PATHOGENICITY OF RENIFORM NEMATODE (ROTYLENCHULUS RENIFORMIS LINFORD AND OLIVEIRA, 1940) ON GINGER (ZINGIBER OFFICINALE ROSE)

By

ELIZABETH JOHN

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Pathogenicity of Reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1940) on ginger (*Zingiber officinale* Rose)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or society.

Vellanikkara,
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ELIZABETH JOHN

CERTIFICATE

Certified that this thesis entitled "Pathogenicity of Reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1940) on ginger (*Zingiber officinale* Rose)" is a record of research work done independently by Smt. ELIZABETH JOHN under my guidance and supervision, and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellanikkara,
10 -- 4--1989

Dr. T.S. Venkitesan
(Chairman, Advisory Committe
Professor (Nematology)
College of Horticulture

CERTIFICATE

We the undersigned, members of the Advisory Committee of Smt. ELIZABETH JOHN, a candidate for the degree of Master of Science in Agriculture, agree that the thesis entitled "Pathogenicity of Reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1940) on ginger (*Zingiber officinale* Rose)" may be submitted by Smt. Elizabeth John in partial fulfilment of the requirements for the degree.

Dr. T.S. Venkitesan
Professor (Nematology)
(Chairman)

Dr. K.M. Rajan
Professor (Plant pathology)
(Member)

27.5.85

Dr. C. C. Abraham
Associate Dean
(Member)

Sri. V.K.G. Unnithan
Associate Professor (Agrl. Statistics)
(Member)

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10-4-1989

ELIZABETH JOHN

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INTRODUCTION

Ginger (Zingiber officianale) an economically important spice is considered to be a native of tropical Asia. The use of ginger in India and China has been known from very ancient times. The country's production is about 67,000 tonnes from an area of 34,000 hectares which is nearly half of world's production (Venkataraman, 1982) and 10-15 por cent of the production is exported to other countries. Kerala contributes 40 per cent of India's production. In Kerala it is cultivated in an area of 12,976 ha with an annual production of 34,388 tonnes. Ginger contributes about 9.8 per cent of the export earnings from spices to our country.

Among the various constraints to realisation of the production potential of ginger varieties and cultivars, the incidence of several diseases, nematodes and pests are considered to be the most serious. The reniform nematode Rotylenchulus reniformis is associated with ginger (Swarup, Nath and Sethi 1967). Huang (1966) reported that Meloidogyne incognita infects rhizomes as well as fibrous and fleshy roots of ginger in Hawaii causing heavy crop loss due to rhizome infection.

Both the reniform nematode R. reniformis and root-knot nematode M. incognita are widely present on the ginger crop grown all over Kerala in diverse soil types (Sundarraju et al., 1979; Charles and Kurian, 1979). The pathogenicity of the reniform nematode R. reniformis and its relative pathogenic effect in conjunction with the root-knot nematode M. incognita on ginger has not been studied so far. Hence the present study was undertaken with a view to understand the nature and degree of pathogenic effect caused by reniform nematode on ginger individually and to determine if any pathogenic interaction existed between the two species R. reniformis and M. incognita when they occur concurrently on the same crop.

REVIEW OF LITERATURE

The reniform nematode was first described from the Hawaian islands as an obligate parasite on cowpea roots by Linford and Oliveira (1940).

In India the first record of the nematode was by Prasad (1960). The widespread occurrence of this nematode in South India was reported by Seshadri and Sivakumar (1963). Khan (1976) revealed the widespread occurrence of the nematode in several types of crops.

1. Damage by reniform nematodes to plants

The first pathogenicity work of this organism was that of Ayala (1962) on six different plant species. Two varieties of pineapple and pigeonpea, tobacco, coffee and tomato crops were found to be susceptable to the nematode. Sugarcane was found to be completely resistant. Length of stem, number of leaves, number of side branches, weight of stem and roots were low in the infected coffee plants. The stems were narrower and the leaves yellowish. All the infected plants had weaker root system.

Twenty four varieties of cotton were tested for infection and reproduction by reniform nematode and all

the varieties were susceptable to varying degrees (Birchfield and Brister 1963).

In the case of castor, Sivakumar and Seshadri (1971) reported poor growth, shedding of leaves, early flowering, poor quality seed and reduction in quality and quantity of oil.

Pathogenic reactions of cowpea to the reniform nematode at varying levels of inoculation was reported by Villanueva and Castillo (1976). Cowpea seedlings inoculated with 1000, 2500, 7500, 10,000 numbers of R. reniformis extracted from soil, on examination after two months, numerous females were feeding on the roots but there was no sigh of necrosis. There were no significant reduction in top weights. But root weights, at the three highest inoculum levels and yields of dried seeds at the two highest inoculum levels were reduced. Nematode multiplication rate was greatest with an inoculum of 2500 nematodes and least at the two highest inoculum levels.

According to Hameed et al. (1977), as the inoculum level of R. reniformis on two months old onion seedlings increased, nematode reproduction rate decreased. Reduction in plant growth was possitively correlated with the increase

in inoculum. The cortical cells of infected roots became hypertrophic and acted as feeding sites.

Rebois (1978) noticed development of reniform nematode on the potato roots but not on tubers. Tuber yield was adequate but the dry weights of plant tops and roots were significantly reduced by its parasitism.

Pathogenicity of R. reniformis on brinjal was studied in pots by Singh and Khera (1979). Symptoms like chlorosis, stunted growth, curling of central crown leaves, promature fall of flowers and sparsely developed roots were observed during the experiment.

Gupta and Yadav (1980) reported that 100 larvae or more/pot of R. reniformis gave significant reduction in height and fresh shoot and root weights of Vigna unguiculata. The yield of snap beans (Phaseolus vulgaris) was negatively correlated with soil populations of R. reniformis Mc Sorley (1980).

Gupta and Yadav (1982) studied the pathogenicity of green gram variety to R. reniformis. Vigna radiata cv H-70-16 was inoculated with 10, 100, 1000, 10,000 R. reniformis per pot. Plant height, fresh shoot weight, fresh root weight and number of nodules per plant were all reduced

with increasing inoculum density. Reproduction was maximum with 100 inoculum density.

R. reniformis parasitises soybean and reproduces rapidly on soybean roots. It also infects Rhizobium root nodules (Mereedith et al. 1983).

The effect and reproduction of R. reniformis on 10 sweet potato selections were observed by Clark and Wright (1983). Reproduction was not related to the effect of the nematode on yield. Goldrush supported least reproduction but was most affected by the nematode, while centennial supported most reproduction but was least affected. Yields were significantly reduced when initial population were high. The thresh hold level of damage by R. reniformis in cotton under green house conditions was 1000 young females/1000 cc of soil (Sud et al. 1984)

According to Misra and Padhi (1985), level of 1000 nematodes/pot caused 35.0, 30.9, 54.2 and 35.9 per cent reductions in respect of shoot and root length, shoot and root dry weights respectively over control when applied to the root zone of 10 day old plants.

2. Damage to plants by root-knot nematodes

Nagakura (1930) was the first worker to report Meloidogyne sp on ginger. M. incognita has been reported

to infect the rhizome, fibrous and fleshy roots of ginger in Hawai causing heavy crop loss due to the rhizome infection (Huang 1966).

Several workers have reported the damage and crop loss caused by M. incognita. They are blackening and drooping off of growing tips followed by wilting in betelvine (Dhande and Sulaiman 1961) reduction in shoot, root and plant weight of egg plant, chillie and tomato (Chidambaranathan and Rangaswami 1965), stunting, bunching of petioles, narrowing and yellowing of leaves in banana (Claudio and Davide 1968), galling and stunting of root tips of coconut palms (Pizarrow 1969), 75 per cent and 48 per cent reduction in foliage and root weight respectively (Mayol and Bergeson 1970) and 85 per cent yield loss in tomato (Ducusin and Davide 1972), severe wilt symptoms of betelvine (Mammen 1974), drying and shedding of leaves and poor pod formation in Cicer arietinum (Reddy 1975), decrease in dry plant weight, stem diameter and plant height in cocoa (Theobroma cocao) (Sharma and Maia 1975). Yield losses of 91, 46, and 27 por centage in the case of tomato, brinjal, okra, respectively were reported by Bhatte and Jain (1979).

Root weight of Solanun melongena seedlings was

significantly less than controls when inoculated with 10 larvae of M. incognita per Kg soil, whereas nematode multiplication rate and gall number was highest with 100 larvae per Kg soil (Dhavan and Sethi 1978).

Charles (1978) reported that root-knot nematode M. incognita is an important pest of ginger in Kerala and it has a wide distribution in the state and cause up to 46.4 per cent loss in the yield.

Pathogenicity of M. incognita to edible ginger was reported by (Cheng and Tu 1979). Second stage larvae of M. incognita infect the ginger (Zingiber officinale) rhizome through the axil of the leaf sheath at the shoot apex, resulting in the formation of extensive internal lesions. No visible galls were formed on the rhizome. Larvae infect the root system through the root tips of the fibrous and fresh roots aggregating in the vascular tissue, giant cells and galls were formed.

In a glasshhouse experiment conducted by Reddy (1981), purple variety of passion fruit Passiflora edulis inoculated with M. incognita showed significant reduction in plant height and shoot weight at 1000 inoculum level. Maximum root-knot index was observed with 10,000 juveniles per plant. M. incognita had no significant effect on plant growth of yellow variety.

Influence of initial population densities of *M. incognita* on three chilli cultivars was investigated by Lindsey and Clayshulte (1982). All the three cultivars were susceptible to *M. incognita* and reacted simultaneously to different initial populations. Severe stunting and yield suppressions occurred at all initial *M. incognita* densities tested ranging from 385 to 4200 eggs and larvae per 50 cm^3 soil.

According to Bora and Phukan (1982) there was a progressive decrease in the growth of jute plant as the inoculum level of *M. incognita* increased. A population density of 1000 larvae/3.5 Kg soil significantly reduced the plant growth. The multiplication rate of the nematode was higher with initial density of ten larvae per pot. Older seedlings of jute were less prone to attack by *M. incognita* as compared to younger and tender seedlings.

The pathogenic effect of root-knot nematode *M. incognita* to Patchouli (*Pogostemon cablin*) were investigated by Prasad and Reddy (1984) at inoculum levels of 100, 1000 and 10,000 nematodes per plant under pot culture. Significant reduction in root and top weights were recorded. Sudha Sukumaran (1986) reported that *M. incognita*

inoculated plants exhibited stunted growth with reduced vigour. A reduction of 74.1 per cent of rhizome weight was obtained at an initial inoculum of 10,000 nematode/plant over a period of six months.

3. Root-knot and reniform nematodes, their association, pathogenicity and interaction studies

Sundarraju et al. (1979) examined soil and root samples collected from the root zone of ginger in Kerala and recorded both M. incognita and R. reniformis associated with ginger. Charles and Kurian (1979) also observed both these nematodes associated with ginger. Reniform and root-knot nematodes are reported as the predominant genera of phyto parasitic nematodes in the rhizosphere of grapewine (Rao and Tami 1979).

On tomato R. reniformis occuring alone causes greater damage than M. javanica alone or both species together in equal densities (Rao and Prasad 1971). Three months after inoculation, separately or together, significant damage to the underground as well as aerial parts of plants was noticed. The population of R. reniformis was found to have increased significantly, 60 days after the treatment.

The percentage penetration of Jupiter soybean

seedlings by M. incognita or R. reniformis was reported to be significantly reduced with increasing inoculum level ten to twenty days after inoculation (Singh, 1976). In mixed species infection significant reduction was found at the higher inoculum levels twenty days after inoculation. M. incognita and R. reniformis singly and in combination significantly reduced top and root dry weights of soybean, ten weeks after inoculation. Simultaneous inoculation with M. incognita inhibited increase of reniformis but M. incognita was little affected by the presence of R. reniformis.

Six thousand juveniles of M. incognita and R. reniformis were inoculated alone or in combination by Ferraz and Sharma (1979) on to two month old rooted cuttings of Piper nigrum grown in pots. Fifteen months after inoculation, assessment of root and shoot growth indicated that M. incognita alone or in combination with R. reniformis was highly pathogenic, whereas R. reniformis alone slightly promoted growth. Plants inoculated with a combination of both showed better growth than plants infected only with M. incognita. The number of M. incognita root galls and egg masses per gram root were significantly reduced when

both species were inoculated together; compared with the numbers in plants inoculated with M. incognita, alone.

The pathogenicity studies of M. incognita and R. reniformis on moth bean by Mishra and Gaur (1981) indicate that both the nematodes are independently pathogenic to moth bean and can cause significant growth reduction at an initial population below one infective individual per g of soil.

Relationship between the different population densities of M. incognita and R. reniformis individually and concomitantly along with growth of black gram were studied by Mishra and Gaur (1981) under pot culture conditions. Both species caused significant growth reduction at the level of one infective individual per cc. of soil. In concomitant inoculations, the extent of growth reduction was relatively less than individual effects. The fresh shoot weight was more sensitive to injury than shoot length.

Thomas and Clark (1982) studied interaction of M. incognita and R. reniformis on sweat potato. Results indicate that a competitive interaction exist with each species capable of inhibiting the other and becoming the

dominant population. The nematodes had no apparent effect on yield at the inoculum densities used, either alone or mixed. Both populations increased cracking of sweet potatoes, but mixed population did not differ in incidence of cracking from either R. reniformis or M. incognita alone.

MATERIALS AND METHODS

The nature and degree of pathogenic effect caused by reniform nematode (Rotylenchulus reniformis) individually and in combination with root-knot nematode (Meloidogyne incognita), at varying inoculum levels on ginger (Zingiber officinale) was studied by conducting two sets of pot culture experiments at the College of Horticulture, Vellanikkara, Trichur.

1. Preparation of soil mixture

The soil mixture for the pot experiments was prepared by mixing red soil, sand and well decomposed farmyard manure in the ratio 2:1:1.

2. Sterilization of pot mixture

The pot mixture was denematized by applying five per cent formalin under cover of polythene sheets. After three days, the soil was stirred well and aerated. Again it was covered with polythene sheet and aerated after three days. Representative samples were drawn from the denematized pot soil mixture and following the method of Cobb's decanting and sieving technique; presence of nematode was checked and found no nematodes in the soil mixture. The denematised

soil mixture was filled in clay pots of 30 Kg capacity.

3. Pure culture of test organisms

Pure culture of R. reniformis was maintained on cowpea plants raised in denematized soil by inoculating with infective pre-adult females obtained from the nucleus culture maintained in the nematology laboratory in the Department of Entomology.

Pure culture of M. incognita was maintained on Coleus plants raised in denematized soil by inoculating surface sterilized egg mass collected from the roots of plants maintained as nucleus culture. Sub-culturing was done periodically to ensure the availability of sufficient larval population for both experiments.

4. Raising of test plant

Original seed rhizome of ginger cv Thodupuzha local was obtained from the nucleus stock maintained in the Department of Plantation Crops, College of Horticulture, Vellanikkara. From this seed Rhizome, bits of uniform size weighing 15 g were selected. These seed rhizomes were planted in pots at a depth of four to five cm and watered and the pots were kept in partial shade. After about a month when the ginger rhizome sprouts emerged a few sprouts

were sacrificed and the number grown was observed. The seed rhizomes put forth three above ground sprouts and four to six number of roots after 45 days of planting.

5. Preparing nematode inoculum and inoculation

Inoculum of the test organism for both the experiments were prepared in the following way. Egg masses from the roots of pure culture plants were collected and transferred to sterile water in cavity blocks and incubated. The hatched out larvae were collected in a 250 ml beaker. The number of larvae per ml of suspension was determined by counting the population from three aliquotes and striking the average per ml of suspension.

6. Treatments

The following treatments were adopted:-

6.a. Pathogenicity test with Rotylenchulus reniformis

T_0	No nematode
T_1	50 Nos per plant (Pre-adult females)
T_2	100 Nos per plant "
T_3	200 Nos per plant "
T_4	500 Nos per plant "
T_5	1000 Nos per plant "
T_6	5000 Nos per plant "

6.b. Interaction on relative pathogenicity of Rotylenchulus reniformis with Meloidogyne incognita

T_0 No nematode

T_1 1000 Nos R. reniformis alone/plant

T_2 1000 Nos M. incognita alone/plant

T_3 Mixture of 500 R. reniformis+500 M. incognita/plant

T_4 Mixture of 1000 R. reniformis+1000 M. incognita/plant

Inoculation in both cases was done by boring five holes in each pot (except control) at 5-10 cm depths away from the stem. The required quantity of suspension was pipetted out and poured equally into the holes and plugged immediately with sterile soil. Pots were watered regularly to keep the soil just moist. No chemical fungicides or pesticides were added to the pots. The experimental pots were maintained in partial shade. The experiment was concluded after 120 days of inoculation.

The treatments were replicated four times.

7. Observations

In both the experiments, observations on tiller productions, leaf production, height of tillers, length of leaves and width of leaves were taken before inoculation.

All the above observations were recorded at monthly intervals 30, 60, 90 and 120 days after inoculation. The increase in growth parameters after 30, 60, 90 and 120 days were recorded by finding the difference between the observations recorded before and after inoculations.

7.1. Number of tillers

Observations on the number of tillers before inoculation and four observations after inoculations were taken at an interval of 30 days. Even very small sprouts that emerged were counted. The difference before and after inoculation was recorded for each replication as additional tiller production after 30, 60, 90 and 120 days of inoculation.

7.2. Number of leaves

Number of leaves on all the tillers were counted and the average number of leaves per tiller recorded for each replication before inoculation and at intervals of 30, 60,90 and 120 days of inoculation. Increase in leaf production 30 days after inoculation was worked out by subtracting the number of leaves before inoculation from the number of leaves after inoculation. Similarly the additional number of leaves produced after 60,90 and

120 days of inoculation was worked out by finding the difference in the number of leaves before and after inoculation.

7.3. Height of tillers

Height of all the tillers were taken from the base of the tiller ie. from the ground level to the base of the last leaf and the average height of tillers found for each replication before inoculation and at 30, 60, 90 and 120 days after inoculation. Difference in height before and after inoculation at different intervals was worked out.

7.4. Length of middle leaf

Length of middle leaf of all the tillers were measured from the base of the leaf to the leaf tip and the average length found for each replication before inoculation and at 30, 60, 90 and 120 days after inoculation. Increase in length of middle leaf after inoculation at different intervals was recorded by finding the difference before and after inoculation.

7.5. Width of middle leaf

Width of middle leaf of all tillers were taken from the middle of the leaf lamina which gave the highest width.

The average width for each replication before inoculation and at 30, 60, 90 and 120 days after inoculation and increase in width before and after inoculation at different intervals worked out.

7.6. Fresh weight of tops

After 120 days of inoculation the tops of plants were cut off and their fresh weights were taken. Immediately, the tops were cut into small pieces and put in paper cover and kept in air oven running constantly at 80°C. The plant materials were weighed till a constant dry weight was observed.

7.7. Fresh weight of root

Each experimental pot was tilted over a large polythene sheet and after dislodging the adhering soil, the root system and rhizome were put in a bucket full of water. The roots were blotted and fresh weight of roots were recorded.

7.8. Fresh weight of rhizome

As in the case of roots, the rhizomes were separated, washed thoroughly, blotted and fresh weight taken.

7.9. Estimation of population of nematodes in soil

The soil from each pot was thoroughly mixed after removal of the root system with rhizome and a representative sample of 100 g weighed out and processed for extracting the nematodes. Nematodes were extracted, following the modified method of Cobb's decanting and sieving technique (Christie and Perry 1951). Nematode suspension was made up to a constant volume by adding sterile water. Number of nematodes, in an aliquot of one ml pipetted out from the above suspension, were counted under a stereoscopic binocular microscope. Three such aliquotes were counted and average population per ml and in the made up constant volume worked out. The total nematode population in the whole pot was also computed and estimated.

7.10. Estimation of population of nematodes in roots

Five g of cleaned representative root samples were weighed out from each experimental pot, and stained in acid fuchsin (Southey, 1970) and kept overnight in lactophenol for destaining. The roots were then teased and examined under binocular stereo-microscope and the number of developing stages and adult females of both

reniform and root-knot nematodes were counted. The nematode population in the whole roots were also then computed and estimated.

RESULTS

The results obtained from the two pot culture experiments namely, pathogenicity of reniform nematode on ginger and relative pathogenic interaction of the root-knot and reniform nematodes on ginger are presented in this chapter.

1. Pathogenicity of Rotylenchulus reniformis on ginger

1.1. Height of tillers

Observations recorded on height of tillers (Table 1) did not show significant variation between the different

Table 1. Effect of different inoculum levels of reniform nematode on the difference in height of tillers at different intervals after inoculation.

	Difference in height of tillers (Mean of four replications in cm)			
Treatment	30 days	60 days	90 days	120 days
T_0	-2.96	-3.31	7.05	7.70
T_1	-3.45	-4.23	5.13	6.50
T_2	-3.54	-3.63	5.17	5.55
T_3	1.78	-3.65	3.38	2.92
T_4	0.88	-4.38	2.97	1.89
T_5	-5.16	-5.03	2.29	1.26
T_6	-5.73	-4.40	2.12	2.63
CD (5%)	Ns	Ns	Ns	Ns

treatments. A general trend of decrease in the height of tillers was observed with increase in inoculum level. The

percentage reduction range from 15.58 to 83.64 at 120 days of inoculation. The higher percentage of decrease of 83.64 was observed in T_5 after 120 days.

Table 2. Effect of different inoculum levels of reniform nematode on the difference in tiller production at different intervals after inoculation.

	Difference in the number of tillers (Mean of four replications)			
Treatment	30 days	60 days	90 days	120 days
T_o	1.50	4.00	5.50	7.80
T_1	1.50	2.50	3.90	5.30
T_2	1.50	2.80	4.00	5.50
T_3	1.30	3.30	4.30	5.80
T_4	1.25	3.50	4.30	6.00
T_5	1.50	3.80	4.50	6.30
T_6	1.80	3.80	5.80	8.00
CD (5%)	Ns	1.40	Ns	2.30

1.2. Tiller production

The number of tillers produced by the plants under different treatments after 30, 60, 90 and 120 days of inoculation of the nematode are presented in Table 2. Except in the treatments T_6, the number of tillers in all other treatments produced are less than that of the control (T_o). The data indicated significant difference in reduction in tiller production only in the case of T_1 compared to T_o after 60 and 120 days.

1.3. Number of leaves

The data recorded are presented in Table. 3. The increase in the number of leaves produced by the plants 90 and 120 days after inoculation showed significant reduction in

Table 3. Effect of different inoculum levels of reniform nematode on difference in leaf production at different intervals after inoculation

	Difference in the number of leaves (Mean of four replications)			
Treatment	30 days	60 days	90 days	120 days
T_0	3	7	9	10
T_1	1	5	7	8
T_2	2	5	7	8
T_3	3	5	7	8
T_4	4	4	6	7
T_5	2	4	5	7
T_6	1	4	5	6
CD (5%)	Ns	Ns	2.57	2.90

treatments T_4, T_5 and T_6, when compared with control. In all other treatments eventhough the plants did show areduction in the number of leaves, they were statistically insignificant.

The percentage of reduction in leaf production in treatments T_4, T_5 and T_6 works out to 33.33, 44.44, and 44.44 after 90 days and 30.00, 30.00 and 40.00 after 120 days respectively compared to T_0.

1.4. Length of leaf

The data on the increase in leaf length at 30, 60, 90 and 120 days are furnished in Table 4. The increase in inoculation levels show a significant reduction in the length.

Table 4. Effect of different inoculum levels of reniform nematode on difference in length of leaves at different intervals after inoculation

	Difference in the length of leaves (Mean of four replications in cm)			
Treatment	30 days	60 days	90 days	120 days
T_0	2.40	2.58	3.32	5.60
T_1	-0.50	-0.20	0.57	0.94
T_2	-0.75	-1.36	-1.19	0.32
T_3	-0.94	-1.46	-1.30	-0.05
T_4	-0.19	-1.67	-1.24	-0.35
T_5	-1.00	-2.09	-1.90	-0.60
T_6	-1.60	-2.40	-1.80	-1.15
CD(5%)	Ns	Ns	5.08	6.37

of leaves in T_5 and T_6 at 90 days of nematode inoculation compared to T_0. After 120 days, T_6 was significantly differen compared to T_0. The percentage of reduction in the length in the above treatment works out to 157, 155 after 90 days for T_5 and T_6 and 120.5 after 120 days for T_6 compared to T_0.

1.5. Width of leaves

Observations were recorded to assess whether the

pathogenic effect is reflected on the width of leaves. Data were collected at 30, 60, 90 and 120 days of inoculation and presented in Table 5. The data show a general tendency in reduction of the width of the leaves at 30, 60, 90 and

Table 5. Effect of different inoculum levels of reniform nematode on difference in width of leaves at different intervals after inoculation.

Treatment	Difference in width of leaf (Mean of 4 replications in cm)			
	30 days	60 days	90 days	120 days
T_o	0.18	-0.07	-0.23	-0.13
T_1	-0.28	-0.11	-0.28	-0.34
T_2	-0.16	-0.20	-0.33	-0.36
T_3	-0.20	-0.26	-0.35	-0.39
T_4	-0.21	-0.32	-0.36	-0.41
T_5	-0.34	-0.36	-0.43	-0.47
T_6	-0.51	-0.53	-0.43	-0.47
CD (5%)	0.47	Ns	Ns	Ns

120 days after inoculation. Treatments T_5, T_6 differed significantly with control T_o after 30 days.

1.6. Leaf area

The data recorded on the leaf area at 30, 60, 90 and 120 days are presented in Table 6. The data show a significant variation in reduction of leaf area between the different treatments only in the case of observations

Table 6. Effect of different inoculum levels of reniform nematode on difference in leaf area at different levels after inoculation.

Treatments	Difference in leaf area (Mean of 4 replications in Sq cm) 30 days	60 days	90 days	120 days
T_o	7.78	5.23	7.65	8.73
T_1	-2.05	-4.70	-5.35	-1.67
T_2	-3.41	-5.19	-5.83	-2.03
T_3	-4.60	-5.89	-5.71	-5.17
T_4	-5.20	-6.15	-6.46	-5.27
T_5	-5.20	-6.76	-7.88	-5.50
T_6	-6.96	-8.47	-8.39	-11.06
CD (5%)	Ns	Ns	Ns	19.11

120 days after nematode inoculation. This variation is found between the treatments T_6 and T_o. The percentage of reduction in leaf area in T_6 is 226 compared to T_o.

1.7. Top weight (fresh and dry)

The data recorded as fresh and dry top weight of plants under different treatments are presented in Table 7. Though the observations show a decreasing trend in both fresh and dry weight of tops, with the increase in inoculum level, statistically they are not significant. The maximum fresh and dry top weight was recorded in plants under T_o and

Table 7. Effects of different inoculum levels of reniform nematode on top weight.(fresh and dry) after 120 days of inoculation.

Treatment	Top weight (Mean of 4 replications in g) fresh	dry
T_o	73.75	12.50
T_1	67.5	11.00
T_2	64.25	10.00
T_3	64.00	9.75
T_4	53.25	9.25
T_5	51.50	8.75
T_6	49.25	8.25
CD (5%)	Ns	Ns

the least was recorded in plants under T_6.

1.8. Rhizome weight and root weight

The data collected on the fresh weight of rhizomes and roots at 120 days after inoculation are presented in Table 8.

a) Rhizome weight

The maximum rhizome weight was recorded in plants under T_o and the minimum was recorded in T_6. Eventhough there is a clear decreasing trend in the rhizome weight in plants under different treatments, with the increase in inoculum level, the treatment did not show significant difference statistically.

Table 8. Effect of different inoculum levels of reniform nematode on rhizome weight and root weight after 120 days of inoculation.

Treatment	Rhizome weight	Root weight
(Mean of 4 replications in g)		
T_0	139.75	59.25
T_1	120.00	51.00
T_2	113.50	47.25
T_3	101.25	46.25
T_4	98.75	45.25
T_5	98.50	43.25
T_6	92.75	42.75
CD (5%)	Ns	Ns

b) Root weight

The highest and the least fresh root weights were observed in plants under treatments T_0 and T_6 respectively. Though the data indicate a decreasing tendency in root weight of plants under different treatments, they were found statistically insignificant.

1.9. Final nematode population

The observations on the final soil, root and total nematode population at the end of 120 days of inoculation in the pots are presented in Table 9.

a) Nematode population in soil

The nematode population in soil was least in T_1 and highest in T_6 among the inoculated pots. The soil

Table 9. Effect of different inoculum levels of reniform nematode on final population after 120 days of inoculation

Treatment	per pot		Total	Multiplication factor
	Soil	Root		
T_0	0	0	0	0
T_1	67350	676	68026	1360
T_2	73125	1098	74223	742
T_3	75600	1178	76778	384
T_4	92175	1457	93632	187
T_5	95025	1842	96867	97.80
T_6	101475	2427	103902	20.70
CD (5%)	2787.50	713.25	-	-

population in different pots show significant difference between the treatments. The population in treatments T_2 and T_3 were on par, whereas in all the other treatments they differed significantly showing an increase in population with the increase in the initial inoculum level.

b) Root population

The data recorded on the root population separately are given in Table 9. The lowest and the highest population were observed in T_1 and T_6 respectively. In

the case of root population also the different treatments recorded an increasing trend in the order of increase in the initial inoculum level. However the root population were statistically on par in the case of treatments T_1. T_2, T_3 and T_3, T_4, T_5 and in the case of T_4, T_5 and T_6. T_6 differed significantly from T_1, T_2, T_3 and T_4, T_5 from T_1 and T_2, T_4 from T_1.

c) Total population

The observations on the final total population computed are presented (Table 9) which shows an increasing trend in the case of soil and root population. The multiplication factor of the nematode was found maximum in T_1 and least in T_6 indicating 1360 times and 20.7 times respectively.

2. Interaction between Rotylenchulus reniformis and Meloidogyne incognita on ginger.

As in the case of pathogenecity studies, an experiment was laid out to study the effect of individual and combined population of R. reniformis and M. incognita at four inoculum densities on ginger, and the results are reported here. All the parameters indicated in the first experiment were observed and recorded in this test also.

2.1. Height of tillers

The observations recorded on the height of tillers of

Table 10. Effect of individual and combined inoculations of root-knot and reniform nematode on difference in height of tillers at different intervals after inoculations.

	Height of tillers (Mean of 4 replications in cm)			
Treatments	30 days	30 days	30 days	120 days
T_0	8.18	-3.38	7.22	7.25
T_1	-5.74	-5.01	2.90	4.33
T_2	-4.88	-5.87	2.68	-2.67
T_3	-3.02	-4.61	4.23	5.69
T_4	-3.56	-4.41	4.63	5.37
CD, (5%)	13.87	Ns	Ns	Ns

plants under different treatments at 30,60, 90 and 120 days of inoculation are furnished in Table 10. The height of

tillers under T_1 was seen significantly reduced after 30 days of inoculation when compared with control. After 120 days control recorded the maximum height and T_2 the least height of tillers, even though they were statistically insignificant.

2.2. Tiller production

Number of tillers produced by the plants under different treatments after 30, 60, 90 and 120 days of inoculation are presented in Table 11. After 30 days treatments T_2, T_3 and T_4 were on par and differed significantly from control. After 120 days, inoculation

Table 11. Effect of individual and combined inoculations of root-knot and reniform nematode on difference in tiller production at different intervals after inoculation.

Treatments	Number of tillers (Mean of 4 replications)			
	30 days	60 days	90 days	120 days
T_o	2	4	6	8
T_1	2	3	4	6
T_2	1	3	4	4
T_3	1	4	5	6
T_4	1	4	4	5
CD (5%)	0.91	Ns	Ns	3.3

with M. incognita alone (T_2) differed significantly from control (T_o).

Table 12. Effect of individual and combined inoculations of root-knot and reniform nematode on difference in number of leaves at different intervals after inoculation.

	Number of leaves (Mean of 4 replications)			
Treatments	30 days	60 days	90 days	120 days
T_o	3	5	8	10
T_1	1	3	5	6
T_2	1	3	55	7
T_3	1	3	6	8
T_4	1	3	6	7
CD (5%)	Ns	Ns	2.48	Ns

2.3. Leaf production

The observations recorded on leaf productions are presented in Table 12. The number of leaves produced by the plants did not show any significant difference between treatments after 30, 60 and 120 days of nematode inoculation. However the observation on the leaf numbers after 90 days in (T_1) and (T_2) showed homogenity and differed significantly from the plants under check (T_o).

2.4. Length of leaf

The data on length of leaf are presented in Table 13.

Table 13. Effect of individual and combined inoculations of root-knot and reniform nematode on difference in the length of leaves at different intervals after inoculation.

	Difference in length (Mean of 4 replications in cm)			
Treatments	30 days	60 days	90 days	120 days
T_o	1.27	2.58	3.32	5.60
T_1	-1.00	-2.09	-1.90	-0.60
T_2	-1.81	-1.65	-2.87	-1.75
T_3	1.25	-0.28	2.44	2.81
T_4	1.84	-0.51	1.15	2.66
CD (5%)	Ns	4.88	4.76	5.60

The length of leaf showed significant difference in plants after 60, 90 and 120 days of nematode inoculation. T_1 differed significantly from control after 60 days. After 90 days treatments T_1 and T_2 were on par, the reduction in length being -1.9 and -2.87 respectively and differed significantly from check (T_o with 3.32).

Similarly after 120 days of nematode inoculation T_1 and T_2 recorded reduced leaf length of -0.60 and -1.75 respectively which differed from control with length 5.60. The decrease in length of leaf in T_2 was more than T_1 after 90 and 120 days of inoculation.

2.5. Width of middle leaf

The data recorded on the width of leaf are furnished in

Table 14. Effect of individual and combined inoculations of root-knot and reniform nematode on difference in width of middle leaves at different intervals after inoculation

	Width of leaf (Mean of 4 replications in cm)			
Treatments	30 days	60 days	90 days	120 days
T_o	0.18	0.02	-0.23	-0.13
T_1	-0.34	-0.36	-0.41	-0.42
T_2	-0.22	-0.16	-0.23	-0.38
T_3	-0.15	-0.02	-0.08	-0.27
T_4	-0.18	-0.05	-0.04	-0.28
CD (5%)	0.37	Ns	Ns	Ns

Table 14. The leaf width showed significant difference in plants inoculated with the nematodes individually in plants under T_1 and T_2 over the plants in check (T_o) after 30 days. All treatments did not show significant variation on width of middle leaf at other periods.

2.6. Leaf area

The observations recorded on the leaf area are presented in Table 15. The plants under treatment inoculated with M. incognita alone (T_2) was found with reduced leaf area compared to control after 30 days. After 90 days and 120 days the leaf area of the plants under both treatments with R.reniformis and M. incognita alone (T_1 and T_2) were reduced and were on par and differed significantly

Table 15. Effect of individual and combined inoculations of root-knot and reniform nematode on the difference in leaf area at different intervals after inoculation.

	Leaf area (Mean of 4 replications in sq. cm)			
Treatments	30 days	60 days	90 days	120 days
T_o	7.78	5.23	7.65	8.73
T_1	-5.15	-6.76	-7.88	-5.50
T_2	-5.98	-7.16	-7.64	-7.09
T_3	-1.13	-0.99	-2.53	-4.64
T_4	-2.82	-1.30	-3.23	-4.87
CD (5%)	13.19	Ns	13.65	13.21

from control.

2.7. Top weight

a) Fresh

The data are presented in table 16. The plants under check (T_o) were found superior to all other treatments with maximum top weight of 73 g. All other treatments were on par with weights recorded 51.5, 41.1, 44.6, 35.4 for T_1, T_2, $T_3$and T_4 respectively. All of them differed significantly from control. In the case of individual inoculation M. incognita caused more reduction than R.reniformis. In combined inoculation the reduction in top weight increased with increase in inoculum level.

b) Dry

The data are presented in Table 16. The plants under

Table 16. Effect of individual and combined inoculations of root-knot and reniform nematode on top weight (fresh and dry)after 120 days of inoculation

	Mean of 4 replications in g	
Treatments	Top weight fresh	Top weight dry
T_o	73.00	11.50
T_1	51.50	8.75
T_2	45.13	7.00
T_3	44.63	6.75
T_4	35.40	5.00
CD (5%)	18.13	2.57

control recorded the maximum top dry weight with 11.5 which was superior when compared to all other treatments. The least dry weight was recorded in plants with combined inoculations of nematode (T_4) with weight 5 g which differed significantly from T_1 and T_o with weights 8.75g and 11.5 g respectively. Treatments T_1, T_2 and T_3 were homogenous with weights 8.75g, 7.00g, 6.75g respectively and they differed significantly from control.

2.8. Rhizome and root weight

a) Rhizome weight

The data recorded on rhizome and root weights after 120 days of nematode inoculation under different treatments are presented in Table 17. Minimum rhizome weight of 61.25g

Table 17. Effect of individual and combined inoculations of root-knot and reniform nematode on rhizome and root weight after 120 days of inoculation.

	Mean of 4 replications in g	
Treatments	Rhizome weight	Root weight
T_o	140.25	59.25
T_1	100.75	43.00
T_2	80.00	36.25
T_3	69.25	32.75
T_4	61.25	29.00
CD (5%)	48.06	14.96

was recorded in plnts under treatmant T_4 and maximum of 140.25g was in plants under check (T_o). The plants under treatments T_2, T3, and T_4were on par and recorded an average rhizome weight of 80.00g, 69.25g respectively and differed significantly from control. The rhizome weight recorded in plants under treatment T_1 did not differ significantly from control. More reduction was observed in plants inoculated with both the nematodes. The rhizome weight of plants under $T_1$was on par with that of plants under treatment T_o.

b) Root weight

Data are presented in Table 17. The plants under check (T_o) recorded maximum root weight of 59.25g. All other treatmentswere on par and they differed significantly

from control. Root weights recorded in treatments T_1, T_2, and T_3, T_4 were 43.00 g, 36.25 g and 29.00 g respectively. The least root weight was recorded in plants inoculated with thousand larvae of both the nematodes.

8.9. Final population

Observation on the final soil, root and total nematode population at the end of 120 days of inoculation in the pots are presented in Table 18.

a) Nematode population in soil

Nematode population in pots under individual inoculation treatments T_1 and T_2 were on par and differed significantly from combined inoculation treatments T_3 and T_4 which were on par. The nematode populations in soil under different treatments T_1, T_2, T_3 and T_4 were 94950, 97500, 78000 and 88,500 respectively. In the case of individual and combined nematode inoculation soil population of root-knot increased more than that of reinform nematode.

b) Nematode population in roots

In the case of root population also treatments T_1 and T_2 and treatments T_3 and T_4 fall in two homogenous groups which differed significantly between groups but not within groups. Significant reduction in population was noticed in combined inoculation when compared with individual

Table 18. Effect of individual and combined inoculations of root-knot and reniform nematodes on final population after 120 days of inoculation

(Mean of 4 replications)

Treatment	Number per plant								
	T_0	T_1	T_2	T_3			T_4		
		Rr(1000)	Rk(1000)	Rr(500)+	Rk (500)	Total	Rr (1000)+	Rk(1000)	Total	CD(5%)
Soil population	0	94950	97500	26625	51375	78000	26025	56475	82500	4937
Root population	0	1843	1630	303	380	683	285	368	653	43
Total	-	96793	99130	26928	51755	78683	26310	56843	83153	-

inoculation treatments. The population recorded under various treatments are in the order 1843, 1630, 683 and 653 for T_1, T_2, T_3, and T_4 respectively. The population of M. incognita was more in roots in all the cases of plants under treatments T_1 to T_4. The result also shows that when either of the nematode was inoculated alone R. reniformis recorded a higher number of population in roots than M. incognita whereas when both nematodes were inoculated in combination with equal number, M.incognita recorded a higher population level in the roots. This shows that in the presence of root-knot infection reniform nematode will be less when both nematodes occur in the soil.

DISCUSSION

Ginger the important annual spice crop cultivated in our country play a major role in the rural economy of farmers of Assam, Himachal Pradesh and Kerala. The root-knot and reniform nematodes are the two major pests occuring widely in the ginger growing areas of the country. The pathogenic effect and damage caused by the root-knot nematode *M. incognita* on ginger have already been studied (Huang, 1966). The frequent association of the reniform nematode *R. reniformis* in soil where ginger is grown has been reported by a few workers (Swarup *et al.* 1967; Sundarraju *et al.* 1979.) No detailed studies on its pathogenic effect and level of damage caused by it have been conducted and reported. In Kerala ginger is the main crop grown in Western ghat hilly regions and these agricultural lands are infested either by root-knot and reniform nematodes or by both. Hence it was found necessary that a study of this type is to be taken up to gather scientific evidence on the role of these nematodes, in ginger crop cultivation and production. Two experiments were therefore carried out under pot culture conditions to understand the degree of pathogenic effect caused by the reniform nematode *R. reniformis* on ginger

and the relative pathogenic interaction effect caused by it along with the root-knot nematode *M. incognita*.

The pot culture experiment on pathogenic effect of reniform nematode had established that ginger is a very good host for the nematode. The population build up of nematode on the host at the end of 120 days was 1360 times from an initial inoculum of 50 number of infective stages provided. The large number of developing and matured adults found attached on the roots @ 13/g root, to 57/g root (Tables 8,9) at an initial inoculum levels of 50 and 5000, clearly show that the nematode could readily infect the host, develop and multiply. The multiplication of nematode on different hosts has been reported by Villanueva and Castillo (1976) on cowpea, Gupta and Yadev (1982) on green gram, Sivakumar and Seshadri (1971) on castor and in these cases the rate of multiplication were greatest/least in treatments with a lowest and highest inoculum respectively. The reasons for the slow multiplication of the nematode in the plants treated with highest inoculum might be due to the non availability of required quantum of root production by the host plant. In this experiment the growth parameters such as number of tillers, leaf production, leaf length and width of leaves

(Tables 2, 3, 4, 5) were found to be influenced by the higher inoculum levels between 90-120 days of adding the nematode inoculum (Fig. 1) It is also observed tha there is a marked difference in the trend of reduction of the growth parameters (Tables 7 and 8) namely, top weight (fresh and dry), and rhizome and root weight of the nematode inoculated plants, even though the data collected did not show significant difference, between the treatments. The above observations are similar to that reported by Ayala (1962) and that of Sivakumar and Seshadri (1971). The present study had not shown any effect of the nematode on reduction in top weight even after 120 days of nematode inoculation. So the early termination of the experiment (after 120 days of nematode inoculation) may be the reason for not obtaining the required pathogenic effect on the plants as induced by the nematode. Similar results have been reported in the case of reniform nematode on cowpea by Villaneuva and Castillo (1976) who observed that there was no significant reduction in top weights of plants even after two months of nematodes inoculation, including a highest inoculum of 10,000 nematodes/seedling. The crop duration of ginger is 8-9 months (Venkataraman,1982)

Figure 1. Effect of different inoculum levels of reniform nematode on growth parameters of ginger after 120 days of inoculation

T_0 - Check

T_1 (50 reniform nematode/plant)

T_2 (100 reniform nematode/plant)

T_3 (200 reniform nematode/plant)

T_4 (500 reniform nematode/plant)

T_5 (1000 reniform nematode/plant)

T_6 (5000 reniform nematode/plant)


1
T6
T5
T4
T3
T2
T1
T0

and the limited experimental period is inadequate for the realisation of the full pathogenic effect of the nematode inoculated plants.

The pathogenic effect of the nematodes, individually or in combination were observed in the relative pathogenicity experiment in the case of growth parameters, mainly, height of tillers, tiller production, leaf production, length of leaf, width of leaves, leaf area, rhizome weight, root weight, fresh and dry weight of tops (Fig. 2 and 3). However a higher degree of pathogenic effect was found to be caused by the root-knot nematode alone (T_2) inoculated plants and in plants inoculated with both the nematodes in equal numbers (T_3 and T_4). The population of nematodes recorded in the roots in plants under different treatments as indicated by the presence of the adults (including developing stages) reveal that the degree of infectivity is more in the case of root-knot nematodes, than reniform nematodes (Table18). The observations in the second experiment show that when either of the nematode was inoculated alone, R. reniformis recorded a higher number of population in roots than M. incognita whereas, when both nematodes were inoculated in combination simultaneously at equal numbers, M. incognita recorded a higher population level in the roots. This indicate that

Figure 2. Effect of individual and combined inoculations of root-knot and reniform nematode on growth parameters of ginger after 120 days of inoculation

T_0 - Check

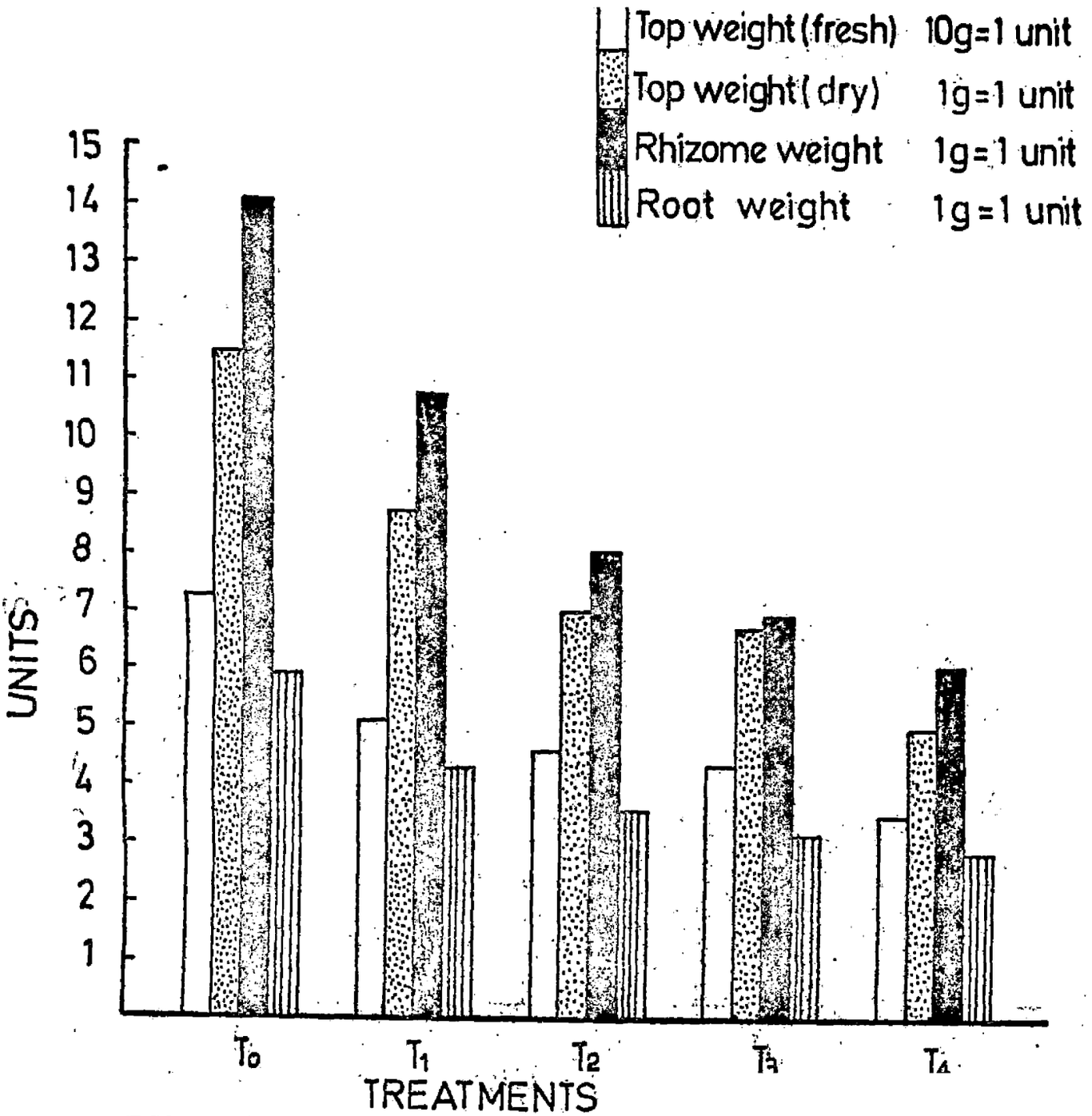
T_1 (1000 Nos. *R. reniformis* alone/plant)

T_2 (1000 Nos. *M. incognita* alone/plant)

T_3 (Mixture of 500 *R. reniformis*+ 500 *M. incognita*/plant)

T_4 (Mixture of 1000 *R. reniformis*+ 1000 *M. incognita*/plant)


2
T4
T3
T2
T1
T0



Effect of inoculum levels of root-knot and reniform nematodes alone or in combination on fresh weight of tops, dry weight of tops, rhizome weight and root weight in ginger 120 days after inoculation.

FIG. 3

in the presence of root-knot infection, reniform nematode infection will be less, when both nematodes occur naturally in soil. The above observation is in conformity with the report of Singh (1976) who found that M. incognita inhibited increase of R. reniformis in soybean when simultaneous inoculation of both were made. This may be the reason for producing a high degree of pathogenic effect by the root-knot nematode or its combination with the reniform nematode in reflecting on the growth parameters. Similar observation has been reported by Ferraz and Sharma (1979) on Piper nigrum. They found that M. incognita alone or in combination with R. reniformis was highly pathogenic, but in respect of the number of root galls and egg mass per gram root, there was significant reduction when both nematodes were inoculated together than when M. incognita was inoculated alone.

The above study reveal that the ginger is a very good host for the reniform nematode R. reniformis and it can cause pathogenic effect to considerable extent, and can adversly affect the tiller and leaf production, leaf length and width. The tops, rhizome and root weight are also affected by the nematode infection at 1000 or 5000 infective stages, per plant or at a threshold level

33 to 165 nematodes per kilogram of soil. However detailed studies covering the entire crop period to maturity has to be taken up for gathering confirmative evidence on the above aspects. In the case of the second experiment the results indicate that the root-knot nematode causes more pathogenic effect on ginger than the reniform nematode.

SUMMARY

Two sets of pot culture experiments were laid out at the Department of Agricultural Entomology, College of Horticulture. (i) to study the pathogenic effect of different inoculum levels of reniform nematode alone on ginger, comprising of seven treatments of 0, 50, 100, 200, 500, 1000 and 5000 nematodes/plant with four replications, (ii) to study the pathogenic effect of different inoculum levels of root-knot and reniform nematode alone or in combination, comprising of five treatments of 0, 1000 reniform alone, 1000 root-knot alone, 500 each of root-knot and reniform and 1000 each of root-knot and reniform/plant replicated four times.

In both the experiments observations on growth parameters were recorded at monthly intervals ie. before and after 30, 60, 90 and 120 days of inoculation.

The results in the experiment on pathogenicity indicated that an initial inoculum level of 1000 or 5000 reniform nematodes/plant seems to have more pathogenic effect on ginger, at the end of 120 days. In the experiment on individual and combined inoculations of reniform and root-knot nematodes revealed, homogeneity in affecting most

of the growth parameters in plants when the nematodes were present alone and differed significantly from plants with no nematodes. The result also indicated that the root-knot nematode was more pathogenic than the reniform nematode whereas the combined inoculations had no effect on external characters. But there was apparent effect on yield. With regard to reduction in tops (fresh and dry) rhizome and root weight, combined inoculations 1000 each of both nematodes gave significant difference over non-inoculated plants.

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PATHOGENICITY OF RENIFORM NEMATODE (ROTYLENCHULUS RENIFORMIS LINFORD AND OLIVEIRA, 1940) ON GINGER (ZINGIBER OFFICINALE ROSE)

By

ELIZABETH JOHN

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COLLEGE OF HORTICULTURE
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ABSTRACT

Two pot culture experiments were laid out (i) to study the pathogenic effect of different inoculum levels of *R. reniformis* on ginger comprising of seven treatments and four replications and (ii) to study the relative pathogenic effect of root-knot and reniform nematodes singly or in combinations on the same crop comprising of five treatments and four replications. The duration of study was 120 days.

The result reveals that ginger is a good host for reniform nematode *R. reniformis* and it can cause pathogenic effect at a considerable extend at higher inoculum levels, within a period of 120 days.

In the experiment with *M. incognita* and *R. reniformis* the pathogenic effect was homogenous in most of the growth parameters when they were present alone and significantly different, whereas in combined inoculum even though there was not much effect on growth parameters, considerable reduction in plant top weight and yield was observed. However root-knot nematode was observed to cause more pathogenic effect than reniform nematode, within study period of 120 days.