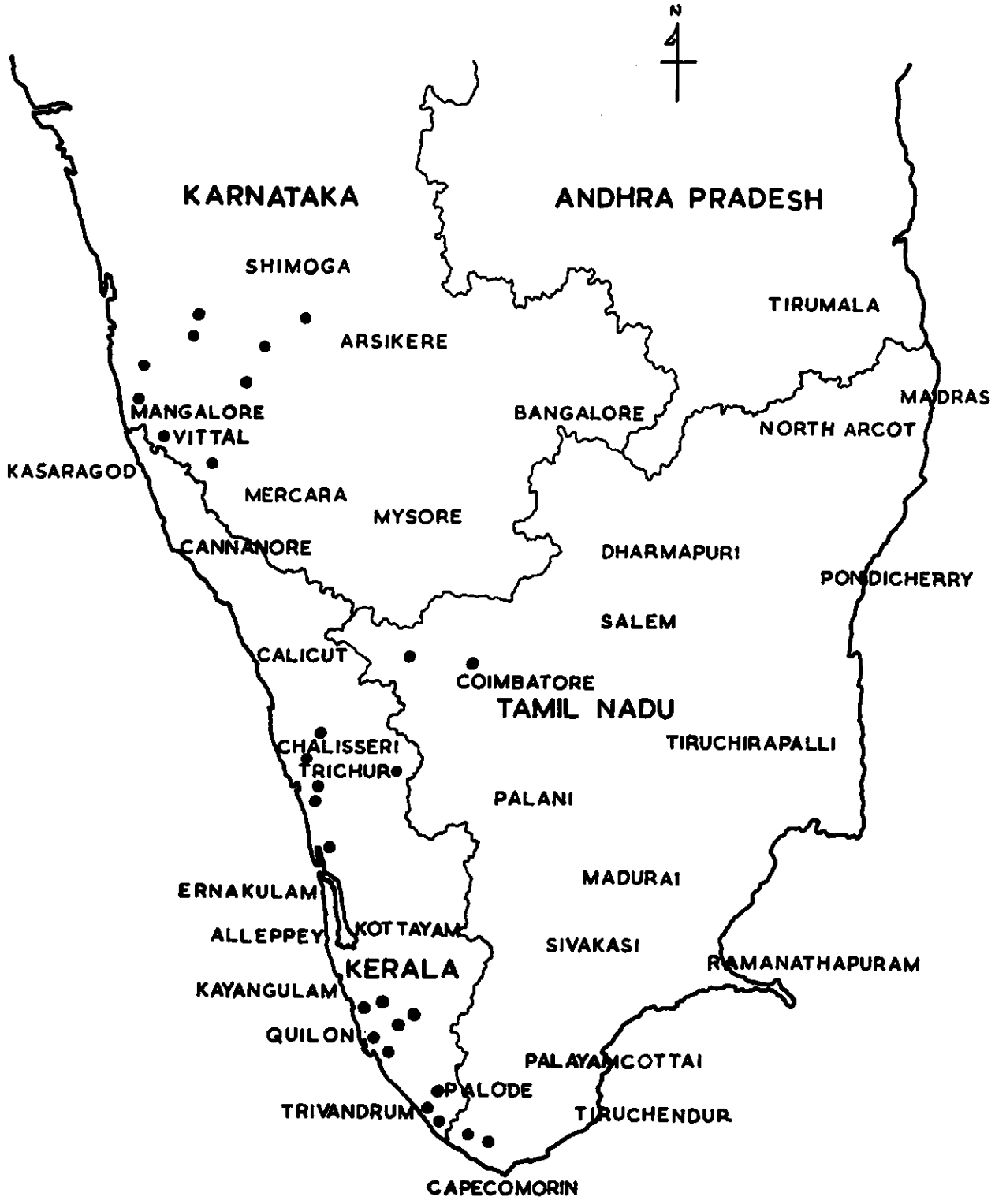
Table 7. Occurrence of Radopholus similis in different soil groups in relation to distribution of yellow leaf disease of arecanut

S. No.	Soil groups	Healthy tract	Diseased tract			
		Healthy	Apparently healthy	Diseased	Total	Sampling site
1.	Sandy loam	6/19[a] (31.58)	15/37 (40.64)	23/41 (56.10)	44/97 (45,36)	28/40 (70.00)
2.	Laterite	40/108 (37.04)	38/113 (33.63)	84/297 (28.28)	162/518 (31.27)	105/167 (62.87)
3.	Alluvial	7/30 (23.33)	13/37 (35.14)	13/44 (29.55)	33/111 (29.73)	37/76 (48.68)
4.	Clayey	3/12 (25.00)	4/37 (10.81)	5/18 (27.78)	12/67 (17.91)	11/21 (52.38)
5.	Red loam	2/13 (15.38)	5/8 (62.50)	6/8 (75.00)	13/29 (44.83)	13/27 (48.14)
	Total	58/182 (31.87)	75/232 (32.33)	131/408 (32.11)	264/822 (32.12)	194/33 (58.61)

Figures in parentheses are the percentages

a = Numerator is number of samples yielded R. similis and denominator is total number of samples collected

Fig.1. Distribution of R. similis on arecanut in South India.



While sampling sites were taken into consideration it was seen that R. similis was recorded from 108/146 (73.97%) sites from YLD-affected palms as compared to 28/53 (52.83%) sites from apparently healthy palms in the YLD tract and 58/132 (43.9%) sites in healthy tract (Tables 6 a, b, c).

The percentage occurrence of R. similis in root samples in relation to sandy loam, laterite, alluvial, red loam and clayey soil groups were 70.0, 62.87, 48.68, 48.14 and 52.38, respectively, when analysed on sampling site basis (Table 7).

Apart from R. similis, 27 genera of plant parasitic nematodes were also observed in soil samples and their frequency of occurrence in different soil groups is shown in Table 8.

The genus Diptherophora and the species Aphelenchoides aligarhiensis, Aphelenchus (Anaphelenchus) isomerus, Caloosia longicaudata, Discocriconemella limitanea, Ecphyadophora teres, Epicharinema keralense, Helicotylenchus dihystera, Hemicriconemoides mangiferae, Hoplplaimus seinhorsti, Longidorus saginus, Macroposthonia ornata, Pratylenchus zeae, Scutellonema unum, Tylenchorhynchus coffeae and Xiphinema inaequale are reported for the first time on arecanut in India.

Table 8. Occurrence of plant parasitic nematodes on arecanut in relation to different soil groups

S. No.	Nematode species	Soil groups					
		Sandy loam 97*	Laterite 518*	Alluvial 111*	Clayey 67*	Red loam 29*	Total 822*
1.	Aphelenchoides aligarhiensis Siddiqi, Hussain and Khan, 1967	3	18	3	1	3	28
2.	Aphelenchus (Anaphelenchus) isomerus Anderson and Hooper, 1980	22	25	24	6	7	84
3.	Brachydorus swarupi Koshy, Raski and Sosamma, 1981	-	4	7	-	-	11
4.	Caloosia longicaudata (Loos, 1948) Siddiqi and Goodey, 1964	11	60	21	5	4	101
5.	Criconemoides sp.	6	30	6	-	2	44
6.	Diptherophora sp.	-	2	3	-	-	5
7.	Ditylenchus sp.	12	12	3	1	-	28
8.	Discocriconemella limitanea (Luc, 1959) De Grisse and Loof, 1965	2	-	-	-	2	4
9.	Ecphyadophora teres Raski, Koshy and Sosamma, 1982	10	4	8	4	1	27

Contd...

Table 8 (Contd..)

S. No.	Nematode species	Soil groups					
		Sandy loam 97*	Laterite 518*	Alluvial 111*	Clayey 67*	Red loam 29*	Total 822*
10.	Epicharinema keralense Raski, Koshy, Maggenti and Sosamma, 1980	8	4	17	2	-	31
11.	Helicotylenchus dihystera (Cobb, 1893) Sher, 1961	46	255	66	27	21	415
12.	Hemicriconemoides mangiferae Siddiqi, 1961	23	80	25	11	7	146
13.	Hoplolaimus seinhorsti Luc, 1958	21	37	25	8	7	98
14.	Longidorus saginus Khan, Seshadri, Weischer and Mathen, 1971	-	8	3	1	-	12
15.	Macroposthonia ornata (Raski, 1958) De Grisse and Loof, 1965	4	25	6	-	3	38
16.	Meloidogyne sp.	21	65	35	10	4	135
17.	Paralongidorus sp.	-	1	1	-	-	2
18.	Paratylenchus sp.	6	26	14	2	4	52
19.	Pratylenchus zeae Graham, 1951	1	10	5	2	-	18

Contd...

Table 8 (Contd..)

S. No.	Nematode species	Soil groups					
		Sandy loam 97*	Laterite 518*	Alluvial 111*	Clayey 67*	Red loam 29*	Total 822*
20.	Psilenchus sp.	-	1	-	-	-	1
21.	Radopholus similis (Cobb, 1893) Thorne, 1949	44	156	33	12	11	256
22.	Rotylenchulus reniformis Linford and Oliveira, 1940	40	224	64	28	20	376
23.	Rotylenchus sp.	1	2	4	1	-	8
24.	Scutellonema unum Sher, 1964	-	2	-	-	-	2
25.	Trichodorus sp.	-	4	2	-	1	7
26.	Tylenchorhynchus coffeae Siddiqi and Basir, 1959	31	48	43	8	5	135
27.	Tylenchus sp.	2	19	18	1	9	49
28.	Xiphinema inaequale Khan and Ahmed, 1975	7	39	8	2	2	58

*Total number of soil samples under each category.

- Not yielded nematodes.

Three nematode species, H. dihystera, R. reniformis and R. similis were recorded in maximum number of samples and their percentage occurrences were 50.49, 45.74 and 32.12, respectively. With reference to the different soil groups, the percentage occurrence of H. dihystera was 47.42, 49.23, 59.46, 40.30 and 72.41 in sandy loam, laterite, alluvial, clayey and red loam, respectively. Similarly the frequencies of occurrences of R. reniformis and R. similis recorded were 41.24, 43.24, 57.66, 41.79 and 68.97 and 45.36, 31.27, 29.73, 17.91 and 44.83 per cent respectively. Among the other species C. longicaudata, H. mangiferae, Meloidogyne sp. and T. coffeae were recorded in more than 15 per cent of the total samples collected. The juveniles of Meloidogyne sp. were noticed in 135 soil samples, however, root galling was not observed. R. reniformis recorded the highest population density (upto 4800 nematodes per 250 cm^3 of soil) in most of the fields sampled, in all soil types. However, no gravid females could be observed on the roots.

Occurrence of R. similis population in arecanut gardens with and without intercrops

When samples, collected from the states of Kerala, Karnataka and Tamil Nadu were considered, R. similis was observed in 56.29 per cent of samples from arecanut palms

mixcropped with cardamom and 51.00, 38.10 and 29.80 per cent when inter/mixcropped with banana, black pepper and coconut, respectively, whereas in pure areca plantations, the percentage occurrence was only 25.37. It is also seen that in Karnataka where the crops are irrigated the per cent occurrence of R. similis was more irrespective of the crop combinations involved (Table 9).

Root samples were collected also from banana, black pepper, cardamom and coconut, grown in arecanut gardens wherever possible. Maximum percentage occurrence of R. similis was noticed in banana (64.45%) compared to black pepper (25.0%), cardamom (18.18%) and coconut (16.77%).

Pathogenicity of R. similis on arecanut seedlings in pots under field conditions

The pathogenicity of R. similis on arecanut seedlings was studied by using different levels of inoculum (10, 100, 1,000 and 10,000 per plant) to gather information on the role of this nematode in causing damage, expressed in terms of plant growth parameters. The growth characters like shoot length, shoot weight, number of leaves, root length, root weight and girth at collar region were recorded. The nematode inoculated plants exhibited general yellowing and visible reduction in growth and vigour compared to control plants (Plate 4a).

Table 9. Effect of intercrops on occurrence of Radopholus similis in arecanut palms

S. No.	Locality (State)	Arecanut + Banana	Arecanut + Black pepper	Arecanut + Cardamom	Arecanut + Coconut	Arecanut alone	Total No. of arecanut samples
1.	Kerala	71/160[a] (44.38)	20/55 (36.36)	-	70/215 (32.56)	40/186 (21.51)	201/616 (32.63)
2.	Karnataka	52/79 (65.82)	4/8 (50.00)	18/32 (56.25)	5/36 (13.89)	12/15 (80.00)	91/170 (53.53)
3.	Tamil Nadu	5/12 (41.67)	-	-	6/20 (30.00)	0/4 (0.00)	11/36 (30.56)
	Total	128/251 (51.00)	24/63 (38.10)	18/13 (56.29)	81/271 (29.89)	52/205 ((25.37)	308/822 (36.86)

Figures in parentheses are the percentages

a = Numerator is number of samples yielded R. similis and denominator is total number of samples collected

Shoot length

A progressive reduction in plant height was evident in the seedlings with increase in initial inoculum levels. The average shoot length of control plants was 225.80 cm as compared to 191.61, 174.40, 161.00 and 157.80 cm of the plants inoculated with 10, 100, 1,000 and 10,000 nematodes, respectively. Maximum reduction (30.12%) in shoot growth was noticed with 10,000 nematodes whereas in other inoculum levels it ranged from 15.15 to 28.70 per cent. Treatment differences were significant at one per cent level. The regression equation for the height of the seedlings was $y = 215.79-16.7602 \log (x + 1)$ $R^2 = 0.9003$ (Fig. 2a and Table 10).

Shoot weight

Data in Table 10 show corresponding decrease in shoot weight of the seedlings with increase in initial inoculum levels. The percentage reduction of shoot weight over control was 11.75, 38.81, 50.40 and 52.86 in plants inoculated with 10, 100, 1,000 and 10,000 nematodes, respectively. The regression equation for shoot weight was $y = 2477.90-364.8673 \log (x + 1)$ $R^2 = 0.9257$ (Fig. 2a).

Number of leaves

The plants inoculated with 10, 100, 1,000 and 10,000 nematodes produced an average of 12.12, 13.12, 11.75 and

fig.2a. Effect of different inoculum levels of R. similis on shoot growth of arecanut seedlings in pots under field conditions.

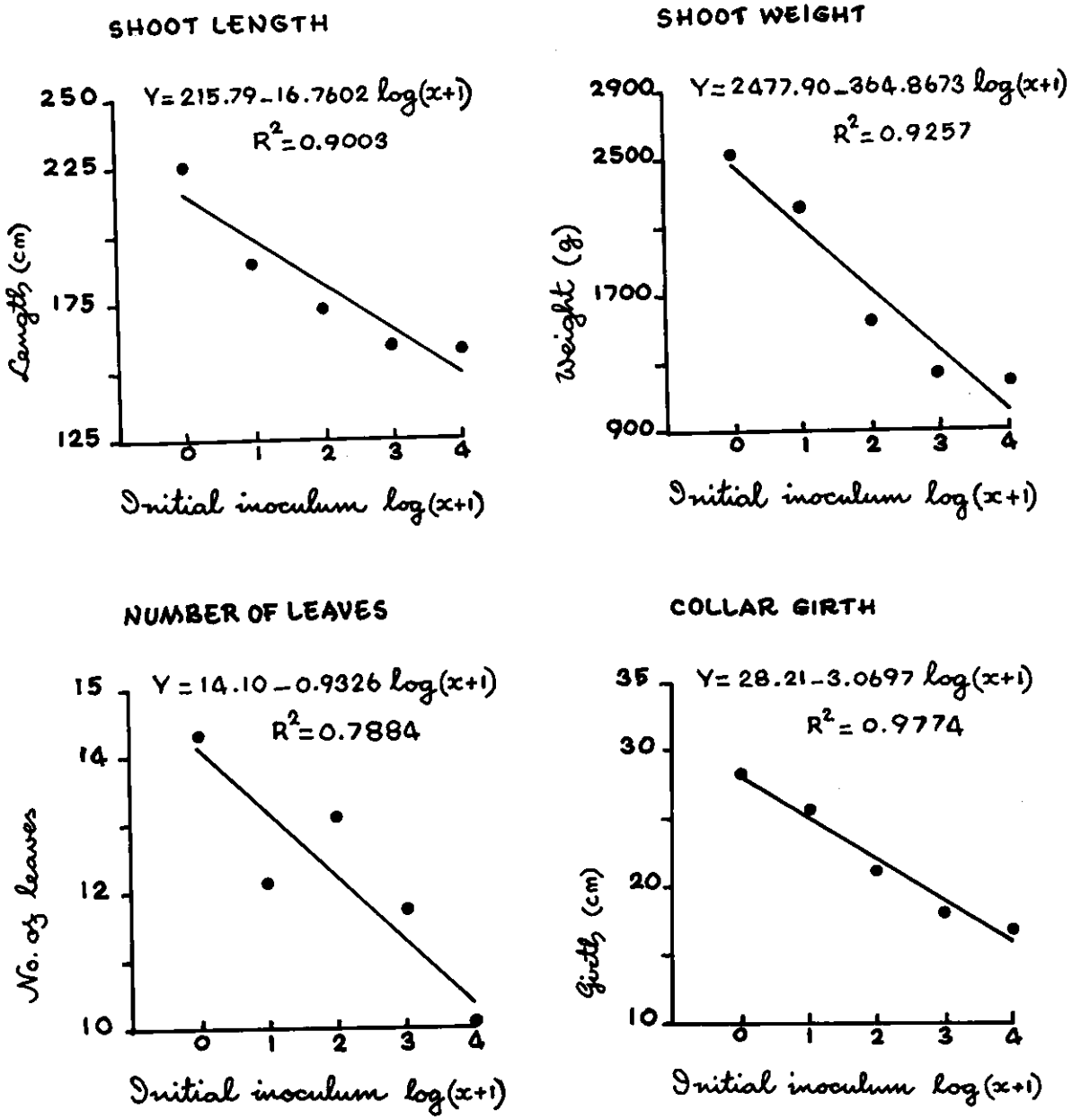


Table 10. Effect of different inoculum levels of *Radopholus similis* on shoot growth of arecanut seedlings in pots under field conditions (Mean of five replications)

S. No.	Initial inoculum levels	Shoot length (cm)	Per cent reduction over control	Shoot weight (g)	Per cent reduction over control	Number of leaves	Per cent reduction over control	Girth at collar region (cm)	Per cent reduction over control
1.	Check	225.80^{c}		2520^{b}		14.29 (3.7808)		28.20^{c}	
2.	10	191.60^{b}	15.15	2224^{b}	11.75	12.12 (3.4820)	15.28	25.80^{bc}	8.51
3.	100	174.40^{ab}	22.75	1542^{a}	38.81	13.12 (3.6228)	8.33	21.20^{ab}	24.52
4.	1000	161.00^{ab}	28.70	1250^{a}	50.40	11.75 (3.4279)	18.06	18.40^{a}	34.75
5.	10,000	157.80^{a}	30.12	1188^{a}	52.86	9.85 (3.1388)	30.56	16.60^{a}	41.13
	S.Em	10.95		223.07		0.1507		1.87	
	C.D.at 1%	44.06		897.50		NS		7.54	
	C.D.at 5%	32.30		658.06		NS		5.53	

Figures in parentheses are square root transformed values
Means with notations by the same letter do not differ at 5% level.

Analysis of variance — Mean sum of squares

Source	df	Shoot length	Shoot weight	Number of leaves	Girth at collar region
Inoculum	4	3869**	1783186**	0.2867	119.58**
Error	20	6000	248794	0.1135	17.54

9.85 leaves, respectively, compared to control (14.29). The differences were marginal and not significant between control and plants inoculated with nematodes. The inoculated plants showed considerable yellowing of leaves, but the characteristic yellowing of the yellow leaf disease was not observed on any of the inoculated plants. The regression equation for number of leaves was $y = 14.10-0.9326 \log (x + 1)$ $R^2 = 0.7884$ (Fig. 2a and Table 10).

Girth at collar region

Maximum plant girth 28.20 cm was recorded in control seedlings and it was minimum (16.60 cm) in seedlings inoculated with 10,000 nematodes. Treatment differences were significant at one per cent level and plants inoculated with 100, 1,000 and 10,000 nematodes were on a par. The regression equation for girth at collar region was $y = 28.21-3.0697 \log (x + 1)$ $R^2 = 0.9774$ (Fig. 2a and Table 10).

Root length

Significant differences in root length were noticed between treatments. The plants inoculated with 10, 100, 1,000 and 10,000 nematodes recorded 3.56, 19.37, 31.23 and 30.63 per cent reduction in root length respectively. However, the root length measurements in 100, 1,000 and

10,000 nematodes inoculated seedlings were found to be at par. The regression equation for root length was $y = 102.16-9.0182 \log (x + 1)$ $R^2 = 0.9122$ (Fig. 2b and Table 11).

Root weight

The reduction in root weight was directly proportional to increase in nematode population. The maximum root weight of 972 g was recorded in control plants and the least (422 g) in plants inoculated with 10,000 nematodes. The percentage reduction in root weights due to different inoculum levels was 19.96, 38.07, 53.09 and 56.58, respectively, in plants inoculated with 10, 100, 1,000 and 10,000 nematodes compared to the control plants. Root weight in plants inoculated with 100, 1,000 and 10,000 nematodes was at par with each other. The regression equation for root weight was $y = 932.98-142.8326 \log (x + 1)$ $R^2 = 0.9549$ (Fig. 2b and Table 11).

In general, there was considerable reduction in the root growth of inoculated plants particularly with higher inoculum levels of nematodes in comparison to control plants. Severe reduction in the number of lateral roots occurred in inoculated plants, whereas in control plants the lateral roots formed the major mass of the root system (Plate 4b).

Fig.2b. Effect of different inoculum levels of R. similis on population build up, host infestation and root growth of arecanut seedlings in pots under field conditions.

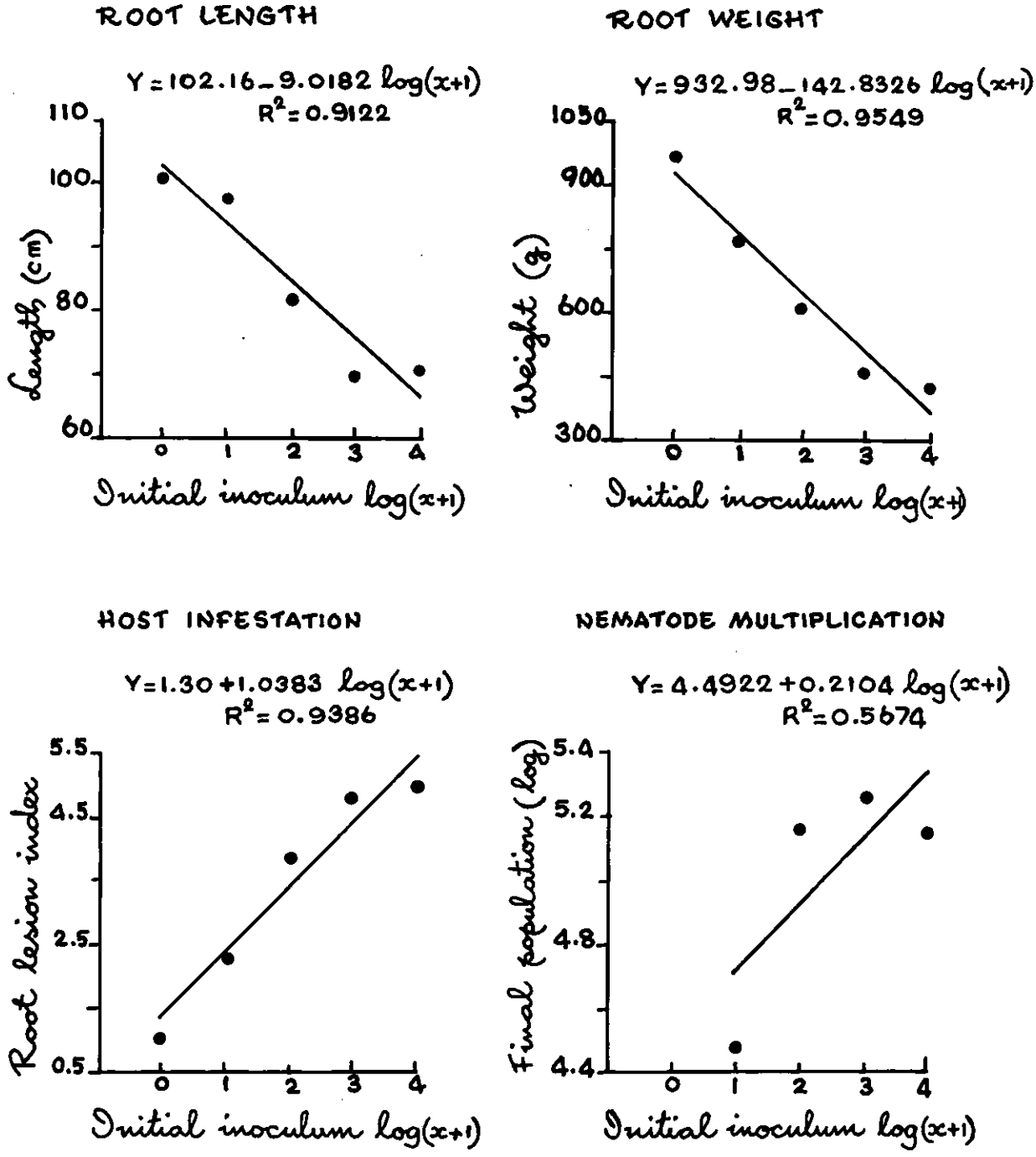


Plate 4

a. Effect on the shoot growth of arecanut seedlings inoculated with different levels of Radopholus similis under field conditions in comparison to uninocuated control

A. Radopholus similis - 10,000
B. " " - 1,000
C. " " - 100
D. " " - 10
E. Control

b. Effect on the shoot and root growth of arecanut seedlings inoculated with different levels of Radopholus similis in comparison to uninoculated control

A. Radopholus similis - 10,000
B. " " - 1,000
C. " " - 100
D. " " 10
E. Control

Plate. 4

a

b

Table 11. Effect of different inoculum levels of Radopholus similis on root growth of arecanut seedlings in pots under field conditions (Mean of five replications)

S. No.	Initial inoculum levels	Root length (cm)	Per cent reduction over control	Root weight (g)	Per cent reduction over control	Root lesion index
1.	Check	101.20^{c}		972.00^{c}		1.00 (1.0)^{c}
2.	10	97.60^{bc}	3.56	778.00^{bc}	19.96	2.35 (1.5314)^{bc}
3.	100	81.60^{ab}	19.37	602.00^{ab}	38.07	3.87 (1.9664)^{ab}
4.	1000	69.60^{a}	31.23	456.00^{a}	53.09	4.77 (2.1843)^{ab}
5.	10,000	70.20^{a}	30.63	422.00^{a}	56.58	4.96 (2.2270)^{a}
	S.Em	6.46		87.94		0.1135
	C.D. at 1%	26.04		353.83		3.35
	C.D. at 5%	19.09		259.43		2.46

Figures in parentheses are square root values
Means with notations by the same letter do not differ at 5% level

Analysis of variance — Mean sum of squares

Source	df	Root length	Root weight	Root lesion index
Inoculum	4	1105**	264890**	1.3353**
Error	20	209	38669	0.0644

An increase in initial inoculum from 10 to 10,000 nematodes resulted in corresponding increase in root lesions, rotting and blackening of root tips. The maximum root lesion index (4.96) was recorded in plants which had an initial inoculum level of 10,000 nematodes against 2.35 in plants inoculated with 10 nematodes per plant. Root lesion indices in seedlings inoculated with 100, 1,000 and 10,000 nematodes were at par with each other. The regression equation for root lesion index was $y = 1.30 + 1.0383 \log (x+1)$ $R^2 = 0.9386$ (Fig. 2b and Table 11).

Reproduction rate and final nematode population

The population build up of the nematode with different levels of initial inocula are given in Table 12. The highest rate of multiplication (3040) was obtained in plants inoculated with 10 nematodes and it was minimum (14) in the pots which had an initial inoculum of 10,000 nematodes. The rate of increase in the other treatments viz. 100 and 1,000 nematodes/plant was 1,477 and 185, respectively. Maximum number of nematodes, was recorded both in soil and roots of the plants inoculated with 1,000 nematodes. The calculated regression equations and the reproduction factors are presented in Table 12 and Fig. 2b.

Fig.3. Effect of different inoculum levels of R. similis on growth of arecanut seedlings in pots under field conditions.

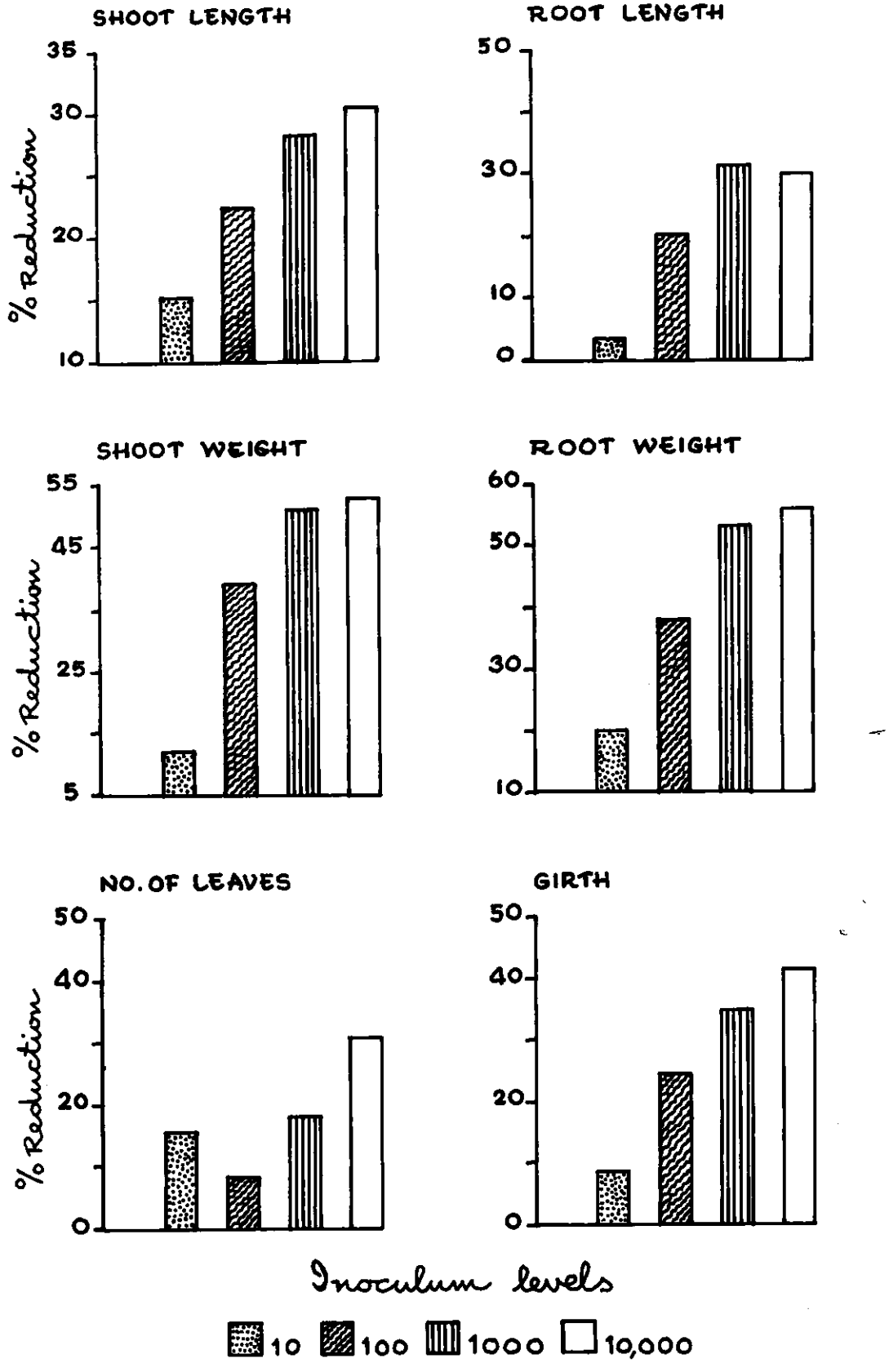


Table 12. Effect of different inoculum levels on population build up of Radopholus similis on arecanut seedlings in pots under field conditions (Mean of five replications)

S. No.	Initial inoculum levels	Nematode population			Multiplication factor
		Soil	Root	Total multiplication	
1.	Check	0	0	0	0
2.	10	581.83 (2.7648)	29573 (4.4709)^{b}	30402 (4.4829)^{b}	3040
3.	100	450.40 (2.6536)	146960 (5.1672)^{a}	147775 (5.1696)^{a}	1477
4.	1000	786.68 (2.8958)	189583 (5.2778)^{a}	189823 (5.2691)^{a}	185
5.	10,000	323.44 (2.5098)	141189 (5.1498)^{a}	141579 (5.1510)^{a}	14
	S.Em	0.1622	0.1426	0.1386	
	C.D. at 1%	NS	0.4276	0.4154	
	C.D. at 5%	NS	0.5892	0.5724	

Check was not included for analysis

Figures in parentheses are log transformed values

Means with notations by the same letter do not differ at 5% level

Analysis of variance

Source	df	Mean sum of squares		
		Soil	Root	Total multiplication
Inoculum	3	0.1346	0.6773**	0.6501**
Error	16	0.1316	0.1017	0.0960

Regression equation:

Soil Y = 2.8367-0.0523 log (x+1) (R^2=0.1693)

Root Y = 4.4796+0.2147 log (x+1) (R^2=0.5672)

Total multiplication Y = 4.4922+0.2104 log (x+1) (R^2=0.5674)

Histopathology of Radopholus similis infested arecanut roots

Examination of longitudinal and transverse sections of R. similis infested roots revealed considerable damage to root tissues. Longitudinal burrows developed underneath the outer cortical cell layers and nematodes and their eggs could be located here (Plate 5). Nematodes were also seen in both inter and intra-cellular positions although intercellular orientation was more common. In no case the nematodes were seen intruding stelar tissues. Necrotic changes occurred around the head of the nematodes and the burrows harbouring them (Plate 6a). The nematode feeding disintegrated the cytoplasm and cell wall of the host, and coalescence of these led to the formation of cavities or burrows in which the nematodes bred and multiplied (Plate 6b).

Axenic culturing of Radopholus similis

Axenic culturing of R. similis was found successful with the R. similis population from arecanut. Carrot discs placed on one per cent agar after 20 to 30 days developed gradual brown discolouration of the callus. Complete discolouration was observed after two months. Discolouration of the carrot discs was taken as a sign of multiplication of the nematode. The nematode population

Plate 5

Longitudinal section of an arecanut root inoculated with *Radopholus similis* showing the orientation of nematodes in the cortical tissue

B - Burrow

N - Nematode

OC - Outer Cortex

Plate. 5

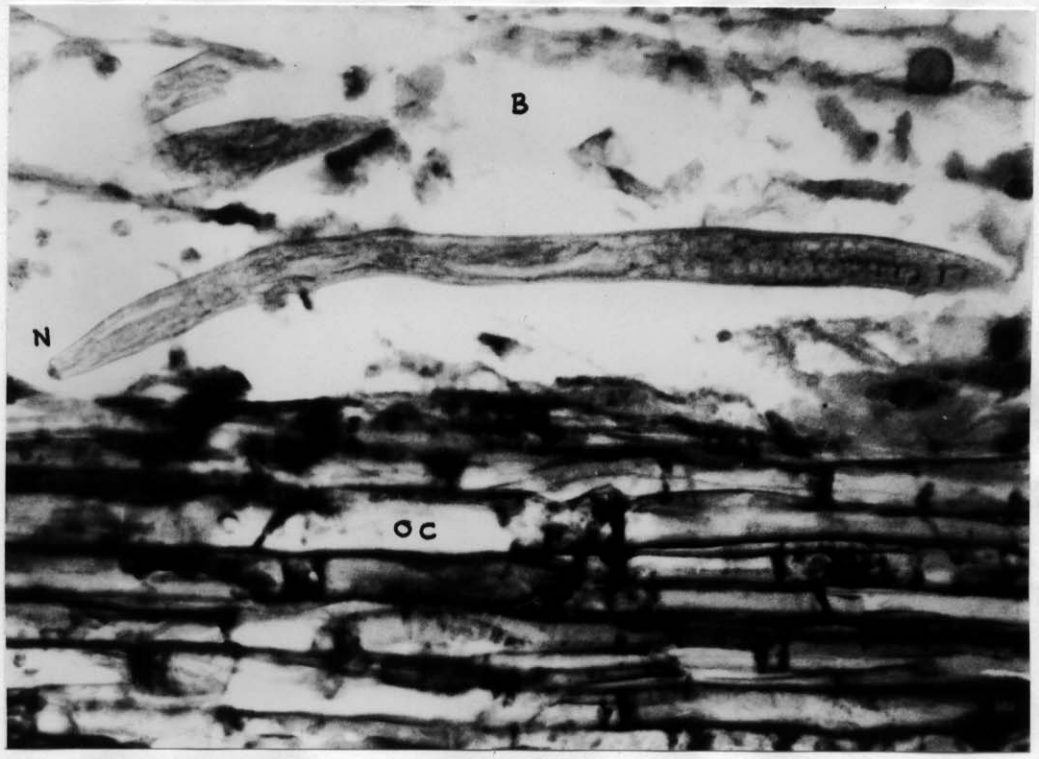


Plate 6

a. Transverse section of an arecanut root inoculated with Radopholus similis. Nematodes are located in the cortical burrows

B - Burrow

E - Epidermis

N - Nematode

OC - Outer Cortex

b. Transverse section of infected root showing the formation of burrows and extent of tissue damage

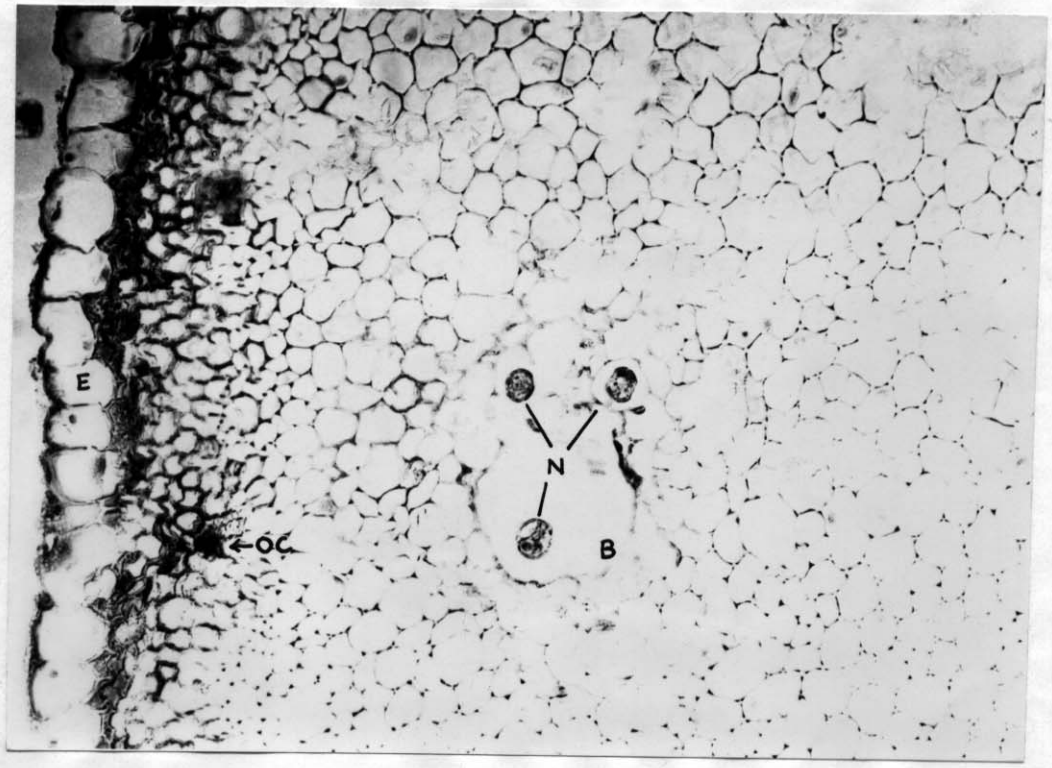
B - Burrow

DC - Damage Cortex

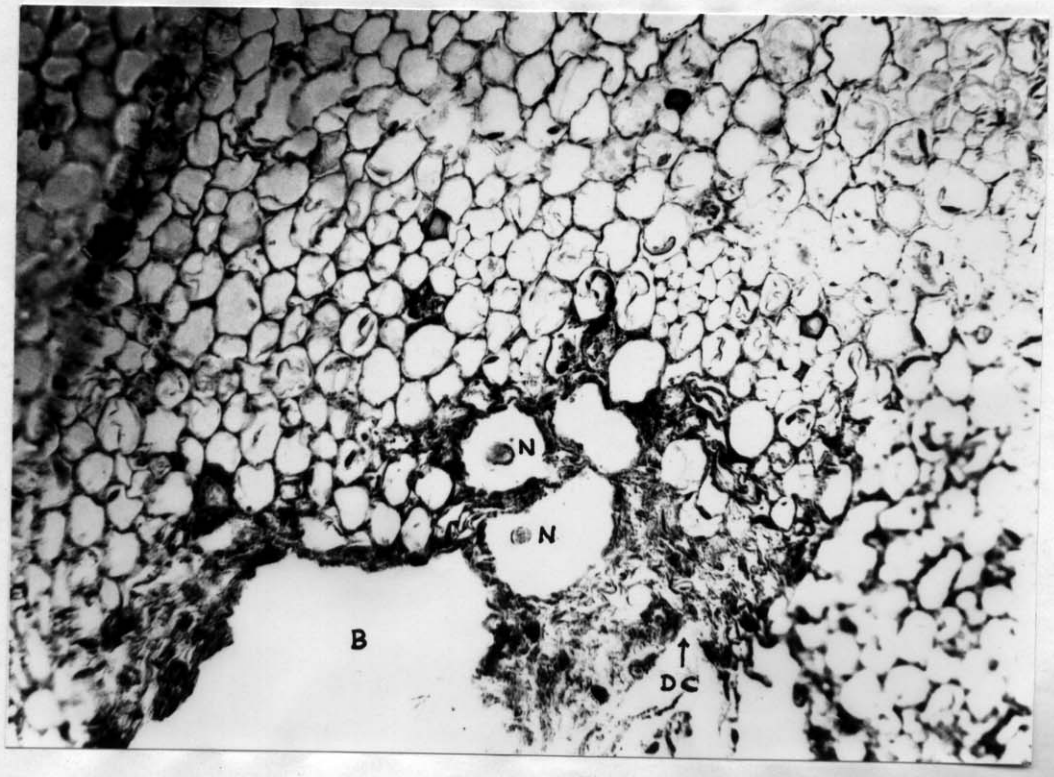
N - Nematode

Plate. 6

a



b



in each disc varied, but a maximum of 80,000 nematodes could be recovered from a single flask after two months of inoculation. Populations of R. similis in a disc ranged from 500 to 80,000. Large numbers of eggs could also be recovered from these discs. The populations reduced gradually after two months. Subculturing was done after 45 days by adding sterile water on to the infested carrot disc and transferring the resultant suspension with a sterile hypodermic needle and syringe on to another flask containing fresh carrot discs. At every subculturing, nematodes extracted from carrot discs were inoculated on to roots of arecanut seedlings raised in sterile soil in greenhouse. Seedlings inoculated with this population produced characteristic lesions, rotting and build up of very high populations after two months. This clearly indicated that the ability of nematodes to parasitise arecanut roots was not lost due to continuous subculturing.

Isolation and identification of fungal organism associated with lesions of Radopholus similis on arecanut roots.

Roots of areca palms showing characteristic R. similis lesions were collected from YLD-affected palms at CPCRI (RC), Palode. Newly formed lesions from the white portions of the main roots were chosen for isolation. Consistent association of a fungus was recorded on PDA

medium from cortex tissues surrounding the lesions. The fungus was identified in the laboratory at generic level as Cylindrocarpon sp. Purified culture was sent to the Commonwealth Mycological Institute, England and it was identified as Cylindrocarpon obtusisporum (Cooke and Harkness) Wollenw. No isolations could be made from the stelar region of the root that had recorded the fungus from epidermis and cortex.

Population fluctuation of Radopholus similis on arecanut

Observations made on the population fluctuation of the nematode during 1977-80 has revealed distinct increase of population during the months of September, October and November which reduced to negligible level or total absence in March, April and May. Reasonably good population (10/g of root) was found to occur from August to December. While looking into the populations recovered from individual palms it is seen that variability in populations occurred between palms for maximum and minimum populations within the same garden (Table 13 and Fig. 4).

Effect of various nematicides in the control of Radopholus similis on arecanut seedlings in pots under field conditions

Data on the efficacy of aldicarb, aldicarb sulfone, carbofuran and fensulfothion applied @ 1 g ai/plant, thrice a year for a period of three years against R. similis on

fig. 4. Population fluctuation of R. similis in arecanut roots from May, 1977 to April, 1980.

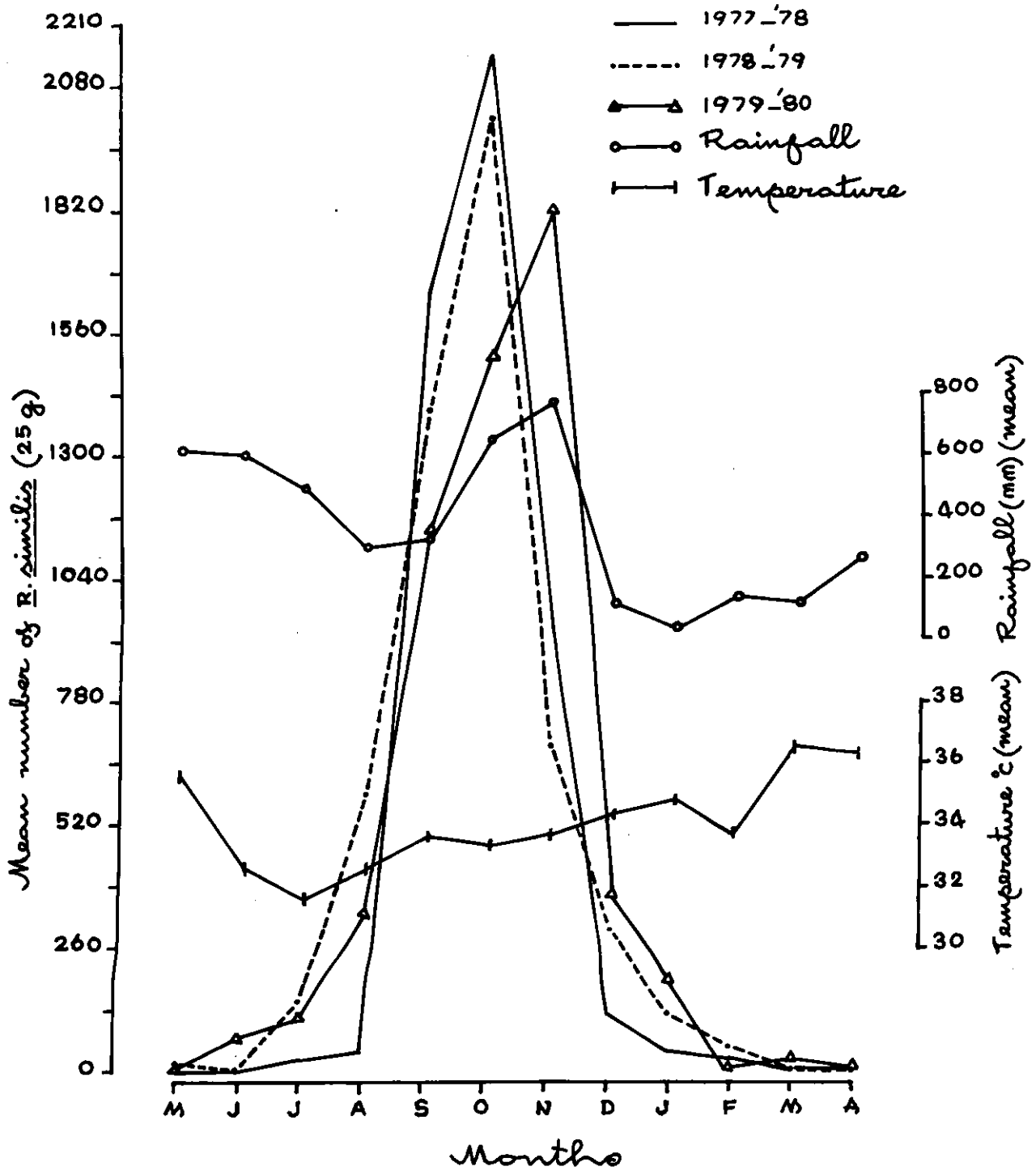


Table 13. Population fluctuation of *Radopholus similis* on arecanut (Mean of four palms)

Period (Month)	*R. similis* population in 25 g roots			Mean
	1977-78	1978-79	1979-80	
May	3.0	12.0	Nil	5.0
June	Nil	3.0	63.0	22.0
July	24.0	141.0	108.0	91.0
August	45.0	581.0	346.0	324.0
September	1636.0	1400.0	1150.0	1395.3
October	2147.0	2005.0	1515.0	1889.0
November	940.0	685.0	1820.0	1148.3
December	125.0	299.0	375.0	266.3
January	43.0	120.0	195.0	119.3
February	25.0	55.0	13.0	31.0
March	4.0	7.0	26.0	12.3
April	7.0	Nil	9.0	5.3

arecanut seedlings are furnished in Tables 14 and 15. The plant growth parameters like shoot length, shoot weight, number of leaves, girth at collar region, root length, root weight and root lesion indices were recorded with respect to the treatments adopted.

Shoot length

The maximum shoot length was obtained in fensulfothion treatment, followed by aldicarb sulfone, aldicarb and carbofuran (Plate 7a). The percentage increase in shoot length over control was 24.5, 33.9, 39.0 and 45.5 with respect to carbofuran, aldicarb, aldicarb sulfone and fensulfothion treatments compared to control plants. However, the differences in shoot length were not statistically significant (Table 14 and Fig. 5).

Shoot weight

It is seen from Table 14 and Fig.5 that the maximum shoot weight was recorded in plants treated with fensulfothion, followed by aldicarb sulfone, aldicarb and carbofuran. Increase in shoot weight due to fensulfothion treatment was 168 per cent, which differed significantly from other treatments. The plants treated with aldicarb sulfone, aldicarb and carbofuran were on par.

Number of leaves

Seedlings grown in infested soil (control) produced

Table 14. Effect of various nematicides on shoot growth of potted arecanut seedlings in Radopholus similis infested soil under field conditions (Means of five replications)

S. No.	Treatments	Shoot length (cm)	Per cent increase over control	Shoot weight (g)	Per cent increase over control	Number of leaves	Per cent increase over control	Girth at collar region (cm)	Per cent increase over control
1.	Control	185.0		1752^{c}		12.80 (3.5762)^{b}		24.4^{b}	
2.	Carbofuran	230.4	24.5	4018^{b}	129.3	13.96 (3.7369)^{ab}	9.4	30.8^{a}	26.2
3.	Aldicarb	247.8	33.9	4150^{b}	136.8	14.97 (3.8687)^{ab}	17.2	32.4^{a}	32.8
4.	Aldicarb sulfone	257.0	39.0	4200^{b}	139.7	14.76 (3.8424)^{ab}	15.6	33.6^{a}	37.7
5.	Fensulfothion	269.2	45.5	4700^{a}	168.3	15.99 (3.9984)^{a}	25.0	34.4^{a}	41.0
	S.Em	20.21		663.52		0.08		1.88	
	C.D.at 1%			-		-		7.59	
	C.D.at 5%	NS		1957		2.37			

Means with notations by the same letter do not differ at 5% level.
Figures in parentheses are square root values.

Analysis of variance

Source	df	Mean sum of squares: Shoot length	Shoot weight	Number of leaves	Girth at collar region
Inoculum	4	5371	6659810*	0.1248*	79.76**
Error	20	2042	2201308	0.03218	17.78

on an average 12.8 leaves compared to 15.99 leaves produced by seedlings maintained in fensulfothion treated soil. The treatment differences were significant at five per cent level only (Table 14 and Fig. 5).

Girth at collar region

The data on collar girth measurement show that in fensulfothion treatment the seedlings have recorded an average of 34.4 cm girth compared to 24.4 cm in the seedlings grown in nematode infested soil. Differences in treatments were highly significant and the per cent increase in girth ranged from 26.2 to 41.0 in the nematicides treated plants (Table 14 and Fig. 5).

Root length

Maximum root length (84.0 cm) was obtained in case of fensulfothion treatment followed by aldicarb (83.0 cm) and carbofuran (79.0 cm) and the least in aldicarb sulfone, (75.4 cm). It was minimum (63.0 cm) in the plants not treated with nematicide (control). The percentage increase ranged from 25.4 to 33.3 in the nematicides treated plants. Treatment differences, however, were not significant (Table 15 and Fig. 5).

Root weight

Application of nematicides had also significantly increased the root weight of arecanut seedlings. However,

fig.5. Effect of various nematicides on the growth of potted arecanut seedlings in R. similis infested soil under field conditions.

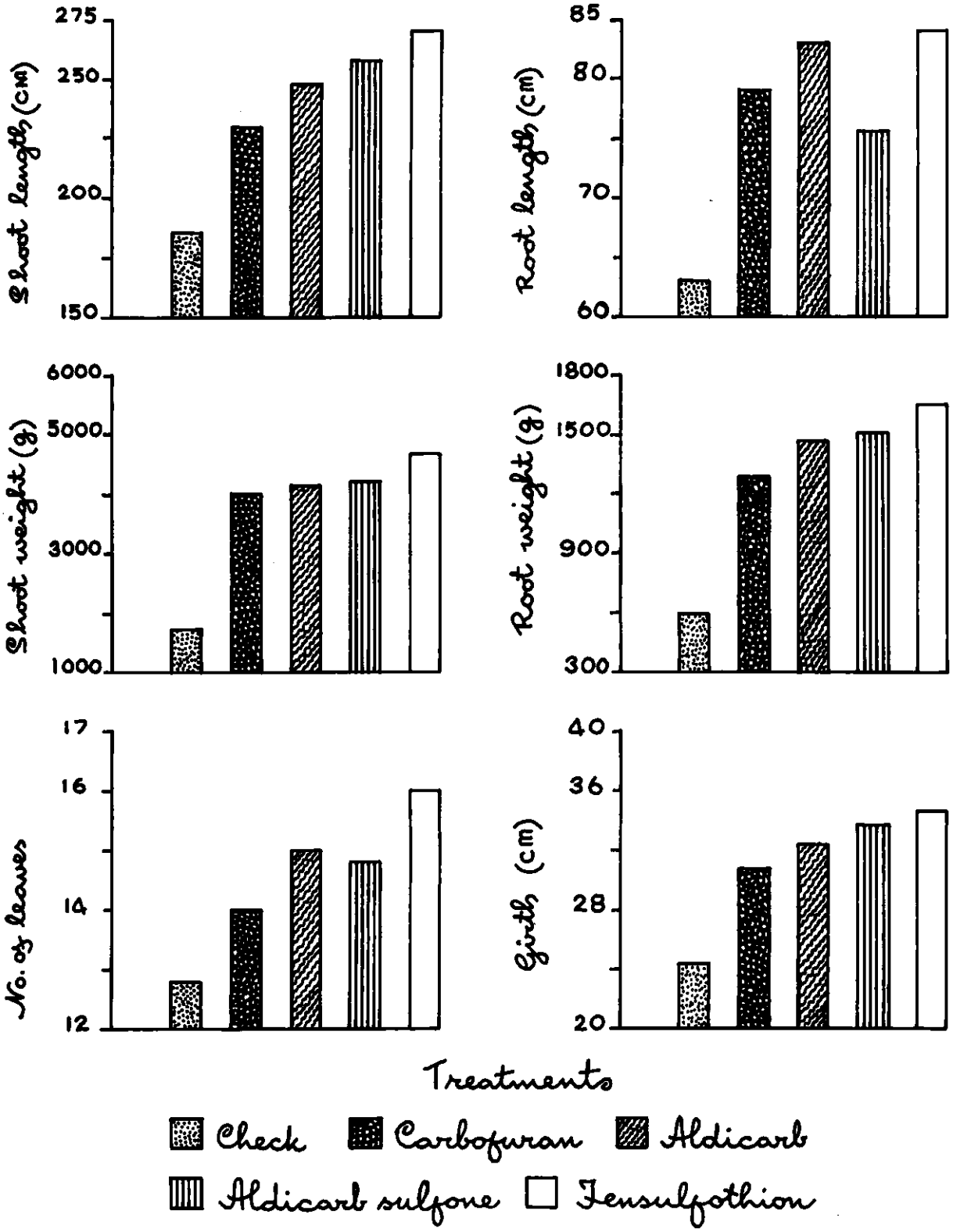


Table 15. Effect of various nematicides on population build up and host infestation of Radopholus similis and their influence on root growth of potted arecanut seedlings in infested soil under field conditions (Mean of five replications)

S. No.	Treatments	Root length (cm)	Per cent increase over control	Root weight (g)	Per cent increase over control	Root lesion index	Total nematode population (soil+root)
1.	Control	63.0		602.0[b]		4.93 (2.2207)[b]	93,488
2.	Carbofuran	79.0	25.4	1270.0[a]	110.9	1.36 (1.1657)[a]	4,018
3.	Aldicarb	83.0	31.8	1468.0[a]	143.8	1.00 (1.0000)[a]	0
4.	Aldicarb sulfone	75.4	19.7	1500.0[a]	149.2	1.17 (1.0828)[a]	1,412
5.	Fensulfothion	84.0	33.3	1644.0[a]	173.1	1.00 (1.0000)[a]	0
	S.Em	10.95		221.71		0.083	
	C.D. at 1%	NS		-		-	
	C.D. at 5%	NS		654		0.24	

Figures in parentheses are square root values
Means with notations by the same letter do not differ at 5% level

Analysis of variance

Source	df	Mean sum of squares: Root length	Root weight	Root lesion index
Inoculum	4	359	843266**	1.36**
Error	20	600	245774	0.0344

differences between nematicides were not significant. Fensulfothion treatment had recorded the maximum root weight (1644 g) in contrast to the minimum root weight (602 g) in control (Table 15 and Fig. 5).

Lesions, rotting and blackening of root tips could not be observed on the root system of any of the seedlings treated with fensulfothion and aldicarb. Plants treated with carbofuran and aldicarb sulfone were also relatively free from root lesions compared to plants grown in untreated soil (Plate 7b) which had recorded the highest root lesion index of 4.93 (Table 15).

Effect on the nematode population in soil and roots

The data on the total nematode population from both soil and roots recorded at the end of the experiment are summarised here in Table 15. Treatment with fensulfothion and aldicarb resulted in absolute control of *R. similis* population while in the case of aldicarb sulfone and carbofuran it was considerably reduced.

Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in YLD affected arecanut palms

The purpose of the study was to find out the effect of nematicides and neem oil cake in the amelioration of YLD symptoms. Three nematicides, viz., fensulfothion @ 50 g ai/palm, aldicarb @ 10 g ai/palm, DBCP @ 10 ml ai/palm and

Plate 7

a. Effect of various nematicides on the shoot growth of arecanut seedlings in Radopholus similis infested soil in comparison to untreated control

A. - Control
B - Carbofuran
C - Fensulfothion
D - Aldicarb sulfone
E - Aldicarb

b. Effect of various nematicides on the shoot and root growth of arecanut seedlings in comparison to the untreated control

A - Control
B - Carbofuran
C - Fensulfothion
D - Aldicarb sulfone
E - Aldicarb

Plate. 7

a

b

neem oil cake @ 1.5 kg/palm were applied three times a year against R. similis on arecanut palms affected by YLD. Initial nematode populations varied from 9 to 61 per g of root. Pretreatment populations were assessed every year during October because of the availability of maximum population during that period. Nematode population in October, 1976, did not show any significant difference, nor were there differences in yield after treatment (Tables 16 and 17). However, the population was reduced considerably after application of nematicides compared to control palms. In the second year (1977) the nematode population in palms treated with fensulfothion, aldicarb, DBCP and neem oil cake was significantly less than in control however, better yield response was noticed only in fensulfothion and neem oil cake treatments. During 1978 also significant reduction in the nematode population was observed and interestingly in the succeeding two years, 1979 and 1980, no R. similis could be recovered from the treated palms whereas the untreated palms (control) recorded 172 to 425 nematodes per 25 g of roots. Increase in yield was noticed in 1978 in fensulfothion, aldicarb and neem oil cake treatments compared to control and DBCP treatments. Though absolute control of R. similis was observed in all treatments during 1979, yield response was more only in the case of fensulfothion and aldicarb treatments. Highly

Table 16. Effect of different nematicides and neem oil cake in the control of Radopholus similis in YLD affected arecanut palms: (a) Nematode population in the roots during different years (Mean of five replications)

S. No.	Treatments	Dosage	Initial population	Nematode population in 25 g of roots				
				1976	1977	1978	1979	1980
1.	Aldicarb	@ 10 g ai/palm	1525	180	48	15	0	0
2.	DBCP	@ 10ml ai/palm	1350	152	4	0	0	0
3.	Fensulfothion	@ 50 g ai/palm	865	125	7	5	0	0
4.	Neem oil cake	@ 1.5 kg/palm	242	120	135	50	0	0
5.	Control		625	648	380	425	472	205

Table 17. Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in YLD affected arecanut palms: (b) yield of nuts during, different years (Mean of five replications)

S. No.	Treatment and dosage	Fruit yield (Number of nuts and nut weight)									
		1976-'77		1977-'78		1978-'79		1979-'80		1980-'81	
		No.of nuts	Nut weight (g)	No.of nuts	Nut weight (g)	No.of nuts	Nut weight (g)	No.of nuts	Nut weight (g)	No.of nuts	Nut weight (g)
1.	Aldicarb @ 10 g ai/palm	163.10 (12.77)	3466	126.52 (11.25)	2520	147.04 (12.13)	4624	172.40 (13.13)	4488	250.91 (15.84)	6205
2.	DBCP @ 10ml ai/palm	209.21 (14.46)	4542	81.42 (9.02)	1680	149.28 (12.22)	3078	185.34 (13.61)	3990	111.64 (10.57)	3034
3.	Fensulfothion @ 50 g ai/palm	331.26 (18.20)	7210	121.23 (11.01)	3360	241.93 (15.55)	6360	234.62 (15.32)	5838	347.90 (18.65)	7958
4.	Neem oil cake @ 1.5 kg/palm	224.36 (14.98)	4640	125.86 (11.22)	4490	200.34 (14.15)	4926	167.73 (12.95)	4230	140.09 (11.84)	3066
5.	Control	137.76 (11.74)	3376	68.71 (8.29)	1868	125.35 (11.20)	2964	88.85 (9.43)	2292	36.48 (6.04)	1066
	C.D. at 5%	NS		NS		NS		NS		5.78	3646.95

Figures in parentheses are square root transformed values

Analysis of variance — Mean sum of squares

Source	df	1976-76 No.of nuts	1976-76 Nut weight	1977-78 No.of nuts	1977-78 Nut weight	1978-79 No.of nuts	1978-79 Nut weight	1979-80 No.of nuts	1979-80 Nut weight	1980-81 No.of nuts	1980-81 Nut weight
Treatment	4	30.660	11988	9.7740	6712	15.5902	9972	23.0931	8058	118.5974**	3872**
Error	20	18.5692	6814	31.5521	10150	17.8451	7713	19.1471	6720	19.1674	7641

significant yield increase was noticed in 1980. Fensulfothion treatment had yielded more number of nuts (344.90) and total nut weight per palm (7958 g), closely followed by aldicarb. Treatments with DBCP and neem oil cake were at par with each other.

The results with respect to disease indices of arecanut palms in different treatments are given in Table 18 The analysis of the data showed that disease incidence did not differ significantly between the treatments. Considerable reduction of disease indices was noticed in the palms treated with fensulfothion and aldicarb compared to untreated (control) palms during 1980. Decrease in disease incidence and increase in yield were observed in all treatments compared to control in the fifth year (1980). While comparing the severity of disease symptoms before and after application of nematicides, it is interesting to note that a gradual decrease in disease incidence was seen in treated palms as compared to that in untreated (control) palms.

Effect of application of various nematicides on population build up of Radopholus similis and incidence of YLD symptoms on arecanut seedlings in field

The data on nematode population recorded in the roots at the commencement of experiment and thereafter once a

Table 18. Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in YLD affected arecanut palms: (c) Disease indices assessed during different years (Mean of five replications)

S. No.	Treatments	Dosage	Pre-treatment disease index	Disease indices during different years					Mean
				1976	1977	1978	1979	1980	
1.	Aldicarb	@ 10 g ai/palm	37.80	28.80	29.80	24.80	24.20	21.00	25.72
2.	DBCP	@ 10ml ai/palm	33.60	30.40	26.20	27.20	30.80	34.20	23.72
3.	Fensulfothion	@ 50 g ai/palm	28.00	22.60	20.80	21.60	24.80	21.20	22.20
4.	Neem oil cake	@ 1.5 kg/palm	35.40	31.80	30.40	23.80	23.40	27.00	27.28
5.	Control		25.20	33.60	35.20	36.20	37.20	43.00	37.04
	C.D. at 5%		NS	NS	NS	NS	NS	NS	

Analysis of variance		Mean sum of squares					
Source	df	1975	1976	1977	1978	1979	1980
Treatments	4	550.00	354.96	573.84	742.64	601.44	2038.24
Error	16	1285.20	1194.64	1983.36	1233.36	2229.84	3254.84

year during 1981-83 as well as expression of YLD symptoms on arecanut seedlings are summarised in Table 19. There was considerable reduction of nematode population in the nematicides treated palms but the differences between treatments were not significant. Population of R. similis could be recovered from all treatments. This is indicative of the fact that the dosage was not adequate enough to eliminate the nematode populations.

Observations recorded on growth parameters revealed that there was marked improvement with regard to shoot length, number of leaves and collar girth in the treated plants compared to untreated check and nematode inoculated plants.

The treatment of aldicarb @ 3 g ai/plant controlled R. similis effectively in 1982 but, however, population was noticed in 1983. In the case of phenamiphos, phorate and DBCP treatments a steady decrease of nematode population was observed. The plants in DBCP treatment exhibited severe phytotoxic symptoms. Plants inoculated with 10,000 nematodes through infested roots as well as from axenic carrot culture supported steady increase in nematode population from 1981 to 1983. The population fluctuations occurred on carbofuran and untreated control plants.

With regard to YLD symptoms maximum percentage of disease incidence occurred in untreated control plants

Table 19. Effect of application of various nematicides on population build up of *Radopholus similis* and incidence of YLD symptoms on arecanut seedlings in field (Mean of eight replications)

S. No.	Nematicide	Dosage	Population in 25 g roots				Per centage of disease incidence	
			Initial population 1980	1981	1982	1983	1982	1983
1.	Aldicarb	@ 3 g ai/plant	44	30	0	12	12.5	12.5
2.	Carbofuran	@ 3 g ai/plant	165	68	45	75	50.0	50.0
3.	Phenamiphos	@ 3 g ai/plant	215	85	48	13	62,5	50.0
4.	Phorate	@ 3 g ai/plant	318	110	55	21	25.0	25.0
5.	DBCP	@ 3ml ai/plant	195	116	80	31	25.0	25.0
6.	Inoculation with nematodes (Infested roots)	10,000 nematodes per seedling	28	168	345	525	62.5	62.5
7.	Inoculation with axenic culture of *R. similis* population	"	126	372	590	625	12.5	25.0
8.	Control		95	285	275	252	25.0	66.6

(66.6%), closely followed by seedlings inoculated with nematodes (62.5%). In general, the percentage incidence of disease was comparatively less in treated plants than untreated control or on inoculation. The percentage incidence of YLD symptoms was significantly less in aldicarb treatment compared to other treatments. It is also interesting to note that aldicarb was superior to all other nematicides tested, because it had controlled nematode population significantly with least percentage of disease incidence (12.5%).

Screening of Areca germplasm for resistance to Radopholus similis

Fortysix Areca germplasm collections available at CPCRI (RS), Vittal were screened for locating their resistance, if any, against R. similis in two experiments. Each cultivar was raised in earthen pots of 35 cm diameter. Plants in five pots were inoculated with 1,500 R. similis each and the remaining five kept without inoculation served as controls. The observations on plant growth parameters like shoot length, shoot weight, number of leaves, girth at collar region, root length, root weight and root lesion indices in fortysix cultivars are presented in Table 20.

None of the fortysix arecanut cultivars screened exhibited immune or a high degree of resistance to R. similis. The first experiment was conducted during

Table 20. Screening of Areca germplasm for resistance to Radopholus similis : Plant growth characters, root lesion index and nematode population (Mean of five replications)

S. No.	Cultivar	Accession number	Per cent reduction of growth characters over control						Root lesion index	Nemas/g of root
			Shoot length	Shoot weight	Collar girth	No.of leaves	Root length	Root weight		
	Experiment I									
A.	Exotic Types									
1.	Fiji	VTL-1	1.3	8.0	2.1	11.5	6.2	26.3	1.9	232
2.	China (Mangala)	VTL-3	11.0	28.2	15.7	2.0	3.9	32.4	2.5	782
3.	Sri Lanka-1	VTL-5	12.6	12.3	9.1	5.8	5.3	16.8	2.0	484
4.	Indonesia-6	VTL-11	8.5	13.6	1.9	+3.8	+4.9	2.6	1.4	776
5.	Saigon-1	VTL-12	1.9	3.4	+6.0	+1.9	19.9	4.1	1.4	146
6.	Saigon-2	VTL-13	2.5	5.0	5.8	11.8	20.4	42.7	1.6	133
7.	Saigon-3	VTL-14	0.8	2.4	+2.0	+3.9	24.2	7.9	2.2	324
8.	Singapore	VTL-17	3.1	11.2	6.0	2.0	12.4	16.8	1.0	19
9.	Solomon Islands-2	VTL-18[b]	1.0	3.0	4.3	4.1	8.2	1.6	1.0	56
10.	Solomon Islands-3	VTL-18[c]	9.3	25.1	2.0	+4.2	20.0	12.9	1.2	13

Contd...

Table 20 (Contd...)

S. No.	Cultivar	Accession number	Per cent reduction of growth characters over control						Root lesion index	Nemas/g of root
			Shoot length	Shoot weight	Collar girth	No.of leaves	Root length	Root weight		
11.	Mauritius (*Areca triandra*)	VTL-2	27.5	43.5	21.4	1.6	21.6	50.7	0.4	179
12.	Indonesia-1 (*A. triandra*)	VTL-6	26.6	58.5	0.0	14.0	48.5	37.1	3.0	97
13.	Indonesia-2 (*A. triandra*)	VTL-7	23.5	48.0	41.5	2.5	10.4	50.8	0.8	575
14.	Australia (*A. normanbyii*)	VTL-23	14.6	51.0	3.6	22.9	21.1	22.5	1.4	624
15.	Saigon (*A. calapparia*)	VTL-27	5.8	12.9	7.3	12.2	6.5	19.3	1.0	3
16.	Fiji (*A. langlosiana*)	VTL-33	5.8	28.7	41.5	8.6	11.4	23.8	1.0	177
17.	New Ireland (*A. macrocalyx*)	VTL-43	3.4	21.6	23.3	4.6	7.2	46.3	3.0	686
B.	Indigenous Types									
18.	Assam	-	3.5	4.2	24.1	0.0	41.7	17.7	3.0	228
19.	Chickmagalur	-	21.6	48.9	10.4	23.1	34.7	41.0	2.0	754
20.	Dapoli	-	14.4	31.0	1.6	9.3	24.6	19.5	1.8	634
21.	Hirehalli		27.2	42.0	28.6	19.6	5.5	22.7	0.4	53

Contd...

Table 20 (Contd...)

S. No.	Cultivar	Accession number	Per cent reduction of growth characters over control						Root lesion index	Nemas/g of root
			Shoot length	Shoot weight	Collar girth	No.of leaves	Root length	Root weight		
22.	Local (Vittal)	-	27.2	42.0	28.6	19.6	5.5	22.7	0.4	53
23.	Mahuva-A	-	2.1	2.3	13.2	0.0	7.4	6.8	2.0	42
24.	Mahuva-B	-	10.9	15.6	4.9	9.1	10.8	19.1	2.1	555
25.	Mettupalayam	-	9.3	7.8	13.0	19.6	25.4	60.7	0.9	253
26.	Mohitnagar	-	8.6	12.4	+6.0	7.1	17.0	16.0	1.1	155
27.	South Kanara	-	28.9	58.6	23.5	14.0	14.2	11.8	1.7	39
28.	Sreevardhan	-	50.4	78.9	51.8	8.1	40.0	71.1	1.9	267
29.	Thirthahalli	-	10.2	29.5	31.9	4.2	5.6	58.4	1.0	145
30.	Thirthahalli oblong	-	15.2	33.3	39.7	7.1	10.7	38.5	2.0	44
31.	Thirthahalli oval	-	10.4	28.7	8.7	15.8	20.5	9.5	1.6	8
Experiment II										
A. Exotic Types										
1.	Sri Lanka-2	VTL-15	6.7	41.6	9.1	33.3	11.7	27.9	3.0	143
2.	Sri Lanka-3	VTL-21	32.4	51.4	34.2	31.6	52.4	39.3	1.2	103

Contd...

Table 20 (Contd...)

S. No.	Cultivar	Accession number	Per cent reduction of growth characters over control						Root lesion index	Nemas/g of root
			Shoot length	Shoot weight	Collar girth	No.of leaves	Root length	Root weight		
3.	Sri Lanka-4	VTL-22	30.3	36.3	16.9	25.0	28.8	53.3	4.6	242
4.	Saigon-1	VTL-28[a]	26.7	20.8	14.5	7.7	3.2	46.9	3.0	136
5.	Saigon-2	VTL-28[b]	36.9	30.3	21.2	20.7	25.3	43.8	2.8	128
6.	Saigon-3	VTL-28[c]	5.1	23.9	10.3	7.1	29.7	6.3	4.0	88
7.	Fiji	VTL-26	21.3	25.1	5.4	21.4	40.7	39.2	3.8	612
8.	Andaman-1	VTL-29[a]	19.3	15.4	8.2	0.0	11.8	41.0	3.2	220
9.	Andaman-2	VTL-29[b]	13.8	3.0	22.1	25.9	38.9	32.7	3.8	189
10.	Andaman-3	VTL-29[c]	17.3	10.1	8.9	4.2	20.8	19.2	4.2	200
11.	Andaman-4	VTL-29[d]	13.8	12.8	12.4	3.6	16.1	19.0	3.2	408
12.	Andaman-5	VTL-29[e]	4.5	4.8	4.9	4.0	47.3	17.0	3.6	384
B.	Indigenous types									
13.	Indica	-	3.4	62.2	18.8	51.7	3.4	58.5	1.2	116
14.	Peechi	-	20.7	17.9	5.6	40.0	38.0	22.7	1.2	105
15.	Sweet areca	-	29.9	52.9	7.3	16.0	36.3	64.9	2.0	95

1977-1979 and 31 Areca germplasm of both exotic and indigenous types were screened. A perusal of the data in Table 20 indicates that all the 31 cultivars of arecanut were susceptible to R. similis, but differed in their degree of susceptibility. Root lesion indices varied from 0.4 in Hirehalli to 3.0 in Assam, Indonesia-1, and New Ireland in the scale of 0.0 to 3.0. Low record of root lesion indices and nematode population in the root occurred in cultivars Singapore, Solomon Islands-2, Solomon Islands-3, Hirehalli, Thirthahalli oval, local variety of Areca catechu and Saigon variety of A. calapparia. Minimum reduction of plant growth characters was also observed in these cultivars. Root lesion indices were high in cultivars Assam and Indonesia-1, but they supported only moderate level of nematode population in the roots. Maximum reduction in plant growth characters was noticed in Indonesia-1 compared to Assam. The cultivars Mangala (782) (Plate 8), Chikmagalur (754), New Ireland (686), Mahuva-B (555) and Indonesia-6 (776) recorded very high nematode populations and root lesion indices with high percentage reduction in plant growth characters which clearly established their highly susceptible nature to R. similis. The cultivars Indonesia-6 and Mahuva-B though recorded high nematode population and root lesion indices in the roots, plant growths were comparatively less damaged, thereby exhibiting a tolerant

Plate 8

Radopholus similis infested arecanut seedling var. Mangala showing lesions, rotting and blackening of root tips

Plate. 8

reaction to the burrowing nematode. Most of the other cultivars were intermediate for root lesion indices and number of nematodes per g of roots. Root systems of control plants exhibited no lesions and yielded no R. similis.

In the second experiment conducted during 1981-83, fifteen Areca germplasm collections were screened and the data are furnished in Table 20. All the fifteen collections were susceptible to R. similis but differed in their susceptibility. The root lesion index of individual plants was rated visually on 0 to 5 scale. The root lesion indices and nematode population were distinctly less in CVS. Sri Lamka-3 (VTL-21), Indica, Peechi and Sweet areca. However these cultivars showed considerable plant damage which indicate their high susceptible reaction to R. similis. The cultivars Fiji (VTL-26), Sri Lanka-4 (VTL-22) and Saigon-2 (VTL-28b) had higher root lesion indices and nematodes populations with conspicuous reduction in plant growth characters and therefore, they could be considered as highly susceptible to R. similis. It is interesting to note that the cultivars such as Andaman-1 (VTL-29a), Andaman-3 (VTL-29c) Andaman-4 (VTL-29 d) and Andaman-5 (VTL-29e) though recorded high root lesion indices and nematode population the reduction of plant growth parameters was minimal which prove that they possess a high degree of tolerance to the nematode

infestation. In one of the cultivars Saigon-3 (VTL-28c) in spite of high root lesion index, the nematode population in the roots and the resultant plant damage were found to be conspicuously less which can therefore be designated as less susceptible. The remaining cultivars were rated intermediate because they supported large population of nematodes with moderate plant damage. There was no correlation between root lesion index and the nematode population except in CV. Sri Lanka-3 (VTL-21). In general, the plant growth parameters other than the root weight did not reflect a trend in relation to nematode population and lesion development.

Discussion

DISCUSSION

The burrowing nematode, Radopholus similis is an important migratory endoparasitic nematode known to cause serious damage in several agricultural plantation crops in many parts of the world. The presence of this nematode in association with a coffee disease in South India was first reported by Mayne and Subramhanyam (1933). A quarter century later, an unconfirmed report of its prevalence on sugarcane was made by Srinivasan (1958). Subsequently, its occurrence in South India on banana, coconut and arecanut were reported by Nair et al. (1966), Weischer (1967) and Kumar et al, (1971). In spite of the reports of its occurrence on these important plantation crops, no systematic investigations were carried out on this nematode. Hence, the present studies on the distribution of this nematode in Kerala, Karnataka and Tamil Nadu with special reference to the yellow leaf disease (YLD) of arecanut, its pathogenicity and control were initiated.

The extensive survey involving 822 each of soil and root samples covering the three major arecanut growing states (1,19,500 ha) in South India revealed the widespread occurrence and distribution of R. similis.

Its prevalence even in very remote and inaccessible areas is indicative of the indigenous nature of the nematode and its association with other crops like coconut, arecanut, black pepper and banana which are also cultivated extensively in the three states surveyed further confirm that the existence of nematode may not be of recent origin.

The nematode was found to occur in 73.9 per cent of samples collected from YLD-affected sites as compared to 43.9 per cent from disease-free sites. However, only 32.1 per cent of the YLD-affected palms yielded R. similis against 31.9 per cent of healthy palms from disease-free tracts. Though considerable root damage by way of lesions and rotting could be recorded on arecanut in association with this nematode, no apparent symptoms could be attributed to nematode infestation on arecanut in both the healthy as well as the diseased palms. A maximum population of 440 per gram of root with an average of 84 was recorded in YLD-affected tracts, as against a maximum of 48 with an average of seven in the healthy areas. More number of nematodes per gram of root was recorded from the roots of palms showing initial stages of YLD than from palms in the middle and advanced stages of the disease. Similar trend was reported in the spreading

decline of citrus by DuCharme and Price (1966). From these results no direct correlation on the involvement of the burrowing nematode in the incidence of YLD could be drawn. However, it suggests the involvement of R. similis as a predisposing factor in YLD on attaining a minimum population build up.

Some of the difficulties encountered during the survey were difficulty in drawing soil and root samples from the root zone of arecanut especially in laterite soil under low moisture conditions, lack of availability of young succulent roots specially in older as well as in palms with advanced stages of disease, non-prevalence of detectable levels of population all through the year, delay in the transport of samples collected to the laboratory, physical labour involved in the processing of root samples, presence of high tannin content in arecanut roots and the need for low temperature (10 to 20°C) conditions for the extraction of nematode populations from the roots. Similar difficulties were also experienced by earlier workers on citrus (Suit and DuCharme, 1953), banana (Vilardebo, 1976) and coconut (Koshy et al., 1975).

Radopholus similis was found to occur more in sandy loam soil than in other soil groups, which obviously indicates its preference for loose well drained soil.

This is in conformity with the findings of Stover and Fielding (1958), O'Bannon and Tomerlin (1971) and Tomerlin and O'Bannon (1974) on the association of R. similis with the spreading decline of citrus in Florida.

Generally arecanut is inter/mixcropped with either one or more crops like banana, cardamom, black pepper, coconut etc. It is interesting to note that 56.29 and 51.0 per cent root samples of arecanut collected from plantations inter cropped with cardamom and banana, respectively, yielded R. similis compared to 25.37 per cent occurrence in root samples from plantations where mono-cropping of arecanut was practised. The crop combinations of arecanut, banana and cardamom were practised only in Karnataka, whereas inter cropping of arecanut and banana is practised more or less in all plantations. Another interesting factor was the influence of irrigation in areca gardens in Karnataka area and the resultant increased per cent occurrence of R. similis irrespective of the crop combinations involved. It was seen that high percentage occurrence of R. similis was recorded from the roots of banana (64.45%), followed by black pepper (25.0%), cardamom (18.18%) and coconut (16.77%) grown in arecanut gardens.

Banana and black pepper are favoured hosts of the burrowing nematode which has been reported as the causal

agent of the root rot disease of banana (Blake, 1961) and yellows of pepper (van der Vecht, 1950). The above findings have clearly brought out the fact that crop combination of susceptible crops especially of perennials is likely to cause severe damage to both the main crop and the subsidiary crops. Though growing together of banana and arecanut happens to be an age old practice, it is preferable to avoid this in the infested gardens. Distribution of planting materials of the main crop and the subsidiary crops to newer areas must have been the major reason for the widespread occurrence and distribution of this nematode. Hence this brings out the need for using nematode-free planting materials and application of suitable nematicides for successful cultivation of these crops together to meet the local requirements of planters as it is inevitable to have multispecies cropping systems to maximise the production per unit area.

Radopholus similis was recorded from 264 out of 822 (32.1%) root samples collected. Similarly the presence of the nematode was noticed in 256 soil samples. In certain locations soil samples collected around infested roots did not yield R. similis. At the same time, in certain other locations root samples did not yield R. similis whereas the nematode population was recovered

from the soil samples. This brings out the need for collection of both the soil and root samples in crucial detection surveys.

Though the survey was conducted mainly to study the distribution of R. similis, the analysis of soil samples recorded 27 genera of plant parasitic nematodes other than R. similis on arecanut. The dominant species were Helicotylenchus dihystera, Rotylenchulus reniformis, Caloosia longicaudata and Hemicriconemoides mangiferae. Though R. reniformis was recorded in 45.74 per cent of soil samples, no gravid females were observed on arecanut roots. Large populations of juveniles of Meloidogyne sp. were also recovered from the root zone of arecanut. However, no galling or adult females were noticed on arecanut roots. Probably these populations are surviving on weed hosts in the root zone of arecanut. Thus, the results of the present studies indicate that the burrowing nematode, R. similis is the only major nematode disease on arecanut. The genus Diptherophora and the species Aphelenchoides aligarhiensis, Aphelenchus (Anaphelenchus) isomerus, Caloosia longicaudata, Discocriconemella limitanea, Ecphyadophora teres, Epicharinema keralense, Helicotylenchus dihystera, Hemicriconemoides mangiferae, Hoplolaimus seinhorsti, Longidorus saginus, Macroposthonia ornata, Pratylenchus zeae,

Scutellonema unum, Tylenchorhynchus coffeae and Xiphinema inaequale are new records on arecanut.

The burrowing nematode, R. similis is notorious for its potential as a plant pathogen specially in the spreading decline of citrus in Florida, pepper yellows in Indonesia and in root rot of banana. The experiment carried out in the cement pots under field conditions had confirmed the potential of this nematode as a pathogen on arecanut. An initial inoculum of 100 nematodes per seedling was found to cause significant reduction in shoot length, shoot weight, girth at collar region, root length and root weight. Thus, the inoculum threshold/damaging level of inoculum was found to be 100 nematodes per seedling or one nematode in 800 g of laterite soil. Though the increase in initial inoculum levels had resulted in corresponding decrease in plant growth parameters, the differences were not significant above the inoculum level of 1000 nematodes per plant. The most conspicuous symptom of nematode parasitization was seen on the root system. The nematodes produced small, elongate lesions on the young succulent creamy white to light orange coloured portions of the main and the lateral roots. Subsequently, the adjoining lesions coalesced and caused extensive root rot. Blackening of root tips was an important symptom noticed in

arecanut unlike in coconut (Koshy et al., 1975). Drastic reduction of root mass and volume could be obtained with inoculum levels of 100 and above. Similar results have been reported on coconut by Koshy and Sosamma (1983). However, no such pathogenicity experiments appear to have been carried out on citrus or banana. The potential of the burrowing nematode as a pathogen on black pepper (Venkitesan, 1976), ginger (Sundararaju et al., 1979) and turmeric (Sosamma et al., 1979) has been established.

The maximum rate of multiplication (3040 times) was obtained in the pots inoculated with 10 nematodes per plant over a period of 36 months and it was minimum (185 times) in pots which had received an initial inoculum of 10,000 nematodes per plant. The reduction in the rate of multiplication with increasing initial densities is a well recognised phenomenon. Similar reports have been made in case of R. similis on coconut (Koshy and Sosamma, 1983). This phenomenon can occur only when the reproductive potential of the pathogen is dependent on and related to the availability of nutrition and substratum.

Though the present results established the pathogenicity of the burrowing nematode on arecanut, its exact role in the causation of YLD could not be fully established. The inoculated plants exhibited considerable

yellowing of leaves, which was, however, different from the characteristic yellowing of the YLD of arecanut. Except for yellowing and stunted growth no apparent or above ground symptoms could be obtained. The root damage on inoculation and the increase in root volume obtained on protection with nematicides in the control experiments gives a vivid picture of the damage this nematode could cause to arecanut. Arecanut palms parasitized by R. similis with impaired root system is bound to succumb readily to drought, YLD and other diseases and pests as compared to the uninfested palms.

Although no extensive studies were made on the infection process and histopathological changes, the results obtained here indicate that R. similis can infect areca palms by destroying the parenchymatous cells in the cortex. The inter and intracellular orientation of the nematodes and lysis of cells and formation of longitudinal burrows are observations similar to those obtained by DuCharme (1959) in citrus, Blake (1966) in banana and Govindankutty and Vellaichamy (1976) in coconut palm roots.

Conduct of pathogenicity experiments and screening varieties of plants with massive root systems requires large populations of nematode. O'Bannon and Taylor (1968) cultured the citrus race of R. similis on carrot discs.

Later, Koshy and Sosamma (1980) cultured the banana race of R. similis infesting coconut on carrot discs by modifying the method of O'Bannon and Taylor (1968) slightly to meet the local conditions. An attempt made to culture the banana race population of R. similis from arecanut on carrot discs was quite successful. The population was also found to retain its infectivity even after continuous culturing on carrot discs. The axenic culturing has considerably helped to overcome the difficulties encountered on the availability of adequate populations of nematodes throughout the year for various studies.

Several fungi have been reported in association with R. similis lesions on citrus (Feder and Ford, 1964), banana (Booth and Stover, 1974; Pinochet and Stover, 1980) and coconut (Sosamma and Koshy, 1978, 1983). Unlike in other nematode-fungal complexes, the nematode fungal complex on banana (Pinochet and Stover, 1980) and coconut (Koshy and Sosamma, 1983). with Cylindrocarpon spp. was found to suppress the nematode populations. The fungus C. obtusisporum isolated from the cortical tissues surrounding the nematode lesions on arecanut roots have already been recorded as pathogenic on strawberry by Booth (1966).

Studies carried out on fluctuations of R. similis population in arecanut palms grown in laterite soil

clearly revealed the occurrence of maximum population in the months of October and November. No population could be recorded during June, 1977, April and May, 1979 and generally the minimum populations prevailed from March to June and high populations from August to December. Variations from the general pattern of occu rrence of peak and low populations existed between palms and samples. Similar observations were reported by DuCharme and Suit (1967) on citrus and on coconut (Koshy and Sosamma, 1978). Vilardebo (1976) recorded fluctua-tions of R. similis populations in banana in Cameroon. He had also noticed the maximum population in roots of plants that had flowered and the minimum population in March towards the end of the dry season.

At Palode, Trivandrum district, Kerala where the population fluctuation studied, there are two rainy seasons viz. South West monsoon during June to August with an average monthly rainfall of 464 mm and 18.8 rainy days and North east monsoon during October to December with an average monthly rainfall of 514 mm and 12.2 rainy days (Appendix - II). The average ambient temperature during these years varied from 25.4° to 36.3°C during March to May and 24°C to 33.0°C during September to November (Appendix III). This shows that dry spell which prevails in this region during March to May might have influenced

the population build up and resulted in low population during this period. However, the rainfall in the subsequent months, not only bring down the soil temperature and provide adequate soil moisture, but also induce maximum number of fresh roots which are parasitized by the residual populations available in the soil, ultimately resulting in population increase during August to December.

It was also seen that the nematode population increased gradually soon after the heavy monsoon coupled with low temperature, availability of continuous soil moisture and young succulent, non-suberised feeder roots, reaching its peak in October/November, when all these conditions prevailed even though for a short period of time. The other reason for the rapid dip in the population was the root decomposition and the resultant migration of nematodes from the root. This situation is almost identical to what had been reported on citrus by DuCharme (1969) and on coconut by Koshy and Sosamma (1978).

From these observations, it is clear that the surveys for detection of R. similis may be carried out during September to December and application of nematicides for control of R. similis may also be done in August to September.

The experiments discussed in the preceding paragraphs have clearly indicated that the burrowing nematode is an important root pathogen of arecanut, affecting the plant growth adversely. Soil application of nematicides to control R. similis had been tried by various workers mainly on citrus and banana (O'Bannon and Tomerlin, 1977; O'Bannon and Tarjan, 1979; Beugnon and Vilardebo, 1973; Hasing-Lama et al., 1976; Broadley, 1979a and Figueroa, 1980). An attempt was, therefore, made to evaluate the efficacy of nematicides and neem oil cake as an organic amendment in the control of R. similis population on arecanut seedlings as well as on bearing areca palms.

The pot culture experiment carried out during the present investigation on the control of R. similis indicated that all nematicides (Fensulfothion, aldicarb, aldicarb sulfone and carbofuran) were effective in reducing the nematode population significantly and increasing the growth of arecanut seedlings in comparison to the control plants. No evidence of phytotoxicity was observed with any of the nematicides used. Fensulfothion and aldicarb @ 1 g ai/seedling applied thrice a year for three consecutive years gave absolute control of R. similis both in soil and roots compared to aldicarb sulfone and carbofuran. Similar results were reported by Koshy and Nair (1979) on

coconut seedlings. They reported maximum reduction in population of R. similis in coconut nursery with fensulfothion @ 50 kg ai/ha. The present result is also in agreement with that of Broadley (1979a), who reported that fensulfothion @ 2 g ai/stool, applied to banana three times a year continuously for four years, gave maximum control of R. similis compared to phenamiphos, ethoprophos and oxamyl. Venkitesan (1976) also observed that fensulfothion or aldicarb sulfone at 4 to 8 kg ai/ha completely eliminated R. similis population on black pepper seedlings.

The results of the experiment on YLD-affected areca palms showed that during the fourth and fifth years, no population of R. similis could be recovered from the nematicides and neem oil cake treated palms. Though absolute control of R. similis was observed in all treatments in the fourth year decrease in disease intensity and increase in yield were observed in all treatments only during the fifth year, compared to untreated control. However, the yield response was high only in the case of fensulfothion and aldicarb treatments. It is inferred that fensulfothion and aldicarb were effective both in controlling the nematode and in increasing the yield of arecanut. The effectiveness of fensulfothion and aldicarb

for control of R. similis and increasing the yield had been reported on banana by Nair (1979). Figueroa and Mora (1977) studied the effect of different dosages of aldicarb and DBCP on banana for the control of R. similis. They found that aldicarb @ 9 kg ai/ha (60 g aldicarb 10 G/plant), applied at three months intervals, gave the best results on yield and control of nematodes.

From the studies it was seen that all nematicides viz. fensulfothion, aldicarb, DBCP and neem oil cake applied to arecanut palms thrice a year continously for four years gave absolute control of R. similis.

The yield increase might have been due to the increased root growth, resulting in better uptake of plant nutrients. The disease indices of treated palms were also low which is very encouraging even though the role of this nematode in the etiology of yellow leaf disease could not be fully established. Root rot is one of the major symptoms of YLD and the present result therefore, suggests the role of this nematode as a pre disposing factor in the incidence of YLD of arecanut.

The results of the experiment on new young transplants indicated that treatment with aldicarb @ 3 g ai/ seedling offered better control of R. similis than other nematicides. Similar results with aldicarb were obtained

by various workers on banana (Hasing-Lama et al., 1976; Recavarren-Herrera et al., 1976; Figueroa and Mora, 1977 and Figueroa, 1980). A steady decrease in nematode population was also observed in the case of phenamiphos, phorate and DBCP treatments, but the plants treated with DBCP died due to phytotoxicity. Koshy et al. (1983) recorded complete control of R. similis with phenamiphos and Phorate @ 25 kg ai/ha when applied thrice at intervals of three months in coconut nursery.

It is also seen that seedlings belonging to all treatments had expressed YLD symptoms. However, maximum percentage incidence of disease occurred in untreated control plants (66.6%), against 12.5 per cent in plants treated with aldicarb. Percentage incidence of YLD symptoms and R. similis population were less in aldicarb treatment compared to other treatments. However, total elimination of R. similis was not observed with any of the treatment. This suggests that the dosage tried was not adequate enough to eliminate the burrowing nematode populations.

Results of the screening of germplasm assemblage indicate that none of the arecanut cultivars exhibited immune or a high degree of resistance to R. similis. All of them were found susceptible in varying degrees.

They could be grouped into three categories such as less susceptible, highly susceptible and tolerant based on their reaction to R. similis in relation to reduction of plant growth parameters, rate of multiplication of nematode and root lesion indices.

In the first series, the cultivars Mangala (VTL-3), Chikmagalur, Indonesia-1 (VTL-6) and New Ireland (VTL-43) and in the second series CVS, Fiji (VTL-26), Sri Lanka-4 (VTL-22) and Saigon-2 (VTL-28b) recorded very high nematode population build up and root lesion indices with maximum percentage reduction of plant growth parameters over the control plants, which clearly established their highly susceptible nature to R. similis. The accession Mangala (VTL-3), an introduction from Peking (China), was found to have a number of desirable characters such as earliness in flowering and bearing, higher number of female flowers per inflorescence, higher yields, quicker stabilisation of yield and its semi-tall nature in comparison with the local (South Kanara) cultivar (Bavappa, 1977). This semi-tall cultivar has been released under the varietal name 'Mangala' and has already achieved wide acceptance among the farmers all over the country. However, the present studies have clearly shown its highly susceptible nature to R. similis.

The cultivars Indonesia-6 (VTL-11), Mahuva-B, Andaman-1 (VTL-29a), Andaman-3 (VTL-29c), Andaman-4 (VTL-29d) and Andaman-5 (VTL-29e) had supported high nematode populations and root lesion indices with comparatively less plant damage, thereby exhibiting a tolerant reaction to the burrowing nematode.

The cultivars Singapore (VTL-17), Solomon Islands-2, (VTL-18b), Solomon Islands-3 (VTL-18c), Hirehalli, Thirthahalli oval, Local and Saigon-3 (VTL-28c) of Areca catechu and Saigon (VTL-27) variety of A. calapparia recorded lower root lesion indices and fewer number of nematodes per gram of root with minimum percentage reduction of plant growth parameters, thereby showing least susceptible reaction to R. similis. The exotic cultivars Indonesia-6 (VTL-11) and Singapore (VTL-17) are known to yield 50 per cent more nuts over the local South Kanara cultivar (Anonymous, 1974). It is also encouraging to note that the exotic collection of Indonesia-6 recorded a tolerant reaction and the cultivar Singapore recorded the least susceptibility to R. similis. These cultivars could profitably be recommended for R. similis infested areas.

These studies suggest that finding of high degree of resistance to R. similis in arecanut is a difficult task especially in established cultivars and varieties

as in the other crops. In citrus, more than 1400 clones were evaluated and only 15 were found sufficiently promising for further testing. Three of them namely Milam, Ridge Pine apple and Carrizo orange were found to have high degree of resistance to R. similis (Feder et al., 1958; Ford and Feder, 1964; Hutchison et al., 1972; O'Bannon and Ford, 1976). Similar studies carried out on 70 cultivars of banana by Wehunt et al. (1978), 125 varieties of banana by Charles et al. (1983), 18 cultivars of black pepper by Venkitesan (1976) and 27 cultivars and hybrids of coconut by Sosamma et al. (1980) did not reveal any of the cultivars/varieties/hybrids to be immune or highly resistant to R. similis. Availability of tolerance and resistance to R. similis in some of the accepted cultivars will facilitate evolution of a management schedule for this nematode. This needs to be done with adequate caution as it is rather difficult to extrapolate greenhouse data to field conditions. In field, other factors such as drought, low fertility, status of soil, role of other micro-organisms including nematodes may result in fewer nematodes causing measurable injury to the plant. The ability of R. similis to reduce plant growth in the greenhouse in the absence of other parasitic organisms adds credibility to the concept that this species is a

major contributing factor to plant damage in fields where large populations occur.

In conclusion, it may be stated that the present investigations have clearly indicated that (i) R. similis is widespread on arecanut in South India as well as on coconut, banana, cardamom and black pepper intercropped in the areas surveyed (ii) it is a potential pathogen of arecanut (iii) its population fluctuated from negligible number during dry period of the year (March to May) to high population after monsoon (August to December) (iv) application of nematicides, particularly fensulfothion and aldicarb have effectively reduced the nematode population and YLD disease incidence in the palms and (v) none of the fortysix cultivars/accessions screened were immune or highly resistant to R. similis.

Summary

SUMMARY

Studies were carried out on the role of the burrowing nematode, *Radopholus similis* on arecanut and the salient findings are enumerated below:

Survey carried out in the States of Kerala, Karnataka and Tamil Nadu revealed the widespread distribution of *R. similis* on arecanut. Population of the burrowing nematode was recorded in 73.9 per cent of root samples collected from yellow leaf disease affected sites compared to 43.9 per cent in disease-free sites. Maximum population of 440 with an average of 84 nematodes per gram of root was recorded in yellow leaf disease affected palms compared to a maximum of 48 with an average of seven nematodes in the healthy palms in disease-free areas during the survey.

The examination of soil samples collected during the survey revealed the presence of 28 genera of plant parasitic nematodes in association with arecanut. *R. similis* was the predominant species recorded in 32 per cent of the samples screened. *Helicotylenchus dihystera*, *Rotylenchulus reniformis*, *Caloosia longicaudata* and *Hemicriconemoides mangiferae* were also noticed in fairly good number of samples. *R. similis* was the only endo-parasite observed in the roots although populations of root-knot and reniform nematodes were recorded in the soil.

Radopholus similis was recorded in the root samples of banana, black pepper, cardamom and coconut intercropped with arecanut. High percentage of root samples of both the crops yielded the nematode population in the plantation where banana was intercropped with arecanut. This increased incidence of R. similis on both arecanut and banana stresses the need for avoiding crop combinations of arecanut and banana or other susceptible crops in R. similis infested areas.

Significant reduction of plant growth parameters observed at the end of the pathogenicity experiment, has clearly established this nematode as a potential pathogen of arecanut. An initial inoculum level of 100 nematodes per seedling or one nematode in 800 g of laterite soil was found to be the threshold level of R. similis on arecanut to cause visible damage. Though no direct correlation could be drawn from the results of survey, pathogenicity and control studies on the role of R. similis to the incidence of yellow leaf disease, the enormity of root damage, this nematode could cause to arecanut has been well established.

Histopathological examination of R. similis infested arecanut roots revealed the presence of nematode and their eggs in cavities developed in the cortical

tissues and no nematode could be observed in the stelar tissues.

Arecanut populations of R. similis was successfully multiplied on carrot discs. Continuous culturing of the nematode under axenic condition did not affect its infectivity and multiplication.

The fungus Cylindrocarpon obtusisporum was isolated from the cortical regions of the arecanut roots from the lesions caused by R. similis.

The population density of the burrowing nematode in arecanut was found to fluctuate. The populations varied between samples, type of roots, palms, groves, soil types, months and years with well recognizable annual periods for high and low populations. Highest populations were recorded during September to December and the lowest or nil during March to June.

The experiment conducted in pots under field conditions proved the efficacy of fensulfothion and aldicarb, applied @ 1 g ai/plant thrice a year, offering complete control of R. similis on arecanut seedlings planted in the infested soil.

Complete control of R. similis was obtained on areca palms affected by yellow leaf disease after four

years of continuous application of fensulfothion @ 50 g ai/palm, aldicarb @ 10 g ai/palm, DBCP @ 10 ml ai/palm and neem oil cake @ 1.5 kg/palm. Significant increase in yield and decrease in disease indices of yellow leaf disease-affected palms were recorded only in fifth year.

Three applications of phorate, phenamiphos, aldicarb and carbofuran @ 3 g ai and DBCP @ 3 ml ai/seedling per year were found inadequate for effecting absolute control of R. similis on fresh transplants in the field over a period of three years. Among the nematicides, aldicarb offered the best control of nematode populations. None of the nematicides tested was effective in controlling the incidence of yellow leaf disease. Maximum incidence of yellow leaf disease was noticed in the untreated control plants (66.6%), as against the minimum of 12.5 per cent in aldicarb treated plants.

Fortysix Areca germplasm collections screened for their reactions to the burrowing nematode were found susceptible in varying degrees. The cultivars Mangala (VTL-3), Chikmagalur and Fiji (VTL-26) were highly susceptible, whereas the cultivars Singapore (VTL-17),

Solomon Islands-2 (VTL-18c) and Saigon (VTL-27) were less susceptible to R. similis. Cultivars Indonesia-6 (VTL-11), Mahuva-B and Andaman-5 (VTL-29e) were found to be tolerant to nematode infection.

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Appendices

APPENDIX-I

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE

REGIONAL STATION:

KAYANGULAM, KRISHNAPURAM P. O., KERALA STATE

NEMATODE SURVEY REPORTING FORM

Reporter/Co-operator _____

Sample Number _____ Date of Collection _____

Locality/Village _____ Tehsil/Taluk _____

District _____ State _____

Precise Location _____

_____

_____

Grower's Name and Address _____

_____

Host _____ Variety _____ Crop Stage\Age _____

Condition of Crop Healthy-Diseased _____

Previous Crop/Inter Crop _____ Soil Type _____

Rainfed _____ Irrigated _____ Watertable _____

SYMPTOMS: Above Ground: Stunting ☐ Yellowing ☐ Flaccidity ☐

Necrosis ☐ Wilting ☐ Dieback ☐ Slow Decline ☐

Sudden Collapse ☐ Malformation ☐ (Part _____)

Root System : Galls ☐ Cysts ☐ Decay ☐ Lesions ☐

Curling ☐ Stubby Root ☐ Other Malformation _____

Severity/Disease Index _____

Other : _____

Distribution of affected plants : General ☐ Localised Areas ☐ Scattered Plants ☐

Percent of planting affected _____

Acreage (Size of Field) $< \frac{1}{2}$ ac ☐ $\frac{1}{2}$ ac ☐ 1 ac ☐ 5 ac ☐ 10 ac ☐ $>$ 10 ac ☐

Stand of the Crop : (Culture) Very Good ☐ Good ☐ Fair ☐ Poor ☐

Comments : _____

_____

Remarks : (Flood, Soil treatment' Crop History : Replanting, Underplanting, Mixed Cropping etc.)

_____

_____

Tick thus ☑ if positive.

APPENDIX-II

Rainfall date at Central Plantation Crops Research Institute, Research Centre, Palode for the period from 1977 to 1979

Month	1977		1978		1979	
	Total rain (mm)	No. of rainy days	Total rain (mm)	No. of rainy days	Total rain (mm)	No. of rainy days
January	40.0	2	47.5	1	14.5	1
February	47.0	4	86.0	3	303.0	9
March	229.0	11	52.0	3	61.0	5
April	364.5	17	227.5	7	206.7	10
May	828.5	23	752.5	22	290.0	11
June	633.9	24	373.0	19	790.4	20
July	366.4	19	626.0	22	488.1	21
August	220.5	9	383.5	22	290.2	13
September	335.5	15	78.4	9	569.5	24
October	1081.4	20	387.2	9	471.5	18
November	574.8	17	1143.9	15	613.0	20
December	24.0	3	55.0	2	279.0	6
Total	4745.5	164	4212.5	134	4376.9	158

Source: Annual Report for 1977, 1978 and 1979, Central Plantation Crops Research Institute, Kasaragod, India.

APPENDIX-III.

Temperature (C°) at Central Plantation Crops Research Institute, Research Centre, Palode for the periods from 1977 to 1979

Months	1977		1978		1979	
	Max	Min	Max	Min	Max	Min
January	34.5	21.5	34.5	21.0	35.5	25.0
February	35.5	23.5	35.5	23.0	30.0	24.0
March	36.0	25.0	36.5	24.5	37.0	25.0
April	35.5	-	36.0	26.5	37.5	26.0
May	35.0	-	37.0	25.0	35.0	26.0
June	32.5	-	32.0	24.5	33.5	25.0
July	31.5	-	31.5	24.0	31.5	24.5
August	32.5	-	31.0	24.5	34.0	24.0
September	33.5	24.0	33.0	24.0	34.0	24.0
October	33.0	23.5	33.0	24.5	34.0	24.5
November	33.0	24.0	34.0	23.0	34.0	24.5
December	34.5	23.0	34.0	24.0	34.0	23.5
Total	407.0	164.5	408.0	288.5	410.0	296.0

\- : Not available

Source : Annual Report for 1977, 1978 and 1979, Central Plantation Crops Research Institute, Kasaragod, India.