NEMATOLOGICAL INVESTIGATIONS

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Increasing evidences on soil borne nature of coconut root (wilt) disease led to initiation of nematological investigations at Central Coconut Research Station, Kayangulam (Regional Station of the Central Plantation Crops Research Institute, since 1970) in 1964.

Weischer intensified the early attempts to probe into the role of plant parasitic nematodes in the incidence of this disease. He examined a total of 60 soil samples covering six soil types in the diseased tract and four samples from three soil types from healthy tract during his five weeks' stay at Kayangulam as an FAO Consultant. He reported the occurrence of plant parasitic nematodes belonging to the following genera in the rhizosphere of coconut; Xiphinema, Longidorus, Tylenchorhynchus, Meloidogyne, Hoplolaimus, Helicotylenchus, Dolichodorus, Pratylenchus, Radopholus, Rotylenchulus, Criconema, Hemicycliophora, Hemicriconemoides, Criconemoides and Paratylenchus (Weischer, 1967). Five species recorded in this study were described by Khan, Seshadri, Weischer and Mathen (1971). They are Dolichodorus pulvinus, Macroposthonia oachirai, Discocriconemella recensi, Longidorus saginus and Paralongidorus flexus. Later, Khan, Chawla and Saha (1975) described one more species Macroposthonia cufeum from the same collection. Detailed and systematic studies on phytonematodes of coconut were taken up at CPCRI, Kayangulam since 1972 to survey plant parasitic nematodes associated with coconut and to study the host range, population dynamics and pathogenicity and to devise methods of control of the most potential pathogen among them.

SURVEY

A total of 877 samples of each soil and root were collected from disease-prevalent/disease-free tracts and border areas between the two, covering different soil types during survey trips. Each sample of 250g soil was drawn one metre away from the bole of palm at a depth of 10-50 cm. Root samples

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contained 50-100g cream to orange coloured tender main roots. Thirty seven species of known and suspected plant parasitic nematodes were recorded. The new records other than those reported by earlier workers (Weischer, 1967; Mathen, 1969; Mathen, Kurian and Lal, 1970; Khan et al., 1971 and Khan et al., 1975) are Boleodorus sp., Paurodontus sp., Caloosia sp., Tylenchus sp., Tylenchorhynchus coffeae, Psilenchus sp., Ditylenchus sp., Atylenchus sp., Neotylenchus sp., Rotylenchus sp., Scutellonema sp., Helicotylenchus abunaamai, Hoplolaimus seinhorsti, Hirschmanniella oryzae, Xiphinema elongatum, Trichodorus sp., Paratrichodorus acaudatus, Tylencholaimellus sp., Diphtherophora sp., Epicharinema keralense, Ecphyadophora sp., Ecphyadophoroides sp., Aphelenchus isomerus, and Aphelenchoides sp. (Koshy, Sosamma and Sundararaju, 1979 and Raski, Maggenti, Koshy and Sosamma, 1980). Except for Radopholus similis, Pratylenchus zeae, Longidorus saginus and Dolichodorus pulvinus, others may be feeding only on the roots of large number of weed plants and intercrops raised in coconut gardens, because, in one of the experiments in which the basins of coconut palms were kept free of weeds throughout the year, very few or none of these vagrants could be recovered from soil samples.

The burrowing nematode, Radopholus similis has been recorded from 213 out of 877 root samples collected from 10 districts in Kerala, six districts in Tamil Nadu and one district in Karnataka and two samples each from Andamans, Andhra Pradesh and one sample from Lakshadweep. Of 213 samples yielding R. similis, 115 yielded at least one nematode per gram of root and 57 of such samples yielded ten and above. Ninety three of them were in sandy loam soil. The percentage occurrence of R. similis in sandy loam, laterite, alluvial, clayey and red loam soils was respectively 9.8, 13.0, 10.5, 3.0 and 17.8 in the healthy tract, 53.0, 0.0, 0.0, 31.0 and 0.0 in the apparently healthy, and 28.0, 12.0, 9.5, 15.6 and 0.0 in the diseased. The samples collected from apparently healthy trees yielded more nematodes compared to the diseased in the diseased tract and the healthy of the healthy tract. Regarding association of the nematode with root (wilt) disease of coconut, it is seen that 41.8% samples from apparently healthy palms, 20.5% from diseased palms in diseased tracts and 12.3% from the healthy of disease-free tracts yielded R. similis. Thus, in

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total, 30.2% of the samples from the diseased area yielded R. similis against 12.3% from the healthy tracts. The maximum number of nematode was 745 per gram of root from sandy loam soil in Karthigappally taluk of Alleppey District, Kerala, whereas the highest population recorded including eggs per gram of root was 3941 (Koshy, Sundararaju and Sosamma, 1978).

The problems encountered in the survey of R. similis in coconut roots were sampling difficulty in laterite and reclaimed clay soils during dry periods, need for frequent sampling, abundance of nematodes in a particular type and portion of the root confined to a certain season (September to November), delay in transport of samples to the laboratory and the cumbersome processing of roots and extraction at low temperature. Studies on the standardisation of sampling zone of coconut have shown that only 30% (av. of three palms) samples yielded R. similis from known infested palms.

Although earlier workers recorded R. similis as an endoparasite of coconut root, the typical lesions and severe root-rotting caused by this nematode were brought out only in subsequent studies. Standardisation of extraction techniques showed that among the various methods of processing attempted, peeling off the epidermis and slicing the main roots longitudinally to 8 pieces each of 2-3 cm length yield the maximum population. The semi-hard orange coloured portion of the main root harboured the maximum number of R. similis. Submerging the sliced roots in water at a temperature range of 4 to 14°C resulted in the recovery of larger numbers of very active nematodes (Koshy, Sosamma and Nair, 1975).

Active population of R. similis was recovered from roots of stumps retained in situ even five months after cutting down the palms. This clearly establishes the need for adoption of strict phytosanitary measures in replanting schemes.

HOST RANGE AND POPULATION DYNAMICS

Forty eight species of plants belonging to 44 genera in 17 families were recorded as hosts of R. similis. Twenty eight of them are new host records and include a number of economically important plants (Koshy and Sosamma, 1975; Sosamma and Koshy, 1977 and 1981). The experiments on ginger (Sundararaju, Sosamma and Koshy, 1979) and turmeric (Sosamma, Sundararaju and Koshy, 1979) using the coconut isolate of

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R. similis population vividly established the pathogenicity of the nematode on these crops and its dissemination through seed rhizomes. This would suggest exclusion of susceptible hosts from crop combination in intercropping patterns in coconut gardens so that population of R. similis can be kept under check and further spread of the nematode through planting/to new areas can be prevented. /material

The coconut populations of R. similis from Kayangulam and Kasaragod were inoculated on to 11 citrus cultivars belonging to 8 species of Citrus and Poncirus trifoliata and was identified as the "banana race" not infesting any of the Citrus spp. and infesting banana (Koshy and Sosamma, 1977).

On cross inoculations carried out, populations from coconut, arecanut and banana were found to have no host specificity.

Data collected through fortnightly sampling during the period from 1973 to 1976 showed that infested roots yielded maximum numbers (44 to 150 per gram of root) of R. similis during October to November and minimum or nil (0 to <1 per gram of root) during March to July (Koshy and Sosamma, 1978a). This finding is very important from the point of survey, pathogenicity and application of control methods.

PATHOGENICITY

Inoculation of the burrowing nematode population isolated from coconut roots showing lesions and rotting on to healthy WCT seedlings grown in sterile soil contained in cement tubs of 75 x 80 cm size, produced small, elongate, reddish brown, cortical lesions on roots which later coalesced and caused extensive root rotting, inhibited production and branching of lateral roots. There was considerable reduction in the growth and vigour of the inoculated plants over the control. The reduction in percentage of number of leaves, girth at collar and height were 10, 26 and 17 respectively, 18 months after inoculation. This established the pathogenicity of the nematode on coconut (Koshy, et al., 1975).

An experiment to study the inoculum potential of the nematode and the effect of various levels of R. similis population on the growth of coconut seedlings grown in sterile soil contained in pots of 45 cm size initiated in February 1975

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with 6 levels of inoculum viz. 0, 100, 500, 2500, 12,500 and 62,500 extracted from coconut roots showed root lesions in all the inoculated seedlings and rotting of various intensities six months after initial inoculation. All these plants were planned to be transferred to soil tanks of 6' x 6' x 4' size for proper growth of the seedlings and multiplication of the inoculum for production of symptoms. This could not be done due to the non-availability of soil tanks. Data on nematode population and plant growth characters have been collected for final evaluation.

Fungi Cylindrocarpon effusum and C. lucidum were isolated from the lesions produced by R. similis on coconut roots (Sosamma and Koshy, 1978).

The massive root system and the perennial nature of coconut palms demand huge populations for fruitful pathogenicity experiments. An attempt to raise pure culture of R. similis on carrot discs following the method of O'Bannon and Taylor (1968) with certain modifications was successful in raising large populations of axenic R. similis for pathogenicity experiments (Koshy and Sosamma, 1980).

R. similis population from infested carrot discs on inoculation into the mesocarp (husk) of 4 month old tender nuts by a syringe using a Number 20 needle produced rotting within husk which were 5 to 10 cm long and 2 to 5 cm wide. Increase in population was 44 times after a period of 5 months without affecting the quality and size of the nut (Koshy and Sosamma, unpublished).

As the earlier pathogenicity experiment was done in small size (45 cm) pots with field population, it is necessary to repeat the experiment with axenic nematode inoculum on plants grown in sterile soil contained in sufficiently large soil tanks of 6' x 6' x 4' size to study the role of R. similis in the incidence of root (wilt) disease, if any. The proposed levels of nematode inoculum are 0, 10, 100, 1000, 10,000 and 100,000 with five replications each.

To study the effect of C. effusum and R. similis alone and in combination on the growth of coconut, an experiment is

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planned with the following treatments replicated 5 times in 6' x 6' x 4' soil tanks: (1) Control (2) C. effusum (3) R. similis (10,000) (4) C. effusum + R. similis (5) R. similis followed by C. effusum after 30 days (6) C. effusum followed by R. similis after 30 days.

Coconut sprouts were raised by sowing dehusked seednuts which had the advantage of early and increased percentage of germination and availability of uninjured roots amenable to easy handling in the laboratory (Koshy and Sosamma, 1978c). By inoculating roots of such sprouts, with R. similis, lesions could be detected under a stereoscopic microscope after 24 h. (Koshy and Sosamma, 1981).

Though all the three known nematode virus vectors Xiphinema, Longidorus and Trichodorus have been recorded in a limited number of soil samples collected from coconut root zone, their inconsistent pattern of occurrence and low population density in diseased tract was not convincing to attribute a positive role in the transmission of the disease. However, Longidorus saginus isolated from the root zone of coconut root (wilt) disease affected palms have been inoculated on to five healthy WCT seedlings grown in sterile soil under insect proof house in September, 1976. Samples drawn in 1978 showed increase in nematode population proving the suitability of coconut as a host for multiplication of this nematode. As this experiment had to be abandoned for improper insect proof conditions, the role of nematode in the transmission of root (wilt) disease could not be determined.

CONTROL

Coconut seedlings raised in the nurseries at Kasaragod, Nileshwar (disease-free areas) and Karunagappally were found to harbour large number of R. similis not only in the roots outside the husk but also in portions of the same roots inside the husk. To release nematode-free seedlings from the nursery, a dip treatment in DBCP 1000 ppm concentration for 15 minutes was developed and recommended (Koshy and Sosamma, 1979).

The most important means by which R. similis gets introduced into new geographical areas is through infested planting materials. Establishment of a new infestation at Kidu (Karnataka) from coconut seedlings taken from CPCRI, Kasaragod is an example of spread through coconut seedlings. This warrants strict intra and inter-State regulatory measures against the supply of infested coconut planting materials in India

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to minimise indiscriminate introduction of R. similis in regions free of the nematode (Koshy et al., 1978).

Treatment with fensulfothion @ 50 kg a.i./ha, carbofuran @ 6 kg a.i./ha and DBCP @ 60 l a.i./ha in September, December and March was tried to raise seedlings free of R. similis in the nursery at Kasaragod with untreated nursery beds as controls. Though none of them offered cent per cent control of R. similis, fensulfothion was found to be the best followed by DBCP (Koshy and Nair, 1979).

Control experiments using nematicides and oil cakes to determine their effect in the control of R. similis on growth and yield of coconut palm and their role, if any, in the incidence or intensity of root (wilt) disease are in progress. In all the three experiments pretreatment soil and root populations are assessed during September/October on the basis of the population abundance. Nematicides and oil cakes are applied thrice a year during May/June, September/October and December/January.

The coconut cultivars, Borneo, British Solomon Islands, Dwarf Green, Dwarf Orange, Fiji Rotuma, Fiji Tall, Guam, Jamaica Sanbla, Jamaica Tall, Java, Kenya, Laccadive Ordinary, Laccadive Micro, Malayan Dwarf Green, Malayan Dwarf Orange, Rangon Kobri, Spicata, St. Vincent, West Coast Tall and hybrids, Dwarf x Tall, Java x Gangabondam, Java x MDG, Java x MDO, Java x MDY, Java Giant x MDG, JG x MDO, JG x MDY, MDG x JG, MDO x JG, MDY x JG, Laccadive x San Ramon, San Ramon x Gangabondam, Tall x Dwarf and Tall x Gangabondam were screened and found susceptible to R. similis (Sosamma, Koshy and Rao, 1980). Varieties, San Ramon, Standard Kudat, Blanchissure, Lono, Philippines Ordinary, S.S. Green, Gonthembeli, Kong Thien Young, Kappadam, Zanzibar, Nigerian Dwarf, Andaman Ordinary, Nigerian Tall, King Coconut, Ceylon Tall, FMS, Seychelles, Andaman Giant and Kenthali are under test for resistance.

DISCUSSION

Weischer (1967) concluded: "The low population density of nematodes and wide occurrence and the general distribution pattern of the disease indicate that plant parasitic nematodes can be excluded from being considered as the primary cause of Kerala wilt". However, the notriety of R. similis in citrus

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decline in Florida and pepper yellows in Indonesia warranted detailed study of its role in coconut cultivation as well. A fund of information collected on the abundance of the endoparasite, its seasonal variations, association with fungi and other details has been narrated in the foregoing pages. Weischer's remark that the nematodes associated with coconut can be considered as disease incitants is of significance. Weischer also suggested that presence of Xiphinema in both diseased and healthy areas and of Longidorus only in the diseased area or very near between these two areas could be of importance if viruses were involved in the disease. A viral etiology has not, for certain, been established for coconut root (wilt) disease. Symptomatology studies have brought out root-rot as a component of the disease syndrome. The lesions produced by R. similis on coconut root, eventually leading to rotting of roots is important in this regard. Pathogenicity experiments with R. similis on coconut seedlings in pots failed to produce the diagnostic symptom of flaccidity of leaflets. Nevertheless, it is not possible to rule out the development of aerial symptoms in experimental plants showing rotting of roots as a result of nematode inoculation, before the experiment is carried out in sufficiently large soil tank with the optimum dose of nematode inoculum with other associated microorganisms (Raski,1979). Similarly, the isolation studies have failed to extract the burrowing nematode always in association with the disease. Considering the vast healthy and diseased tracts, the number of samples collected was admittedly not only inadequate, but also did not represent all the soil types equally. It may therefore be not desirable to negatively correlate root (wilt) disease of coconut with R. similis infestation since results of abundance in various samples would strictly be comparable if only all of them were drawn during the peak season (October-November). Transmission studies with nematodes like Longidorus, Xiphinema and Trichodorus for direct evidence on their role in coconut root (wilt) disease demand exhaustive experimental facilities like insect-proof cages as the disease is reported to be transmitted by lace bugs. Indirect evidence on the role of plant parasitic nematodes in the incidence of the disease is expected to yield from the different control experiments now in vogue.

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