CROP LOSS CAUSED BY ROOT-KNOT NEMATODE (*Meloidogyne incognita* Kofoid) INFESTING *Coleus parviflorus* AND ITS CONTROL

BY

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INTRODUCTION

INTRODUCTION

The root knot nematode Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 is an important pest causing serious damage to a large number of the cultivated crops all over the world. Infestations by this nematode have been causing much concern to most vegetable growers (Sen, 1958) in India.

Kurka or Coleus parviflorus Benth., a tuber yielding vegetable, is also found seriously affected by this pest. Coleus is mainly cultivated in South East Asia in homesteads. The plants yield small dark brown tubers with an unusual flavour. It is also commonly called Chinese Potato. Coleus is a seasonal crop cultivated from July to October and harvested after 4½ to 5 months ie. from December to March (Maini, et al. 1975). Very little or no tuber formation has been noticed when Kurka is grown in other seasons. It yields 20 - 80 Q/ha (Hrishi et al., 1972).

Due to the attack of M. incognita on coleus, conspicuous gall-like swellings are formed on roots and tubers. The galled roots soon rot and new root growth is prevented (Nirula, 1961; Thorne, 1961). Severely affected plants are stunted. Plants less seriously affected may survive but have a sickly appearance and yield poorly. Infested

tubers are unfit for sale in the market. As the infected plant wilts, dries up and dies before the tubers have attained maturity the loss caused is often heavy. But sufficient work has not been done on the crop losses caused by the nematode and its control, the histopathology of infected roots and the keeping quality of infested tubers.

The present studies have been carried out with the objectives of studying the crop loss caused by the nematode on coleus, the histopathology of infected roots and the keeping quality of infected tubers, and to evolve a suitable control measure for the nematode under field conditions.

REVIEW OF LITERATURE

1. REVIEW OF LITERATURE

A brief review of the literature on crop loss caused by *M. incognita*, histopathology of infected roots, keeping quality of infested tubers and control of the nematode is presented below.

1.1 Assessment of the effect of different levels of population of *M. incognita* on growth and yield

The root-knot nematode, *M. incognita* is an important plant parasitic nematode damaging almost all the vegetable crops in Kerala (Mammen, 1973). It causes severe losses to Coleus also. (Seshadri, 1965; Sathyarajan *et al.*, 1966; Hrishi and Mohankumar, 1976).

1.1.1 Symptoms on root

Root galls are observed to be formed on roots of Coleus due to infection by the nematode (Pushkarnath and Roychoudhary, 1958; Sathyarajan *et al.*, 1966; Hrishi and Mohankumar, 1976). Similar observations have been made on other crops also (Tarjan, 1952; Krusberg and Nielsen, 1958; Nadakal, 1963; Adesiyan and Odihirin, 1978). Number and size of galls formed on roots of plants depended on the number of larvae per gall and the species of plants and nematodes involved (Dropkin, 1954 & 1955). Sometimes individual galls coalesced into amorphous masses containing large number of nematodes in roots of severely affected plants.

A high positive correlation existed between gall area and number of larvae in the gall (Dropkin, 1955). In sweet potato, primary root penetration by second stage larvae occurred at the tips of young roots, in the region of tissue differention, through loose ruptured cells of enlarging roots or broken surface of root cracks. (Krusberg and Nielsen, 1958). A direct correlation was found to exist between the amounts of inoculum and the resulting number of egg masses (Tarjan, 1952; Phukan et al., 1983).

Infection by M. incognita on plants restricted to the root system in many cases while in few instances it was noticed at the bases of stem below the soil level (Nadakal, 1963). Swelling of the entire root and root tip was caused due to infection by the nematode, through combination of hypertrophy and hyperplasia of cells (Krusberg and Nielsen, 1958; Babatola, 1985). The intensity of root galling in ginger was directly proportional to the increase in nematode inoculum (Phukan et al., 1983; Sudha and Sundararaju,1985) while Jagdale et al., (1985), working on betelvine, reported that increased root galling and final nematode population was not proportional to initial population density. Similar results were also

obtained by Seinhorst (1961). He observed that, in all his experiments the higher the initial densities of population, the lower was the rate of increase. Relatively low final population densities resulting from high initial ones have been ascribed to damage inflicted on the host plant and also the inter- and intra-specific competition for food. He also reported that all initial population densities increased or decreased to a "ceiling level" on good hosts.

Studies by Tarjan (1952) showed that on vegetables the root-knot nematode did not show significantly different effect on plants receiving 1 and 10 egg masses. Plants inoculated with 100 egg masses had significantly lower root weights than plants receiving lesser amounts of inoculum. Sharma and Swarup (1968) reported that one thousand larvae of M. incognita per 400 g soil reduced the root length, shoot length and shoot weights of tomato plants. Significant reduction in plant growth was noted in jute infected with M. incognita (Phukan et al., 1983) at 10,000 juveniles per kg soil. However, the difference in plant growth between inoculum levels of 100 and 1000 juveniles were not significant. As high as 27.9 per cent reduction in root and top weights were recorded with initial inoculum levels of 1000 nematodes per plant over a period

of six months in patchouli by Prasad *et al.*,(1984). Sudha and Sundararaju (1985), while working on ginger infected by *M. incognita* found that an initial inoculum level of 100 nematodes per plant was the marginal threshold level for damaging plant growth six months after inoculation. Pathogenicity of *Capsicum anuum* infected by *M. incognita* was studied by Varela *et al.*,(1986). They observed significant difference in plant growth and dry weight of shoots and roots between control plants and inoculated plants at all inoculum levels. Root weights of different crops have been found to be reduced due to attack of *M. incognita* (Tarjan, 1952; Brodie and Cooper, 1964; Rajagopalan, 1972; Prasad and Reddy, 1984; Sudha and Sundararaju, 1985).

Reduction in root length has been reported in different crops resulting from the attack by *M. incognita* (Sharma and Swarup, 1968; Gunasekharan *et al.*, 1972; Rajagopalan, 1972; Chandramathy, 1973; Sudha and Sundararaju, 1985; Anwar, 1986).

Rotting of roots infected by M. incognita was reported in vegetables (Golden and Vangundy, 1975; Sudha and Sundararaju, 1985). Golden and Vangundy (1975), observed that roots infected by M. incognita were highly susceptible to Rhizoctonia solani in the field. Root decay by fungus occurred 4 to 5 weeks after nematode infection. Fungal sclerotia were found only on nematode gall tissues. Fungus penetrated either directly or through ruptures in the root. R. solani colonised giant cells and root xylem cells.

1.1.2 Symptoms on shoot

Above ground symptoms of root-knot nematode infection on potato were difficult to detect in the field in the early stages of infection (Pushkarnath and Roychoudhary, 1958). Chlorotic leaves on infected plants have been reported in coleus and other crops (Pushkarnath and Roychoudhary, 1958; Nadakal, 1963; Franklin, 1964; Anon, 1978; Babatola, 1985; Melakeberhan et al., 1985). Reduction in size of leaves in infected plants has been reported (Babatola, 1985; Jagdale, 1985).

Infected plants also showed reduction in leaf number in different crops (Jagdale et al., 1985; Sudha and Sundararaju, 1985).

The shoot length and shoot weight of plants infected with M. incognita was found to be less than that of uninfected plants (Pushkarnath and Roychoudhary, 1958; Nadakal, 1963; Brodie and Cooper, 1964; Franklin, 1964; Bergeson, 1968; Sharma and Swarup, 1968; Rajagopalan, 1972; Anon, 1978, Caveness, 1982, Anwar, 1986).

Tarjan (1952) observed that increasingly greater amounts of inoculum resulted in significantly lower top weights. Similar observations were also made by Gunasekharan and Kalyanaraman, 1972; Phukan et al., 1983; Prasad and Reddy, 1984; Jagdale et al., 1985; Melakeberhan et al., 1985; Sudha and Sundararaju, 1985). Studies of Tarjan (1952) and Chapman (1960) showed that M. incognita infection causes higher root-knot indices and more severe reduction in dry weight of top shoot than other species of the same nematode. Wilting, drooping of leaves and other premature symptoms of ageing have been observed in several crops infected by M. incognita (Pushkarnath and Roychoudhary, 1958; Franklin, 1964; Pillai, 1976; Mjuge and Estey, 1978). Mjuge and Estey (1978) suggested that drooping of leaves can be explained by impaired absorption of water from soil

by damaged root system, thus leading to water deficit.

1.1.3 Symptoms on tubers

Coleus tubers infected with M. incognita became malformed and hypertrophied due to heavy galling and become unsuitable for consumption and marketing (Pushkarnath and Roychoudhary, 1958; Sathyarajan et al., 1966; Pillai, 1976). Uneven knobbly appearance with small necrotic spots around nematodes have been observed in infected tuber crops like yams. Rotting of tubers were also recorded (Anon., 1978).

According to Griffin (1985), root-knot nematode affectes the quality rather than quantity of potato tubers. Fatemy and Evans (1986), working with Globodera rostochiensis on potato found that water stress resulted in smaller shoot/root ratioes and decreased tuber production and decreased total P, K and Mg uptake. Poor or no yield have been reported in all crops infected severely by M. incognita (Nadakal, 1963; Olthof and Potter, 1972; Barker et al., 1976; Prasad and Reddy, 1984; Reddy, 1985). According to Melakeberhan et al., (1985) reduction in growth parameters and yield in infected plants might be due to inhibited photosynthetic processes and enzyme systems, and the interference caused by M. incognita in the translocation systems.

1.1.4 Biochemical changes in plants due to infection by M. incognita

Beevers (1976) observed that there was a decrease in chlorophyll, protein and RNA levels in plants during senescence. Symptoms similar to premature ageing have been noticed on plants infected by M. incognita (Mjuge and Estey, 1978; Melakeberhan, 1985). Khan and Haseeb (1984) detected increased peroxidase activity in roots of okra infected with M. incognita. Biochemical studies by Agarwal et al., (1985) on okra seedlings infected with M. incognita showed that there was significant enhancement in protein and total free amino acids with simultaneous increase in protease activity, increased reducing sugar contents, D-amylase and invertase activity, decreased levels of starch, non reducing sugars and total sugars and increased levels of phenolic compound and IAA. An increased production of growth inhibitors was observed by Khan and Iftikharuddin (1985) in plants infected with M. incognita when compared to healthy plants. Glazer et al. (1986) noted increased ethylene production by nematodes along with high level of IAA in infected roots than in uninfected roots.

1.1.5 Growth of coleus

Maini et al.,(1975) reported that the duration of coleus crop is about 4½ months and that its vegetative growth is maximum at the third month.

Vijayakumar and Shanmugavelu (1984) observed that tuber initiation of coleus occurred 80 days after planting. Growth of tuber continued till 150 days of planting. The increase in tuber yield by weight after initiation depended primarily on the aerial parts, to synthesise the needed assimilates for the rapid development of tubers, which act as a "sink". The general decline in growth rate observed after 100th day may possibly be attributed to the drain of assimilates from the aerial portion of the plant to the developing tubers.

1.2 Histopathology of roots inoculated with M. incognita

Saffranin was observed to be a suitable stain for studying endoparasitic nematodes in root tissues (McBeth et al., 1941; Krikpatrick et al., 1957; Taylor, 1976).

Penetration of root-knot larvae was directly through the root epidermis near the root tip, destroying some epidermal cells during penetration (Krusberg, 1963). Once in the cortex, larvae migrated intracellularly. Formation of characteristic giant cells on susceptible

plants due to infection by M. incognita have been reported by several workers (Crittenden, 1958; Dropkin and Nelson, 1960; Littrell, 1966; Kozhokaru, 1985). There is difference of opinion regarding the tissue from which giant cells originate. According to Littrell (1966), they originated from the provascular strand while other workers (Birchfield, 1964; Taylor, 1976) reported that giant cells are formed from phloem. Some workers found the formation of giant cells from xylem and phloem parenchyma (Molina and Nelson, 1983), while some noted giant cell formation from the xylem (Taylor, 1976; Jacob, 1977). Crittenden (1958) reported that giant cells occurred frequently in the region of the pericycle. Giant cells were generally found adjacent to the head of nematode (Crittenden, 1958; Ferver and Crittenden, 1958; Dropkin and Nelson, 1960; Littrell,1966; Taylor, 1976, Kozhokaru, 1985).

The number of giant cells initiated by M. incognita in different crops showed variations (Orr et al., 1978). In pepper 4 to 6 giant cells were observed (Jacob, 1977); while in vegetables 8 to 9 giant cells were usually seen (Kozhokaru, 1985). Giant cells were observed to be larger than the surrounding cells, had very dense cytoplasm with a large number of nuclei in each cell (Crittenden, 1958; Dropkin and Nelson, 1960; Birchfield, 1964; Taylor, 1976;

Molina and Nelson, 1983; Sosa Moss et al., 1983; Kozhokaru, 1985).

Increase in size of giant cells was reported to be due to incorporation of surrounding parenchyma cells (Dropkin and Nelson, 1960; Birchfield, 1964; Littrell, 1966).

Birchfield (1964) observed progressive cell wall dissolution in advance of the nematode which was followed by the formation of thick walls around the feeding area. The nuceli of dissolved cells aggregated within cyncytia and maintained nuclear membranes intact.

According to Littrell (1966), multinucleate cells were noted in plants 72 hours after inoculation with the nematode. He also observed mitosis without cell division. A similar observation was made by Jones and Payne (1978) who also found that cell plate alignment in the giant cells proceeded normally, but cytokinesis was unsuccessful. They did not find any evidence of wall break down. Electron microscope studies by Kozhokaru (1985) showed that nuclei of giant cells had lobate contours. Root-knot nematodes were found with its head embedded in the stele and body in the cortex. (Ferver and Crittenden, 1958; Krusberg and Nielsen, 1958).

Enlargement of the stele and cortex due to infection by M. incognita was reported by several workers (Ferver and Crittenden, 1958; Krusberg and Nielsen, 1958; Taylor, 1976; Vovlas et al., 1986) Hyperplasia also occurred in the pericycle (Littrell, 1966; Dropkin and Nelson, 1960). In infected roots, vascular tissue differentiation was not observed. (Dropkin and Nelson, 1960). Prominent disruptions in the stele, cortex and pericycle were observed due to extensive hypertrophy and hyperplasia in roots infected by M. incognita (Ferver and Crittenden, 1958; Dropkin and Nelson, 1960; Birchfield, 1964; Taylor, 1976; Sosa-Moss et al., 1983; Vovlas et al., 1986).

Akhthar et al. (1983) found that certain enzymes were secreted by the nematodes which reduced the concentration of lignin in the cell facilitating the movement of the nematode within the host.

In late stages of root infection by M. incognita, normal development was severely disturbed so that only remnants of the xylem and phloem remained (Dropkin and Nelson, 1960). Abnormal xylem formation in infected root cells was reported by Dropkin and Nelson (1960), Littrell (1966) and Orr et al. (1978).

Necrosis was observed in the cortex 30 days after infection (Dropkin and Nelson, 1960; Veech, 1970; Sosa-Moss et al., 1983; Kozhokaru, 1985).

According to Krusberg and Nielsen (1958), as the nematodes matured after oviposition, it either died or stopped feeding as the giant cell protoplasm often disintegrated and disappeared especially in young enlarging roots where the giant cells usually collapsed. Studies by Birchfield (1964) showed that older syncytia became necrotic, hard and crumbly. Dropkin and Nelson (1960) found that the cortex in galls of older roots sloughed off; galls observed in mature soyabean plant contained little or no cortex tissue.

1.3 Field experiment on the control of M. incognita infesting coleus and other crops

Burning of dried vegetable cover in the field was found to be an efficient means for reducing the population of plant parasitic nematodes in soil (Arjunlal et al., 1983; Venkitesan, 1984).

Furadan was found to give good control of M. incognita and reduced gall index significantly (Johnson et al., 1974; Taylor and Sasser, 1978; Anon, 1983; Jagdale et al., 1986) Yield increase and control of the nematode in crops were obtained due to the application of furadan (Johnson and

Cairns, 1972; Sivakumar et al., 1973; Weingartner et al. 1974).

Routaray and Sahoo (1985) worked on the integrated control of root-knot nematode on tomato and found that the application of carbofuran @ 1 kg a.i./ha + neem cake and urea, each at 10 kg N/ha produced maximum yield with lowest gall index and nematode population.

Application of urea was found to reduce the population of plant parasitic nematodes in soil (Lall and Hameed, 1969; Sitaramiah and Singh, 1971). They also recommended crop rotation and summer ploughing as effective control methods.

Fumigants like EDB and Nemagon were found to control root-knot nematodes on potato successfully but a disadvantage was their relatively high cost (Nirula, 1961). Weingartner (1974) observed that non volatile nematicides were generally more effective than soil fumigants. According to Pillai (1976), post planting treatment after one month with Nemagon or Terracur P was highly effective in reducing root-knot infection of coleus thereby leading to higher production of quality tubers. Phorate was also found to give good control of root-knot nematode (Rodriguez -Kabana et al., 1976; Jagdale et al., 1985).

Idicula *et al*. (1988) reported that carbofuran appeared to be more toxic and more effective than aldicarb against second stage larvae of *M. incognita*. Carbofuran treated plots gave maximum yield.

1.4 Insecticide residues in tuber crops

Bacon (1960) could not detect any residue of phorate, applied to seed pieces or to soil at planting time, in potato tubers at harvest. Kathpal *et al*., (1983) found that no residue of phorate was present in tubers of potato at harvest 90 days after planting. Residues from all treatments dissipated completely in a period of 2-3 months.

Misra and Agrawal (1987) observed a residue of 0.123 ppm of carbofuran in tubers at harvest when the nematicide was applied at 3.375 kg a.i. per ha at planting. According to Mithyantha *et al*., (1977), carbofuran residues in potato tubers after the application of dosages ranging from 1.125 to 3.340 kg a.i. per ha to the soil at planting ranged from 0.047 to 0.295 ppm in different treatments. The tolerance limit fixed by W.H.O for carbofuran is 0.5 ppm (Anonymous, 1977).

In sweet potato, Palaniswami (1988) found that the application of insecticides between 50 and 80 days after planting was effective for controlling the sweet potato weevil.

1.5 Assessment of the keeping quality of tubers

Respiration and weight losses of tubers increased with temperature during storage (Butchbaker et al., 1973; Dambroth, 1970).

Damaged Cassava tubers were observed to start rotting earlier than undamaged tubers (Booth, 1974; Maini and Balagopal, 1978). Immersion of Diascoria tubers in hot water at 46.7°C for 65 minutes and 50°C for 3.5 minutes was recommended by Acosta and Ayala (1976), for complete control of the nematodes without seriously impairing viability.

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 Assessment of the effect of different levels of population of *Meloidogyne incognita* on the growth and yield of *Coleus parviflorus*

The experiment was conducted at the College of Agriculture, Vellayani from August 1986 to December 1986.

Thirty earthen pots of 10 litre capacity were taken and filled with potting mixture which was sterilized two weeks earlier with aqueous solution of formaldehyde. The soil used was of red loam type.

A terminal cutting of coleus, 10 cm long, was planted in each pot. Fertilizers were applied at two split doses at 30:60:50 kg N, P and K per hectare at the time of planting and 30 kg N and 50 kg K per hectare 45 days after planting. Forty five days after planting 1 litre sterilized soil per pot was applied at the base of the plant to promote tuber formation.

The soil was inoculated with nematodes 15 days after planting. Inoculation was done @ 0,100, 1000, 2500, 5000 and 10,000 nematodes per pot. The experiment was laid out in completely randomised design with five replications for each treatment.

M. incognita culture was maintained on ornamental coleus roots in greenhouse. At the time of inoculation, egg masses were picked from roots and kept for hatching in cavity dishes containing water.

Larval population in the nematode suspension obtained from cavity dishes was ascertained using a counting dish. This suspension was then diluted with sterile water for getting the desired concentration of nematodes. The suspension was then applied at the root zone of the plant through holes made in soil with a thin stick. In control pots, distilled water was similarly applied at the root zone.

The number of leaves and shoot length in the treatments were observed at monthly intervals, and at the time of harvest the population of M. incognita, and weights of shoot and tubers were also recorded.

The percentage increase/decrease in the number of leaves as compared to control were calculated as follows:-

$$(Y - \frac{X}{Y}) \times 100$$

where Y = number of leaves in control and

X = number of leaves in the different treatments

Similarly percentage increase/decrease in shoot length also were calculated.

Nematodes from soil were extracted by the Cobb's Sieving technique as modified by Christie and Perry (1951).

Split plot analysis was done with levels of treatment as major factor and months after inoculation as minor factor.

Correlations between the tuber yield and the growth characters and soil population of M. incognita were also studied through statistical analysis of the data.

2.2 Deterioration of coleus tubers infected by M. incognita in storage

For the study, tubers having 10-15 galls were selected and kept in storage and the external and internal symptoms were recorded once in 3 days for a period of 15 days; uninfected tubers served as control.

2.3 Histopathology of C. parviflorus inoculated with M. incognita

Ten pots of ten litre capacity, filled with sterilized potting mixture, were planted with terminal cuttings of coleus. Two weeks after planting 10,000 one-day old M. incognita larvae each were inoculated in five pots. The remaining five pots served as control.

Galled roots were collected from each inoculated pot at monthly intervals and microtome sections were taken (Johansen, 1940). Microphotographs of selected sections

were also taken at 25X and 30X magnification.

2.4.1 Field experiment on the control of *M. incognita* infesting *C. parviflorus*

The field experiment was laid out at the Instructional farm, College of Agriculture, Vellayani during 1986 to evolve an effective technology for the control of root-knot incidence on *C. parviflorus*.

The field used for the experiment was kept fallow during the previous season and it was inoculated uniformly with *M. incognita* larvae and egg masses collected from infested ornamental coleus plants in the college garden. Immediately after inoculation, bhindi seeds were sown in the field. After 30 days the plants were uprooted and the roots were chopped and incorporated in the soil uniformly. This ensured adequate population of nematodes uniformly distributed in the experimental plots. Then the land was prepared and plots were laid out. Planting of coleus was done one week after land preparation. The terminal cuttings used for planting were obtained from seed tubers planted in pots containing sterilized potting mixture.

The experiment was laid out in randomised block design with eight treatments each replicated thrice (vide Table 4). The plot size was 2.5 x 2 m. Preplanting counts of nematodes in plots were recorded. In relevant plots 30 kg each of dried plant material was spread uniformly and burnt. All other plots received 5 kg ash to compensate the extra nutrients received by way of burning plant material. After 24 hours, when the soil got cooled planting was done. The cultural operations and fertilizer applications recommended in the Package of Practices were adopted (KAU, 1981). The nematicides required were applied at the root zone and it was raked into the soil. Watering was done immediately after nematicide application. The plants were irrigated daily and harvesting was done 4½ months after planting.

On harvest, the shoot length, shoot weight, tuber yield, soil population of nematodes and root population of nematodes were recorded. Root-knot index was worked out from the data in 0-4 scale.

Data were analysed using analysis of covariance with initial population as independent variable and the observations at harvest as cofactors.

2.4.2 Estimation of insecticides residues in tubers

Tubers selected at random from each treatment were analysed in the laboratory to assess the residue of carbofuran and phorate present, adopting the techniques of Getz and Watts (1964) and Gupta and Dewan (1973) respectively.

2.4.3 Assessment of the keeping quality of stored coleus tubers

Samples of infected tubers (500 g) taken from each treatment in the field experiment and one sample of uninfected tubers were stored in two litre glass jars, covered with muslin cloth and kept in store. One sample each of galled and infected tubers (500 g) were treated in hot water at 55-57°C for five minutes. Then they were dried in shade and also stored as two treatments in the experiment. From the data, percentages of the weight of spoiled tubers in each treatment compared to the weight of tubers in control during the corresponding periods of observation were worked out.

3. RESULTS

3.1 The effect of different levels of population of *M. incognita* on the growth and yield of *C. parviflorus*

M. incognita larvae were found to affect the growth of *C. parviflorus*. The different growth parameters like number of leaves, shoot length, fresh shoot weight and weight of tubers were observed (Plates I A to F).

3.1.1 Number of leaves

The number of leaves per plant in control showed a gradual increase upto the third month. The number in third month was 932. Then the number gradually decreased, and at the time of harvest it was 737 per plant only (Table 1).

The mean number of leaves in different treatments including that of control did not show statistically significant variations. The percentage decrease in the number of leaves compared to the uninoculated check in the treatment where 100 nematodes were inoculated was 3.56 after one month, whereas the percentage reduction was 19.88 in the treatment which received 10,000 nematodes per plant. In plants inoculated with 100 larvae the decrease in leaf number was less than 10 per cent upto

Table 1. Effect of different inoculation levels of *M. incognita* on number of leaves of Coleus at monthly intervals.*

Number of nematodes inoculated	Mean number of leaves observed at monthly intervals				
	1	2	3	4	5
0	337	764	932	759	737
100	325 (3.56)	698 (8.64)	877 (6.00)	523 (31.09)	166 (77.48)
1000	321 (4.75)	682 (10.73)	841 (9.86)	462 (39.13)	153 (79.25)
2500	318 (5.64)	653 (14.53)	834 (10.61)	461 (39.26)	129 (82.50)
5000	285 (15.43)	644 (15.71)	793 (15.01)	428 (43.61)	98 (86.70)
10,000	270 (19.88)	606 (20.60)	716 (23.26)	399 (47.43)	91 (87.65)
C.D	N.S	N.S	N.S	N.S	N.S

* Mean of 5 values

C.D for comparing variations between months = 10.088

Figures in parenthesis represent the percentage decrease when compared to control.

three months after inoculation. Subsequently there was a drastic reduction in leaf number and by the end of the fifth month 77.48 per cent reduction was observed. A similar trend was observed in plants inoculated with 1000 nematodes.

In plants inoculated with 2500 nematodes the reduction in leaf number was more than 10 per cent even from the second month onwards. At the end of the fifth month the reduction reached the level of 82.50 per cent.

In plants given 5000 and 10,000 nematodes the reduction in mean leaf number exceeded 10 per cent in the first month itself. But at the time of harvest the decrease was 86.7 per cent and 87.65 per cent respectively and these were comparable with the effect observed in the treatments with lower levels of nematode populations.

The plants inoculated with different levels of nematode population remained wilted during day time from the third month after inoculation, even with daily irrigation, while no wilting was noticed in control.

3.1.2 Shoot length

The mean shoot length of C. parviflorus in control showed a gradual increase from the first month upto harvest (35.86 to 53 cms). The shoot length observed in pots where 100 nematodes were applied per plant during the first and second months were significantly different (35.08 and 41.74 cms). The shoot length observed during the fourth and fifth months were on par and significantly higher than the shoot length observed during the first month (Table 2).

In all the treatments the shoot length of the plants showed significant increase between the first and second month after inoculation with nematodes. In pots in which the plants were inoculated with 1000 to 5000 larvae the shoot length length from the second to fifth month remained on par. In pots inoculated with 10,000 larvae the shoot length of the plant in the first and third month showed significant differences (26.9 and 37.58 cms) while the latter came on par with the shoot length observed at the fourth and fifth months.

The percentage reduction in the shoot length of the plants in various treatments ranged from 2.18 to 24.99, 7.82 to 30.00, 5.95 to 20.04, 10.59 to 19.64 and 12.08 to 25.09 during the first, second, third, fourth

Table 2. Effect of different inoculation levels of *M. incognita* on shoot length of coleus at monthly intervals *

Number of nematodes inoculated.	Mean shoot length observed at monthly intervals (cm)				
	1	2	3	4	5
0	35.86	45.28	47.00	50.60	53.00
100	35.08 (2.18)	41.74 (7.82)	44.20 (5.95)	45.24 (10.59)	46.60 (12.08)
1000	33.04 (7.86)	40.78 (9.94)	43.80 (6.81)	45.20 (10.67)	45.20 (14.72)
2500	32.98 (8.03)	38.62 (14.71)	42.30 (10.00)	42.80 (5.42)	42.90 (19.06)
5000	29.58 (17.51)	38.24 (15.55)	40.90 (12.98)	41.14 (18.69)	40.80 (23.02)
10,000	26.90 (24.99)	31.34 (30.00)	37.58 (20.04)	40.66 (19.64)	39.70 (25.09)
C.D.	N.S	N.S	N.S	N.S	N.S

* Mean of 5 values

C.D. for comparing variations between months = 5.264

Figures in parenthesis represent the percentage decrease when compared to control.

Plate I. Above ground symptoms on C. parviflorus caused by varying levels of M. incognita larvae inoculated in pots observed at one month and five months after inoculation.

A. Plants observed one month after inoculation.

T_1	- Control	T_4	- 2500 larvae
T_2	- 100 larvae	T_5	- 5000 larvae
T_3	- 1000 larvae	T_6	- 10,000 larvae

B. Plants observed five months after inoculation

T_1	- Control	T_2	- 100 larvae

PLATE. I.

A

B

Plate I. (contd.)

C

T_1 - Control

T_2 - 1000 nematodes per plant

D

T_1 - Control

T_4 - 2500 nematodes per plant

Plate I. (contd.)

E

T_1 - Control

T_5 - 5000 nematodes per plant

F

T_1 - Control

T_6 - 10,000 nematodes per plant

(CONTD)

E

F

(CONTD)

C

A

and fifth months after inoculation respectively. A significant increase in shoot length was observed in control plants during the fifth month as compared to plants inoculated with 10,000 larvae/plant.

3.1.3 Shoot weight

A significant reduction in the mean shoot weight was noticed in the different treatments at the time of harvest (Table 3).

The maximum shoot weight of 167.6 g per plant was recorded in control plants and it was significantly higher than the weights recorded in other treatments. The shoot weights of the plants inoculated with 100, 1000, 2500 and 5000 larvae were on par (108.9 g to 77.4 g) and significantly lower than that of control. The shoot weight of plants inoculated with 10,000 larvae per plant came on par with shoot weight of plants inoculated with 5000 larvae but the former was significantly lower than the shoot weights in the remaining treatments including control.

Table 3. Effect of different inoculum levels of M. incognita on the shoot weight, tuber yield and final population of M. incognita per 100 ml soil.*

Treatment	Number of nematodes inoculated per plant.	Shoot weight per plant (g)	Tuber yield per plant (g)	Final population of M. incognita in soil (100 ml)
T_1	0	167.6	198.92	0 (1.000)
T_2	100	108.9	76.24	312.8 (17.714)
T_3	1000	104.1	57.22	416.4 (20.402)
T_4	2500	90.1	37.24	515.6 (22.710)
T_5	5000	77.4	31.40	939.0 (30.640)
T_6	10,000	40.5	15.44	1522.8 (38.900)
C.D		42.98	102.39	(2.938)

* Mean of 5 values

Figures in parenthesis are $\sqrt{x + 1}$ values.

3.1.4 Tuber yield

The mean tuber yields obtained from the uninoculated plants (198.92 g) was significantly higher than those of inoculated plants (15.44 g to 76.24 g). The variations between yield of inoculated plants were not statistically significant. The yield showed a negative relationship with the number of larvae inoculated. The lowest tuber yield of 15.44 g was recorded in plants receiving an inoculum of 10,000 larvae per pot and highest yield among the treatments was from plants given an inoculum of 100 larvae per pot (76.24 g).

3.1.5 The population of *M. incognita* in soil observed at harvest

A highly significant increase in the mean final population of nematode was observed at the time of harvest of the crop (Table 3).

A maximum mean soil population of 1522.8 nematodes per 100 g soil was recorded in plants given in inoculum of 10,000 larvae. The minimum mean population of 312.8 nematodes was recorded in plants which were given an inoculum of 100 larvae/ plant. There was no *M. incognita* larvae in soil of control. Plants which were given 10,000 larvae had a significantly high population than the plants

Plate II. Root of *C. parviflorus* inoculated with *M. incognita* @ 10,000 larvae per pot observed at different intervals after inoculation.

1. Infected roots observed two months after inoculation.

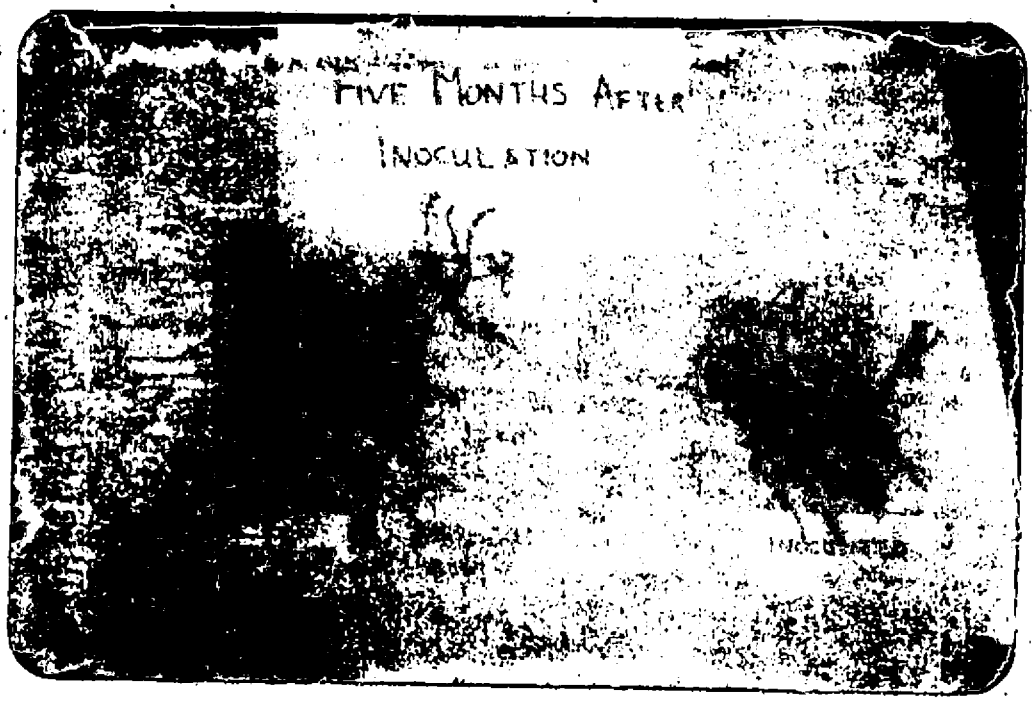
2. Infected roots observed three months after inoculation.

3. Infected roots observed five months after inoculation.

4. Uninfected roots observed five months after inoculation.

PLATE . II .

2 1



4 3

given 100, 1000, 2500 and 5000 nematode larvae. Soil populations of nematodes from plants given 1000 and 2500 nematodes were on par.

3.1.5.1 Correlation Analysis

A significantly high positive correlation of 0.64 and 0.49 was observed between the weight of tuber and the number of leaves and shoot weight of coleus respectively.

On path analysis the number of leaves was found to have direct effect on the weight of tuber while shoot weight had only an indirect effect. Shoot length did not influence tuber yield significantly.

A high negative correlation (-0.50) was observed between tuber weight and the population of M. incognita in soil at harvest. Population of M. incognita in soil at harvest had a direct effect on weight of tuber.

3.1.6 Deterioration of Coleus roots infected by M. incognita

Roots of C. parviflorus observed two months after inoculation with M. incognita showed profuse galling (Plate II 1). Rotting was observed to start by the third

Plate III. Coleus tubers infested by *M. incognita* in storage.

A. Whole tubers B. Cross section

1. Uninfected tubers
2. Infected tubers observed three days after storage.
3. Infected tubers observed six days after storage.
4. Infected tubers observed nine days after storage.

PLATE. III.

A

B

Plate III (contd.)

C.

c - Control (uninfected tubers)

5 - Infected tubers observed 12 days after storage.

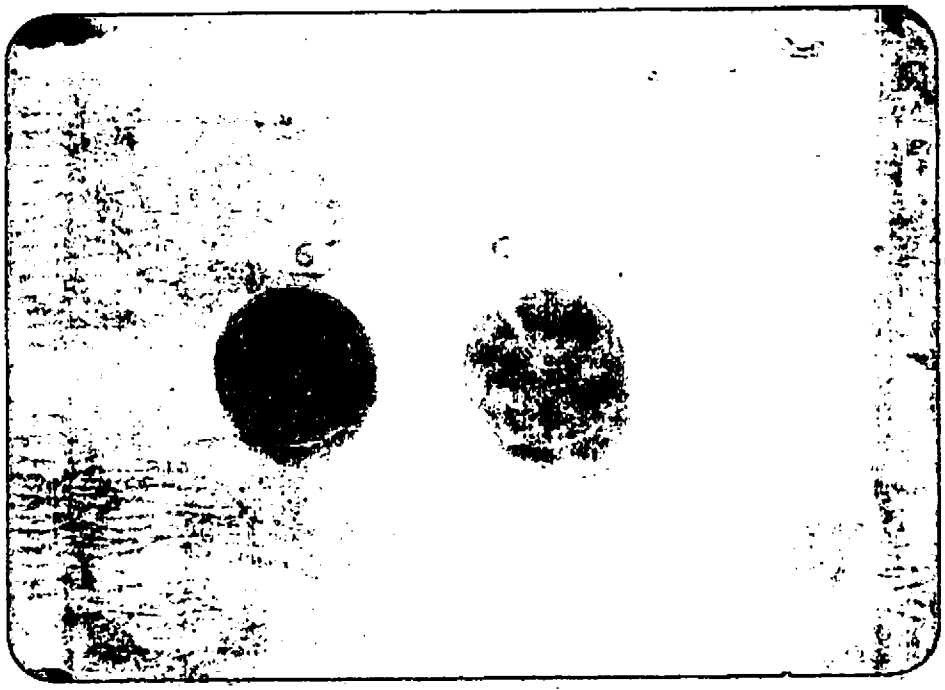
D.

c - control

6 - Infected tubers observed 15 days after storage.

(CONT'D)

C



D

month (Plate II 2) and at harvest, almost no root was present on infected plants (Plate II 3). Uninfected plants observed at harvest had roots and tubers (Plate II 4). Due to extensive decay of roots, samples could not be collected for extraction of nematodes. Therefore nematode population in roots and the root-knot index could not be worked out in the experiment.

3.1.7 Deterioration of Coleus tubers infected by *M. incognita* in storage

For this study tubers having 10-15 galls were selected and kept in storage and observations recorded once in 3 days for a period of 15 days. Uninfected tubers served as control (Plate III).

In the infected tubers, rotting was first noticed on the galls three days after storage. The rotting started as a dark patch on the external surface of the gall (Plate III A 2). When cut open the internal tissues also showed a dark soft patch just below the surface (Plate III B 2). The remaining part of the tuber was free from rot and a marked zone delimiting the healthy and diseased area was noticed (Plate III A 3). On the sixth day the rot was seen extending from the region of the galls to the apparently healthy region of the tuber.

When cut open rotting was seen extended further into the interior region of the tuber covering almost half of the area of the cross section. The tissue which was already rotten on the third day had become softer (Plate III B 3). When observed on the ninth day the rot was seen extended to the whole surface of the tuber (Plate III A 4). In cross section, it was seen that the whole of the internal tissue was also discoloured. Almost half of the tissues in the tuber had turned into a dark brown watery mass with a bad odour (Plate III B 4). Tubers observed on the twelfth day showed that the entire tuber content had turned into a rotten liquid material. The outer peel was intact. When observed after 15 days the peel had ruptured releasing the watery content of the rotten tuber (Plate III D 5). Even in this advanced stage of decay of tubers, rotting of its peel was not noticed.

3.2 Histopathology of *C. parviflorus* inoculated with *M. incognita*

Microtome sections of root of healthy and galled coleus were examined under the microscope. Microphotographs showed that infection by nematodes was in the stelar region.

In the uninfected coleus root, the exodermis, cortex, endodermis, pericycle, xylem and phloem could be observed clearly. All the tissues were arranged in their symmetrical order (Plate III A 1).

One month after inoculation, section of infected root showed mature female nematode and associated egg mass in the region of the cortex. The head of the nematode was seen directed towards the stele. Giant cells, four in number, were observed adjacent to the head of the nematode. The shape of these giant cells was roughly quadrangular. The giant cells were conspicuous since the cytoplasm was granular and more deeply staining. These cells were larger than the surrounding cells. Some cells of the stelar region near the nematode were found to be compressed and distorted. Pericycle and endodermis were not well-defined and could not be differentiated. The number of cells in the stelar region of infected root was higher compared to the uninfected root. Systemmatic alternate arrangement of the xylem and phloem was disturbed but the central cylinder could be distinguished clearly from the cortex.

Sections of normal roots, obtained from control, at two months after the inoculation of M. incognita larvae

Plate IV. Cross sections of healthy and infected roots of C. parviflorus inoculated with M. incognita.

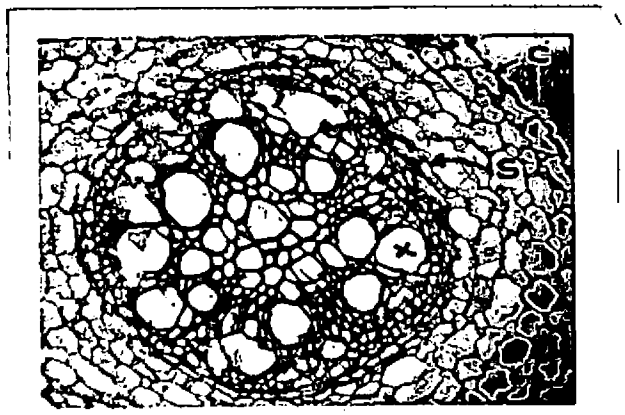
A. Roots observed one month after inoculation.

1. Control

2. Infected root

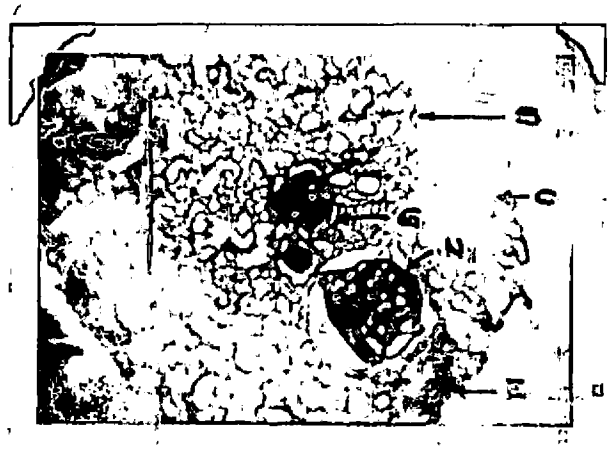
C = Cortex
S = Stele
N = Nematode
G = Giant cell
E = Egg mass
X = Xylem

PLATE. IV. A



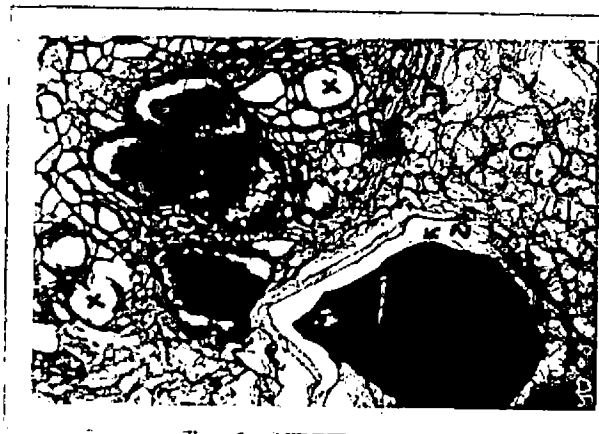
1

25 x



2

25x



2

30x

Plate IV (contd.)

B. Roots observed two months after inoculation.

1. Uninfected root (control)

2. Infected root

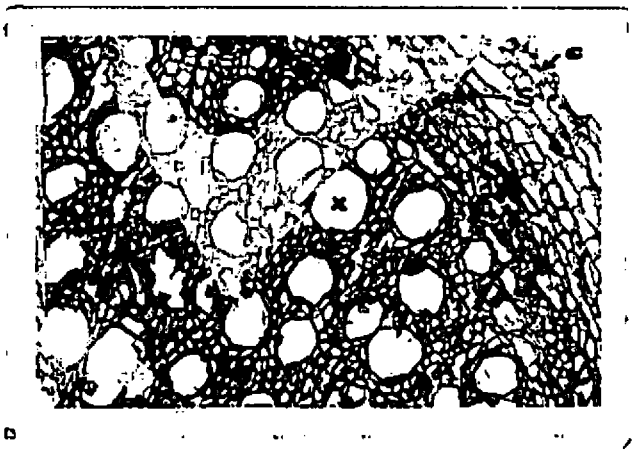
N = Nematode

G = Giant cell

Cy = Cytoplasm

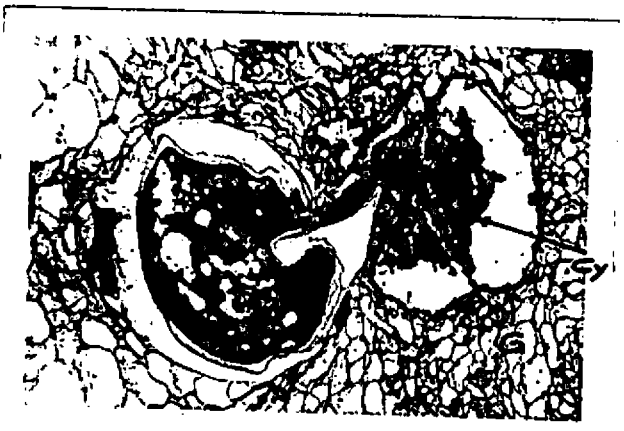
Hp = Hyperplasia

(CONT'D) B



1

30 x



2

30 x

2

30 x

Plate IV (contd.)

C. Roots observed three months after inoculation.

1. Control

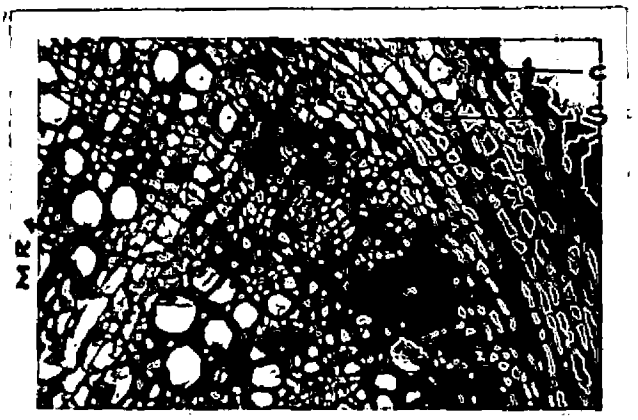
MR = Medullary rays

2. Infected root

Ht = Hypertrophy

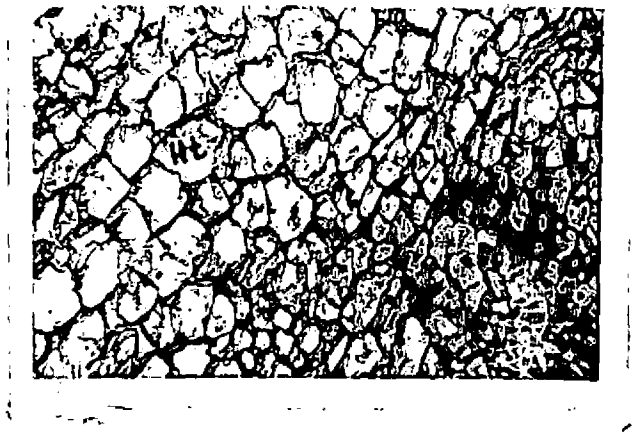
G = Giant cell

(CONT'D) C



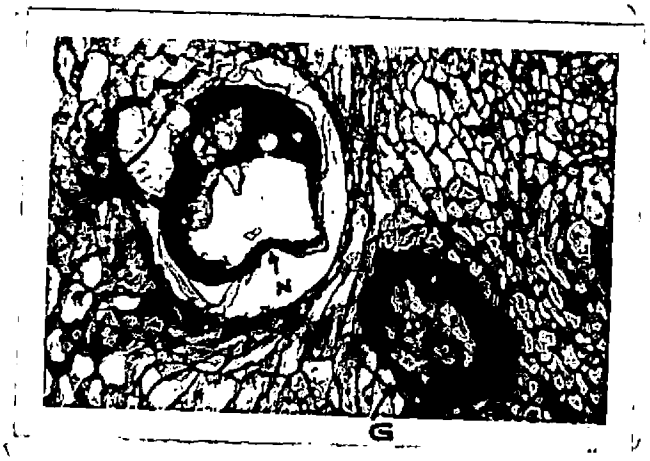
1

30×



2

30×



2

30×

Plate IV (contd.)

D. Roots observed four months after inoculation.

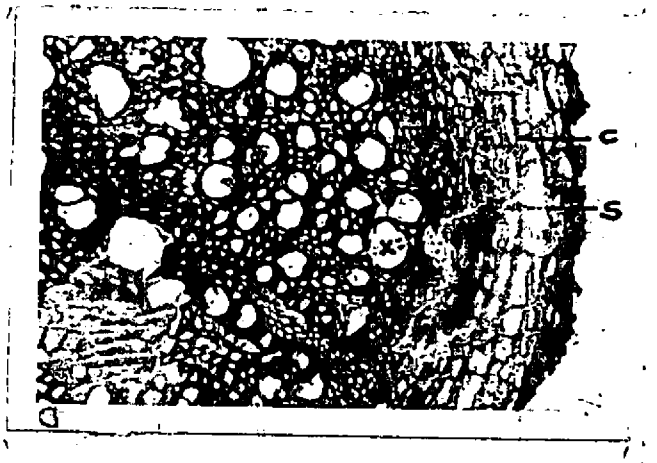
1. Control

2. Infected root

CG = Cavity formed by deterioration of giant cell

Ne = Necrosis

(CONT'D) D



1

30 x.

2

30 x

2

30 x

Plate IV (contd.)

E. Roots observed five months after inoculation.

1. Control

2. Infected root

CN = Cavity formed by the deterioration of the nematodes

Rt = Rotten tissue

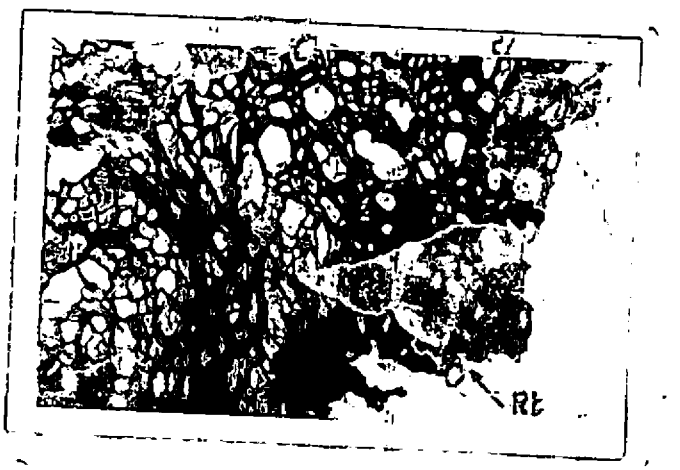
(CONT'D). E

1

30 x

2

30 x



2

30 x

showed very thin cortex consisting of 10-12 layers of rectangular cells and well-defined stele bound by the endodermis and pericycle. In the stele, protoxylem was observed towards the periphery and metaxylem towards the interior. Xylem strands were arranged alternately with phloem. Stele occupied the major portion of the section (Plate IV B 1). In sections of infected roots, many adult females were observed within the tissues. The giant cells were larger than those observed one month after inoculation and fewer in number. Cytoplasm of giant cells were granular and seen aggregated towards the head region of the nematode. The stele was seen completely disrupted without specific arrangement. Endodermis and pericycle were absent. The stele and cortex could not be separately identified. Cortex was greatly modified due to hypertrophy and hyperplasia (Plate IV B 2).

Section of uninfected root collected at the end of three months after inoculation showed the development of medullary rays and secondary xylem. (Plate IV C 1). Sections of galled root observed three months after inoculation showed thick-walled giant cells with dense cytoplasm. Cortex of the nematode-infected root was grossly malformed due to intensive proliferation of cells.

Cortical region occupied the major part of the root sections. Cells of the cortex did not have a regular size, shape or arrangement. Xylem and phloem elements were not distinguishable. Medullary rays were not observed in root sections of infected plants (Plate IV C 2).

Section of uninfected root collected at the fourth month showed a large number of secondary xylem vessels. The stele occupied the major portion of the section with the cortex limited to about ten layers of cells in the periphery (Plate IV D 1). Sections of galled root showed numerous cavities formed by disintegration of giant cells, egg masses and adult females. Necrosis of the root tissue was also observed. Xylem and Phloem elements were found scattered here and there within the root tissue and were not easily distinguishable (Plate IV D 2).

Sections of uninfected roots taken at the fifth month showed intact cortex and stele (Plate IV E 1). All the tissues of galled roots taken five months after inoculation were seen decayed and rotten (Plate IV E 2).

3.3 Field experiment on the control of *M. incognita* infesting *C. parviflorus*

Data relating to the experiment and results of statistical analysis of the same are presented in Tables 4 and 5.

3.3.1 Shoot length

The shoot length of the plants at the time of harvest showed significant variations. The mean shoot length of a plant in control was 40.17 cm while the shoot length of the plants in plots in which burning of dried plant material coupled with application of Carbofuran one month after planting (T_6) was done was 63.7 cm.

T_6 was found to be significantly superior to all other treatments, while T_7, T_4, T_3 and T_5 were on par. Control (T_8) was significantly inferior to all treatments except T_1 which came on par.

3.3.2 Shoot weight

Shoot weights varied significantly in the different treatments. It ranged from 0.978 kg in T_8 to 5.896 kg in T_6. Statistical analysis of the data on shoot weight showed that T_6 was significantly superior to all other treatments. T_7 and T_4 were on par and significantly

Table 4. Effect of different treatments on M. incognita and on the shoot length, shoot weight and yield of C. parviflorus observed in a field experiment

	Treatments	Observations at harvest		
		Shoot length (cm)	Shoot weight (kg)	Yield of marketable tubers/plot of 5 g (kg)
T_1	Burning of dried plant material before planting.	46.63	1.850	2.12
T_2	Carbofuran @ 1 kg a.i./ha at planting (Furadan 3G)	47.90	3.305	2.95
T_3	Phorate @ 1 kg a.i./ha at planting (Thimet 10G)	53.13	3.890	3.68
T_4	Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ha at planting (Furadan 3G)	55.67	4.329	4.96
T_5	Burning of dried plant material before planting + Phorate @ 1 kg.a.i./ha at planting (Thimet 10G)	50.17	3.327	3.78
T_6	Burning of dried plant material before planting + Carbofuran @ 1 kg.a.i./ ha one month after planting (Furadan 3G)	63.70	5.896	9.25
T_7	Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha one month after planting (Thimet 10G)	56.00	4.900	5.78
T_8	Control	40.17	0.973	1.119
	C.D.	7.019	0.746	1.527

superior to treatments T_3, T_5 and T_2. T_1 was inferior to treatments T_2 to T_7 and all treatments were superior to control.

3.3.3 Tuber yield

Significant differences were observed in the yield from the experimental plots. It ranged from 1.119 kg per plot in treatment T_8 to 9.25 kg per plot in T_6. Statistical analysis of the data revealed that T_6 was significantly superior to all other treatments. T_7 and T_4 were on par. T_5 was on par with T_3 and the latter was on par with T_2 and T_1. Treatments T_2, T_1 and T_8 were on par.

3.3.4 Population of *M. incognita* in soil and root of coleus at time of harvest

The population of *M. incognita* in different plots before the commencement of the experiment did not vary significantly. The nematode population observed at harvest in various treatments showed significant differences. Maximum population of nematodes was observed in control (1502.67/100 ml soil). In this plot the population increase was to the tune of 108.31 per cent over the initial population. An increase in population (34.45 per cent) was noticed in T_1 which did not receive any nematicide.

Table 5. Effect of different treatments on the population of M. incognita in soil before and after treatment, the populatinn in roots at harvest and the root-knot index of C. parviflorus observed in the field experiment

Treatments	Nematode population in soil (100 ml) before treatment.	Nematode population in soil (100 ml) at harvest.	Increase in population. (%)	Nematode population in roots (5 g) at harvest.	Root-knot index
T_1 Burning of dried plant material before planting.	901.67	1212.33(34.82)	+34.45	1115.00(33.39)	3.67(1.916)
T_2 Carbofuran @ 1 kg a.i./ha at planting (Furadan 3G)	1361.00	1043.33(32.30)	-23.34	660.00(25.69)	3.33(1.825)
T_3 Phorate @ 1 kg a.i./ha at planting (Thimet 10G)	1281.00	973.00(31.19)	-24.04	540.00(23.24)	3.00(1.732)
T_4 Burning of dried plant material before planting + Carbofurar @ 1 kg a.i./ha at planting (Furadan 3G)	1292.33	830.00(28.81)	-35.78	341.67(18.48)	2.67(1.634)
T_5 Bunring of dried plant material before planting + Phorate @ 1 kg a.i./ha at planting (Thimet 10G)	1290.00	988.00(31.43)	-23.41	650.00(25.49)	3.33(1.825)
T_6 Burning of dried plant material beofre planting + Carbofuran @ 1 kg a.i./ha one month after planting (Furadan 3G)	842.00	282.67(16.81)	-66.43	210.00(14.49)	2.00(1.414)
T_7 Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha one month after planting (Thimet 10G)	861.00	375.67(19.38)	-56.31	286.31(16.93)	2.33(1.520)
T_8 Control	721.67	1502.67(38.76)	+108.31	2216.67(47.08)	4.00(2.000)
C.D.	N.S	12.84		(13.075)	(0.2785)

Figures in parenthesis are $\sqrt{x}$ values.

In all the plots which received nematicide treatment, there was a reduction in population of nematodes. This reduction ranged from 23.34 per cent in T_2 to 66.43 per cent in T_6. Statistical analysis showed that T_6 had the maximum reduction in the population of nematodes, but it was on par with T_7 and T_4. Treatments T_4, T_3, T_5 , T_2, T_1 and T_8 were inferior to the other treatments and on par.

3.3.4 Population of M. incognita in roots

Treatments 1 and 6 which did not receive nematicides had more than 1000 nematodes per 5 g of root. The least number of nematodes (210/5g root) was recorded in T_6. It was on par with all other treatments except T_1 and T_8.

3.3.5 Root knot index

The root-knot index, which is an estimate of the disease caused by root-knot nematode on plants also showed significant variations in the different treatments. Control plot (T_8) showed the maximum root-knot index of 4 which was on par with T_1, T_2, T_5 and T_3. The indices in the above treatments ranged from 3.00 to 3.67. In T_4, T_7 and T_6, the indices were 2.00, 2.33 and 2.67 respectively. Root-knot index recorded in T_6 was the least and it was significantly superior to all other treatments except T_4 and T_7.

3.4 Estimates of nematicides residues in tubers

Samples of tubers collected from different plots did not show nematicide residues at detectable levels.

3.5 The keeping quality of coleus tubers

The data relating to the experiment are presented in Table 6.

Uninfected tubers could be stored without deterioration in quality for a period of 8 weeks and the weight reduction during the period was 42g only from the original weight of 500g. The tubers were fully fit for consumption or marketing even eight weeks after storage.

The percentages of unconsumable tubers by weight compared to uninfected tubers ranged from 0.44 to 5.96 after the first week of storage in the different treatments. While in control where no nematicides were applied, the damage was 17.46 per cent. In tubers treated in hot water, the percentage of unconsumable tubers was 32.11 and 16.51 in infected and uninfected tubers respectively.

The percentage weight of unconsumable tubers was only 1.97 after two weeks of storage in Treatment 5. In other treatments it ranged from 12.54 to 34.51 per cent,

Table 6. Effect of storing tubers harvested from different treatments in the field experiment (500 g each)

Treatments	Percentage of tubers rendered unfit for marketing observed at different periods after storage (weeks) compared to the corresponding weights of healthy tubers.							
	1	2	3	4	5	6	7	8
T_1 Burning of dried plant material before planting.	5.96	34.51	67.54	-	-	-	-	-
T_2 Carbofuran @ 1 kg a.i./ha at planting.	1.07	14.44	21.73	32.63	56.75	67.17	-	-
T_3 Phorate @ 1 kg a.i./ha at planting.	2.44	16.63	41.36	67.32	-	-	-	-
T_4 Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ ha at planting.	4.33	12.54	26.29	66.22	-	-	-	-
T_5 Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha at planting.	0.44	1.97	3.6	12.37	16.19	50.39	70.11	-
T_6 Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ ha one month after planting.	3.78	15.18	32.99	49.30	66.02	-	-	-
T_7 Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha one month after planting.	0.99	13.99	22.90	31.37	65.19	-	-	-
T_8 Control	17.46	39.95	79.36	-	-	-	-	-
T_{10} Infected tubers treated with hot water.	32.11	71.45	-	-	-	-	-	-
T_{11} Uninfected tubers treated with hot water.	16.51	30.31	38.64	64.11	75.63	-	-	-

respectively while in control it was 39.95 per cent. In nematode infected tubers treated in hot water percentage weight of unconsumable tubers was 71.45 after two weeks and in uninfected tubers treated with hot water it was only 30.31 per cent. After 3 weeks of storage more than 25 per cent of the tubers had become unfit for consumption in treatments T_1, T_3, T_4 and T_6. In T_5 only 3.6 per cent of tubers had become unfit for consumption while more than 50 per cent of tubers (67.54 and 79.36 per cent) had become spoiled in T_1 and T_8 respectively.

Percentage of unconsumable tubers was more than 50 in all treatments except Treatments T_2 and T_5 (32.63 per cent and 12.57 per cent respectively) four weeks after storage.

By the fifth week of storage more than 50 per cent of tubers had become unconsumable in all treatments except T_5 which had only 16.19 per cent spoilage. In T_{11} more than 75 per cent of tubers had been spoiled.

In T_5, by the sixth week more than 50 per cent of the tubers had become unfit for consumption. In T_5 by the seventh week 70.11 per cent of the tubers had become unfit for consumption.

Results of hot water trial showed that the keeping quality of uninfected and infected tubers could not be improved by hot water treatment.

DISCUSSION

4. DISCUSSION

4.1 Pathogenicity of *M. incognita* to *C. parviflorus*

The pot culture experiment was carried out for studying the pathogenicity of *M. incognita* to *Coleus parviflorus*. As seen in the table 1, the number of leaves in uninfected plants increased till the third month after planting and there was a gradual decrease in number of leaves till harvest. Similar reduction of leaves due to *M. incognita* infestation had been reported on betelvine (Jagdale *et al.*, 1985) and ginger (Sudha and Sundararaju, 1985). Wilting, yellowing of leaves and other symptoms of premature ageing have been reported in potato infected by *M. incognita* (Pushkarnath and Roy Choudhary, 1958).

There was reduction in shoot length in the nematode infected plants as seen in table 2. In control the shoot length showed a gradual increase from the first month after planting till the fifth month; while in plants inoculated with nematodes the shoot length increased from the first month to the fourth month and then decreased significantly at harvest.

Significant reduction was also noticed in the shoot weight of root-knot nematode infected Coleus plants in comparison with control (Table 3).

The tuber yield which ranged from 76.24 to 15.44 g in plants inoculated with M. incognita at different levels of population differed significantly from the yield in control (198.92 g per pot). There was an yield reduction of 92 per cent in plants inoculated with 10,000 nematode larvae per pot.

Thus the growth of the plants and the yield obtained were seen negatively related to the increase in the initial inoculation levels of the nematode larvae. The infected plants remained wilted during day time inspite of the repeated irrigation given, while the control plants did not show such symptoms. The root showed intense galling by the third month and rotting was noticed during the fourth month after inoculation of larvae. At harvest roots were seen completely damaged in plots inoculated with 10,000 larvae.

The deleterious effect of root-knot nematodes on coleus was reported earlier (Sathyarajan et al., 1966; Hrishi and Mohankumar, 1976). Similar observations have been made on snapdragon (Tarjan, 1952), red clover (Chapman, 1960), cotton (Brodie and Cooper, 1964),

bhindi (Rajagopalan, 1972), tomatoes (Gunasekharan et al., 1972), jute (Phukan et al., 1983), patchouli (Prasad et al., 1984), papaya (Babatola, 1985), betelvine (Jagdale et al., 1985) and Phaseolus vulgaris (Melakeberhan et al., 1985).

The reduction in yield in the plants receiving even the minimum inoculum of 100 nematodes per plant, in comparison with control, showed the high susceptibility of the crop to the nematode infection. The threshold level of M. incognita on coleus could not be established in the experiment, and it may be far below the level of 100 larvae per pot.

Sharma and Swarup (1968) found that a population level of 1000 larvae of M. incognita per 400 g soil was required to reduce the root length, shoot length and shoot weights of tomato.

Significant reduction in the growth of jute was observed with M. incognita at 10,000 juveniles per kg of soil only (Phukan et al., 1983). Prasad et al., (1984) observed 27.9 per cent reduction in root and top weights with initial inoculum levels of 1000 nematodes per plant over a period of 6 months in patchouli. But Sudha and Sundararaju (1985) found that an initial inoculum level of 100 M. incognita per plant was the marginal threshold level for damaging the growth of ginger observed six months after inoculation.

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Root system of coleus infected by M. incognita was found extensively damaged with heavy galling and subsequent decay. The decayed roots fail to absorb water and nutrients required by the plant for photosynthesis and tuber production. Similar observations were made on potato (Nirula, 1961; Thorne, 1961) and other vegetables (Franklin, 1964). Mjuge and Estey (1978) observed that the impairment of the photosynthetic processes affects the normal growth and yield of plants infected by M. incognita.

Golden and Vangundy (1975) observed that the actual rotting of galled roots was caused by other soil pathogens which enter the plants through wounds caused by M. incognita. The giant cells caused by the nematodes were observed as favourable substrate for fungal infection.

The data presented in para 3.1.7 showed that the infected tubers when stored started rotting with a black discolouration of tissues around the female nematode within the tuber. This discolouration extended towards the interior of the tuber and the tissues became soft to touch, and later turned to a dark watery liquid with a bad odour. These symptoms suggest the involvement of some fungal and/ or bacterial organisms as a secondary cause for the decay of tubers. Golden and Vangundy (1975)

observed that in tomato root, decay by fungus occurred 4–5 weeks after nematode infection. Fungus penetrated either directly or through ruptures in the root and colonised giant cells which provided favourable substrate for fungus growth.

The suppression of plant growth did not show a linear relationship with different levels of nematode inoculated (Tables 1, 2 and 3). The growth suppression become evident from the third month after inoculation only. The nematode population would not have reached injurious level till then. During the early periods (upto the third month) the effect of the root damage might not have manifested due to the active growth of the shoot so that root could absorb sufficient quantities of water and nutrients. Maximum suppression of vegetative growth of coleus was recorded at the third month after inoculation of M. incognita.

Seinhorst (1961) also observed that the increase in nematode population after a critical level did not cause further increase in crop loss.

The tuber initiation of the crop commences 80 days after transplanting (Vijayakumar and Shanmughavelu, 1984). Subsequently the food materials produced by the aerial parts are mostly used for the development of tubers rather than for the production of leaves and shoots. This, combined with the deprivation of nutrients by the parasitic nematodes for its own growth and reproduction and the obstruction in the transportation of the food materials at the above critical stage of the crop growth produce the conspicuous aerial symptoms.

4.2 Histopathology of Coleus roots infested by M. incognita

Histopathology of Coleus roots infected by M. incognita was studied for the first time. The nematode and the egg masses were situated within the cortex of the infected roots and the head of the nematode was seen embedded in the stele. Similar observation was made earlier on oats by Ferver and Crittenden (1958). Giant cells caused by M. incognita on coleus was found to be four in number. The giant cells observed in pepper roots ranged from 4 to 6 (Jacob, 1977). In contrast to the usual irregular polygonal shape of giant cells in vegetables (Kozhokaru, 1985) the giant cells in coleus were roughly quadrangular in shape (Plate IV A 2). As observed in soyabeans (Crittenden, 1958; Dropkin and Nelson, 1960), the giant

cells in coleus were much larger than the surrounding cells with thickened cell wall and deeply staining cytoplasm. The giant cells in coleus arose from the cells of the stelar region. Birchfield, (1964) and Taylor (1976) observed that the giant cells in Echinocloa colonum and roselle arose from the phloem tissue. But Jacob (1977) found that in pepper the xylem vessels were preferred by M. incognita.

Two months after the inoculation of M. incognita, enlargement and reduction in the number of giant cells were observed (Plate IV B 2). It might be due to the dissolution of cell walls and merging of the adjacent plant cells. Similar observations were made on soyabean (Dropkin and Nelson 1960) and in E. colonum (Birchfield 1964). Veech (1970) and Agrawal et al. (1985) had observed that such abnormal increase in size and number of cortical cells might be due to the increased levels of IAA, phenolic compounds, other growth regulators and enzyme activity in the plant tissue. The aggregation of granular cytoplasm towards the head region of the nematode was also observed in the section. The observations were in agreement with earlier observations in oats (Ferver and Crittenden, 1958), roselle (Taylor, 1976) and vegetables (Kozhokaru, 1985).

In the sections of the root taken four months after inoculation, large cavities were noticed within the root tissue (Plate IV D 2). As observed by Krusberg and Nielson (1958), these cavities were caused by the deterioration of giant cells and the death of adult females within the tissue. Golden and Vangundy (1975) suggested that the deterioration of giant cells along with the entry of soil borne pathogens through the wounds caused by the larvae promoted root rot.

Tissue differentiation was not observed even after five months in coleus roots infected by M. incognita (Plate IV E2). Dropkin and Nelson (1960) also observed that M. incognita affected the differentiating tissues of the roots in soyabean crop.

4.3 Field experiment on the control of M. incognita infesting coleus

The results presented in para 3.3.1 to 3.3.4 and Figure 1 showed that the yield of marketable tubers obtained from plots in which dried plant material was burnt prior to planting (T_1) did not show significant differences from that of control. The treatments were on par in terms of growth parameters also. The nematode population in the treatment at harvest in the soil and roots, as well as the root-knot indices came on par with those of the control.

Arjunlal *et al*. (1983) and Venkitesan (1984) had observed that burning of plant materials in the field preceding ploughing and land preparation reduced the nematode population due to the lethal action of heat or by reducing the food availability in the field for free-living males of the root-knot nematodes.

The treatment in the present experiment also appears to have given positive effect since the soil heating when combined with insecticide treatments enhanced the effect of the latter. Probably the reduction brought about by the treatment at planting did not persist upto the tuber formation which normally occurs around 80 days after planting. The nematodes which escaped the lethal action of heat might have multiplied at a faster rate due to the lack of competition for space and food and that would have brought the population level on par with that of control at harvest.

The application of carbofuran or phorate at planting increased the yield significantly over control. With reference to the length and weight of shoot also, the treatments were found significantly superior to control. But with reference to the population of nematodes in soil and the root-knot indices at harvest, the treatments did not show significant differences from control. Carbofuran and Phorate had been reported to be effective against root-knot nematodes in in vegetables by many of the earlier workers (Johnson and

Fig.1. Control of *M. incognita* on coleus with chemical and cultural methods.

T_1. Burning of dried plant material before planting.

T_2. Carbofuran @ 1 kg a.i./ha at planting.

T_3. Phorate @ 1 kg a.i./ha at planting.

T_4. Burning of dried plant material before planting + Carbofuran @ 1 kg .a.i. /ha at planting.

T_5. Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha at planting.

T_6. Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ha one month after planting.

T_7. Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha one month after planting.

T_8. Control.

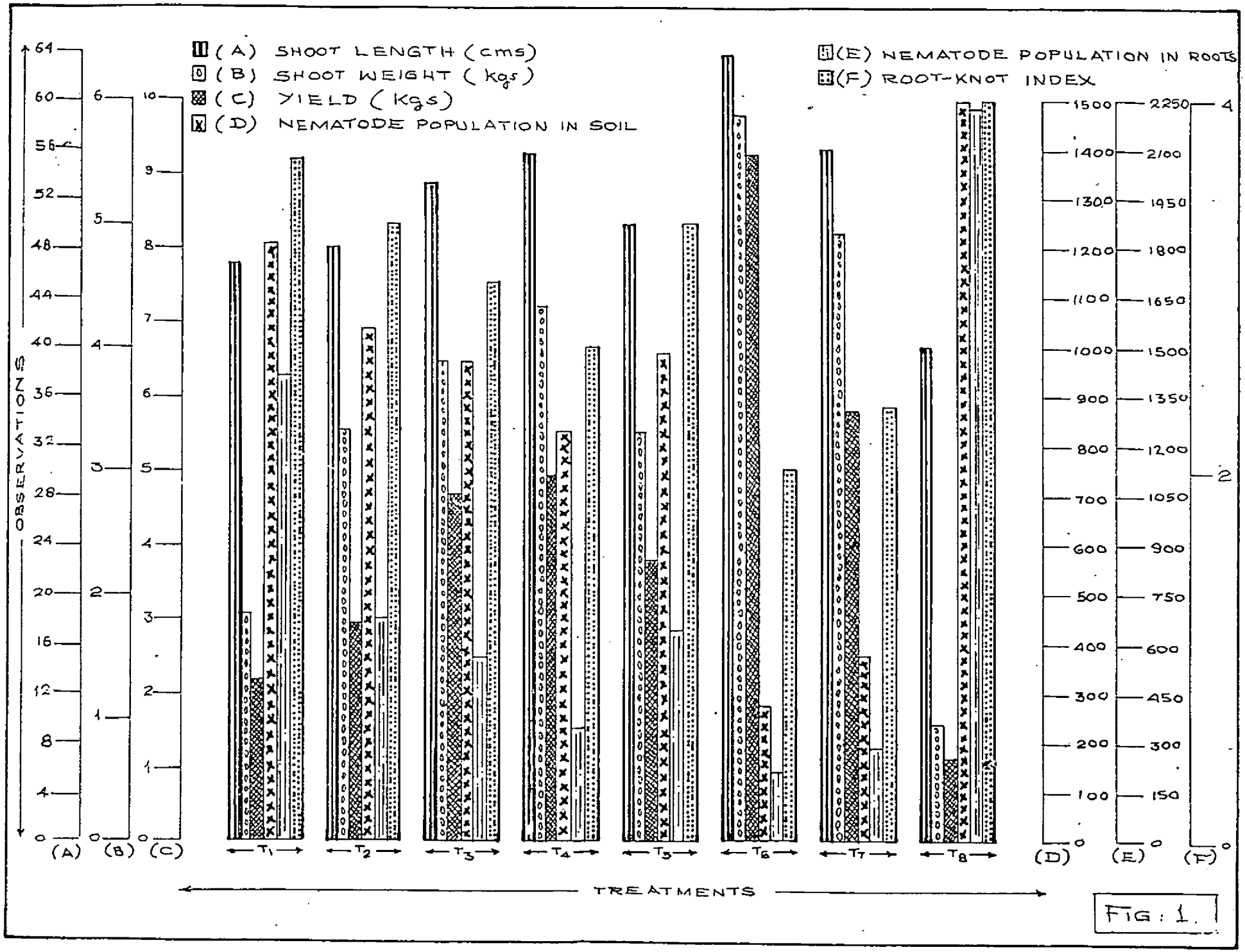
(A) SHOOT LENGTH (cms)
(B) SHOOT WEIGHT (kgs)
(C) YIELD (kgs)
(D) NEMATODE POPULATION IN SOIL
(E) NEMATODE POPULATION IN ROOTS
(F) ROOT-KNOT INDEX
OBSERVATIONS
(A)
(B)
(C)
(D)
(E)
(F)
T1
T2
T3
T4
T5
T6
T7
T8
TREATMENTS

FIG: 1.

Cairns, 1972; Sivakumar et al., 1973; Johnson et al., 1974; Weingartner et al., 1974; Rodriguez-Kabana et al., 1976; Taylor and Sasser, 1978; Anon, 1983; Jagdale et al., 1985 & 1986).

Obviously the persistent effect of the chemical applied at the time of planting was not adequate to protect the crop long enough. The pathogenicity studies (para 3.2) had shown that the symptoms of attack by root-knot nematode on coleus manifests three months after planting. Proper protection at the above susceptible stage might not be possible with one application of the insecticide at planting.

The application of nematicides one month after the planting combined with the burning of plant material in the field prior to the planting were better than the other treatments. The differences were statistically significant on shoot length, shoot weight, yield and root-knot indices. But the differences in the population in soil and in roots between the two types of treatments were not statistically significant. The application of nematicides one month after planting will kill the first generation of nematodes getting built up in the field from the survivals after the lethal effect of the heat treatments, since one life cycle of the nematode is normally completed within a month. The

above reduction in the soil population of nematodes will affect the yield reduction through less root damage.

Between the two nematicides tried, Carbofuran was found to be significantly superior to Phorate for the control of M. incognita (Jagdale et al., 1986).

From the result it could be concluded that burning of dried plant material in the field prior to planting coupled with the application of carbofuran @ 1 kg a.i./ha one month after planting would be the most effective method of controlling M. incognita on coleus.

No residues of insecticides could be detected in tubers collected from the different treatments at harvest. Obviously the application of phorate or carbofuran upto one month after planting of coleus can be considered safe. Bacon (1960) observed that phorate residues was not detectable in potato tubers 74 or 91 days after treatment. Kathpal (1983) did not find any residue of phorate in tubers of potato harvested 90 days after treatment.

In the pathogenicity studies, the marketable tubers were found rotting when kept in storage after harvest. Realising that such deterioration might normally occur since the produce is often disposed of by the farmers after storage, the keeping quality of infected and uninfected tubers was also studied.

Samples drawn from various treatments in the field experiment were stored and the extent of tuber deterioration was studied upto a period of eight weeks. Hot water treatment has been recommended for the killing of nematodes in infested tubers (Acosta and Ayala, 1976) and hence that also was included as a treatment in the above experiment. The data presented in para 3.5 showed that the tubers were heavily damaged in store.

Results in Table 6 and Figure 2 show that the assessment of the treatment effects in a field experiment for the control of M. incognita on coleus should include the subsequent loss of tubers in storage also. The tubers collected from plots treated with phorate at planting preceded by the heating of soil (T_5) showed least deterioration in storage, and it was closely followed by the treatment with carbofuran at the time of planting (T_2). The tubers subjected to hot water treatment deteriorated even faster than those collected from control plots. The tubers when exposed to hot water may kill the nematodes within. As observed in para 4.1 the rotting of tubers might have been caused by the secondary invasion of micro-organisms through the injuries caused by nematode entry and the heat treatment is not likely to close such entry points for the micro-organisms. Moreover, the softening of the tuber content due to hot water treatment may render them more suitable for the multiplication of micro-organisms.

Fig.2. Deterioration of tubers harvest from the different treatments in the control experiments and kept in store.

T_1. Burning of dried plant material before planting.

T_2. Carbofuran @ 1 kg a.i./ha at planting.

T_3. Phorate @ 1 kg a.i./ha at planting.

T_4. Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ha at planting.

T_5. Burning of dried plant material before planting + Phorate @ 1 kg a.i./ha at planting.

T_6. Burning of dried plant material before planting + Carbofuran @ 1 kg a.i./ha one month after planting.

T_7. Burning of dried plant material before planting + Phorate @ 1 kg a.i./ ha one month after planting.

T_8. Control.

T_9. Uninfected tubers

T_{10}. Infected tubers treated with hot water.

T_{11}. Uninfected tubers treated with hot water.

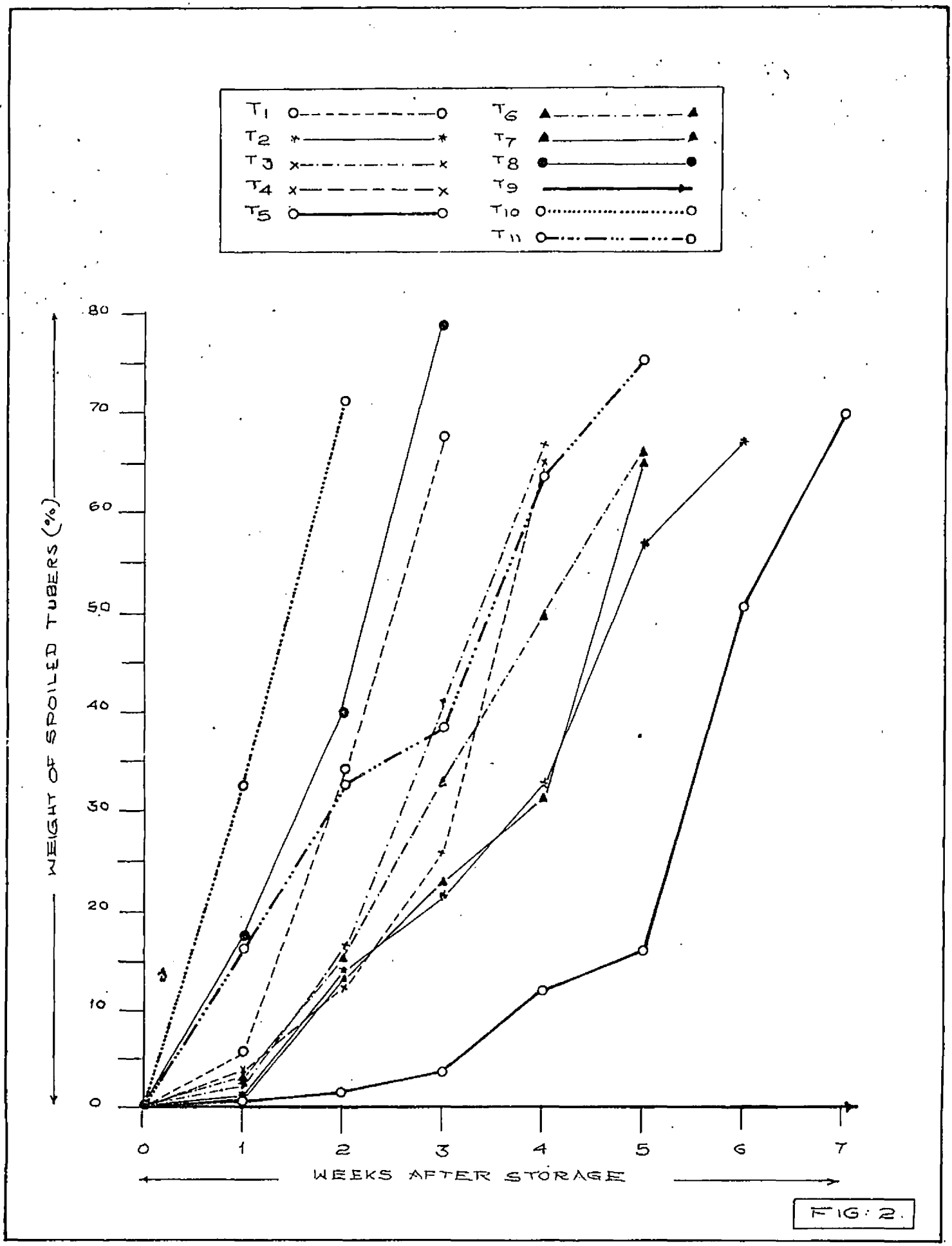
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
WEIGHT OF SPOILED TUBERS (%)
0
10
20
30
40
50
60
70
80
0
1
2
3
4
5
6
7
WEEKS AFTER STORAGE

FIG: 2.

The relative efficacy of the different treatments in the field experiment are not seen reflected in the keeping quality of the samples kept in storage. The erratic trend in the result might be due to the lack of foo-proof techniques in drawing samples of the yield from the different treatments in the experiment. There was significant deterioration of tubers in all the samples from the third week onwards while the uninfected tubers did not deteriorate even after the storage for eight weeks. For reducing this storage loss, more effective treatment of the tubers prior to storage may have to be evolved or the timing of nematicide treatment will have to be postponed to a later date. In the case of sweet potato, normally harvested at 100th day after planting, treatment done between 50 and 180 days after planting was reported to be effective for controlling the incidence of the sweet potato weevil(Palaniswami, 1988). In Coleus the tuberisation is around 80th day after planting and the application of carbofuran one month after planting would not have given protection of the tubers completely. Infected tubers became rotten in store. The results thus indicate that the application of nematicide at a later date (around 80th day) may be more effective in controlling the pest.

SUMMARY

5. SUMMARY

A pot culture experiment and a field experiment were carried out in the Department of Entomology, College of Agriculture, Vellayani, Trivandrum during 1986 to study the crop loss caused by Meloidogyne incognita infesting Coleus parviflorus and to evolve effective methods for the control of the nematode.

In the pot culture experiment, the pathogenicity of Meloidogyne incognita on Coleus parviflorus was studied at five levels of nematode population. The growth characters of the host plant like the number of leaves, shoot length, fresh shoot weight and weight of tubers were recorded. The number of leaves per plant in all cases increased upto the third month and then decreased till the harvest of the crop. In all nematode inoculated plants, the decrease in number of leaves was more pronounced than in control. Same trend was observed in the case of shoot length also. While the shoot length of plants in control increased gradually till harvest, the shoot length of nematode inoculated plants increased till the fourth month only and then there was significant reduction.

A significant reduction was noticed in the shoot weights of nematode infected coleus plants at the time of harvest compared to shoot weight of uninfected plants.

Yield obtained from uninfected plants was significantly higher than those of inoculated plants. The yield and other growth parameters showed a negative correlation with the population of nematodes. The tuber weight showed significant correlation with the number of leaves, and shoot weight of coleus while shoot length did not influence the tuber yield significantly.

Profuse galling was observed on roots two months after planting. Rotting was noted by the third month, and at harvest, no healthy root was present on infected plants. Uninfected plants had both normal roots and tubers at the time of harvest.

Tubers having 10 to 15 galls were stored for 15 days and the changes were recorded once in three days. In the first observation dark patches were noted on the surface of the tubers around the galls. Later these patches extended below the surface and caused the rotting of the internal tissues. On the sixth day, the rotting was seen spreading to the inner region of the tubers. The rotten tissue became softer. By the ninth day, the rotting extended over the entire internal tissues. Half of the tissues had by this time, turned into a dark brown watery liquid with a bad odour. By the fifteenth day, the rotting and liquefaction of the tissues had affected the whole tubers and the peel

had ruptured releasing the foul smelling liquid contents. Even in this advanced stage of decaying the peel was not rotten. The nature of damage indicated the possible involvement of secondary organisms like fungi and bacteria.

In histopathological studies of the roots, it was observed that the infection affected the stelar region of the root. One month after inoculation with nematodes, roughly quadrangular giant cells (four in number) were observed. There was cell proliferation also in the region. Alternate arrangement of the xylem and phloem was disturbed though the central cylinder could be clearly distinguished.

In two months the giant cells became larger and fewer in number and the cytoplasm of the cells became granular and aggregated towards the head region of the nematode. The stele was completely disrupted. The cortex was greatly enlarged due to hypertrophy and hyperplasia of cells. The medullary rays and secondary xylem seen in normal roots in the third month was not distinct in infected roots. Cells of the cortex were grossly malformed and did not have definite size, shape or arrangement. At the fourth month the infected roots showed numerous cavities formed by the disintegration of giant cells, egg masses and adult females. Necrosis of tissue was also observed. At the fifth month all the tissues of infected roots were found decayed.

In the field experiment, two nematicides and a cultural method were tried, alone and in combination, for the control of *M. incognita*. The application of nematicides one month after planting combined with the burning of plant material in the field prior to planting were effective treatments. Between the two nematicides tried, carbofuran @ 1 kg a.i./ha was significantly superior to phorate.

No residue of carbofuran or phorate could be detected in tubers at harvest.

Samples of 500 g tubers taken from each treatment in the experiment kept in store showed that the least deterioration was observed in samples collected from plots treated with phorate following the burning of dried plant material. By the eighth week, over 75 per cent of the tubers (by weight) became unfit for consumption or marketing.

Hot water treatment included in the experiment showed that the method was ineffective in reducing the damage.

The loss caused by the nematode in field and store showed that the treatments included in the experiments were not adequate for the control of the pest and the application of the nematicide at a later stage of the crop (at the commencement of tuber formation) may be necessary.

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* Original not seen

APPENDIX I

Summary of analysis of variance table relating to percentage decrease in the number of leaves and coleus in pot experiment.

Source	df	Mean squares	
		Number of leaves	Shoot length
Treatments (A)	4	463.03	647.61
Error 1	20	201.29	297.84
Months after inoculation (B)	4	11090.91**	277.13*
A x B	16	45.88	28.84
Error 2	80	317.99	86.04

* Significant at 5% level

** Significant at 1% level

APPENDIX II

Summary of analysis of variance table relating to shoot weight, tuber yield and soil population of nematodes in pot experiment

Source	df	Mean squares		
		Shoot weight	Tuber yield	Soil population
Treatments	5	8806.31**	22360.52*	1447903**
Error	24	1804.01	6151.73	14676.58

* Significant at 5% level

** Significant at 1% level

APPENDIX III

Summary of analysis of variance table relating to pre-treatment soil population of nematodes of Field Experiment

Source	df	MSS
Replication	2	226.04
Treatments	7	326.13
Error	14	183.49

APPENDIX IV

Summary of analysis of Covariance table relating to shoot length, shoot weight and tuber yield per plot in field experiment.

Source	df	Mean squares		
		Shoot length	Shoot weight	Tuber yield
Replication	2	11.31 **	0.0805 *	0.1095 **
Treatments	7	152.47	7.58	18.92
Error	14	16.06	0.1814	0.76

* Significant at 5% level

** Significant at 1% level

APPENDIX V

Summary of analysis of covariance table relating to soil population root population of nematodes and root-knot index in the field experiment.

Source	df	Mean squares		
		Soil population.	Root population.	Root-knot index
Replication	2	32521	61791	0.542
Treatments	7	495126.3 *	1294072 *	1.375 **
Error	14	178160.3	134697.7	0.304

* Significant at 5% level

** Significant at 1% level

APPENDIX VI

Weight of tubers rendered unconsumable at weekly intervals (g)

Treatment	1	2	3	4	5	6	7	8
T_1	29.65	170.15	326.90	-	-	-	-	-
T_2	5.30	71.20	105.15	156.30	267.85	311.00	-	-
T_3	12.14	82.00	200.20	322.50	-	-	-	-
T_4	21.50	61.80	127.25	317.20	-	-	-	-
T_5	2.20	9.70	17.40	59.25	76.40	233.30	321.12	-
T_6	18.75	74.82	159.70	236.15	311.60	-	-	-
T_7	4.90	69.00	110.80	150.25	307.72	-	-	-
T_8	86.70	196.95	384.10	-	-	-	-	-
T_{10}	159.50	315.55	256.00	127.90	65.00	-	-	-
T_{11}	82.0	149.45	187.00	307.10	357.00	-	-	-

Weight of uninfected tubers at weekly intervals in storage

496.70	493.00	484.00	479.00	472.00	463.00	458.00	458.00

CROP LOSS CAUSED BY ROOT-KNOT NEMATODE (*Meloidogyne incognita* Kofoid) INFESTING *Coleus parviflorus* AND ITS CONTROL

BY

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ABSTRACT OF A
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ABSTRACT

Crop loss caused by the root-knot nematode infesting Coleus parviflorus and its control was studied in a pot culture experiment and a field experiment in the Department of Entomology, College of Agriculture, Vellayani, Trivandrum, during August to December, 1986.

The number of leaves, shoot length, fresh shoot weight and weight of tubers obtained were less in inoculated plants than in control. The yield and growth parameters showed a negative correlation with the population of nematodes. A high positive correlation was noticed between the tuber weight and the number of leaves and shoot weight of coleus. The shoot length did not influence the tuber yield significantly.

Roots showed profuse galling by the second month. Rotting of roots was observed by the third month, and at harvest, no healthy root was present in infected plants.

Tubers when stored developed dark patches on the surface of galls and these spread inwards covering the whole of the internal tissues within nine days, and by the twelfth day the internal contents had turned into a dark brown watery liquid with a bad odour. The peel of the tubers did not show rotting. The nature of rot indicated the involvement of secondary organisms like bacterial and fungi.

Histopathological studies showed that the nematodes were lodged in the cortex with head in the stelar region of the root. One month after inoculation, quadrangular giant cells (four in number) were observed in roots. There was cell proliferation in the stelar region of the infected roots. Alternate arrangement of the xylem and phloem was disturbed, though the central cylinder and cortex could be distinguished.

Two months after inoculation the giant cells were seen larger and fewer in number. The cytoplasm of giant cells became granular and aggregated towards the head region of the nematode. Stele could not be easily distinguished from the cortex. The cortex was greatly enlarged due to hypertrophy and hyperplasia of cells. The development of medullary rays and secondary xylem noticed in the normal root could not be distinguished in infected roots. Infected roots showed numerous cavities formed by the disintegration of giant cells, egg masses and adult females by end of the fourth month. Necrosis of tissue was also observed. The xylem and phloem elements were not distinguishable. At five months after inoculation the tissues of the roots were completely rotten.

In the field experiment application of nematicides one month after planting preceded by the burning of plant material in the field prior to planting was found better

than the other treatments. Carbofuran @ 1 kg a.i./ha was more effective than phorate for the control of the nematode.

No residue of carbofuran or phorate could be detected in tubers at harvest.

The rotting of tubers obtained from different plots when kept in store indicated the inefficacy of the treatments for giving protection from the nematode and the need for a second application of the nematicide at tuber setting. Hot water treatments of the tubers prior to storage did not reduce the damage.