EFFECT OF ROOT-KNOT NEMATODE *Meloidogyne incognita* ON NODULATION IN COWPEA

By

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THESIS
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Department of Plant Pathology
COLLEGE OF AGRICULTURE
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1986

DECLARATION

I hereby declare that this thesis entitled " Effect of root-knot nematode, Meloidogyne incognita on nodulation in cowpea " 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.

Vellayani
15 /4/1986

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CERTIFICATE

Certified that this thesis entitled " Effect of root-knot nematode, Meloidogyne incognita on nodulation in cowpea", is a record of research work done independently by Smt. NIRMALA MONI.D. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

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INTRODUCTION

INTRODUCTION

Pulses are the principal grain crops of legume family. They have an important place in the diet of people of many developing countries including India, as a high protein food of lesser cost. In India, pulses occupy nearly 21 million hectares with an average yield of about 517 kilograms per hectare. In Kerala, cowpea (*Vigna unguiculata* (L.) Walp), is probably, the most widely cultivated pulse crop. It is grown in uplands throughout the year and in fallows during the third crop season. However, the present yield of cowpea in the state is only about 400 kilograms per hectare. This comparative low yield is due to several factors like neglected production technology, uncertain weather, pests and diseases.

The association of root-knot nematodes with cultivated crops is reported by many workers. The four important *Meloidogyne* spp. which account for nearly 95 per cent of all nematode infestations and cause an average crop loss of about 5 per cent on a world wide basis are *M.incognita* (Kofoid and White) Chitwood, *M.javanica* (Treub) Chitwood, *M.hapla* Chitwood, and *M.arenaria* (Neal) Chitwood (Sasser *et al*. 1983). In Kerala, mainly two species, *M.incognita* and *M.javanica* are seen and between them, *M.incognita* is found to be the more destructive one (Venketesan 1984). It infests various crop plants like cowpea, brinjal, tomato, bhindi, amaranthus, turmeric, ginger and cucumber.

Cowpea infested with M.incognita shows a stunted growth with poor vigour and yield. Its actual effect on nodulation and nitrogen fixation by Rhizobium is yet to be understood well. Therefore, the present investigation was carried out. Three separate pot culture experiments were conducted in order to study the host varietal variations in cowpea to infestation by M.incognita and to understand the effect of inoculation with different levels of this nematode and age of host plant on nodulation, gall formation and other plant characters in cowpea. Following observations on nodule number, nodule fresh weight, gall number, gall fresh weight, fresh and dry weight of shoot, percentage nitrogen content of shoot and the nematode population in infested root and soil were taken for this purpose.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The symptoms of root-knot formation were first described by Berkeley in 1855 who observed gall formation in infested roots of cucumbers in England. However, the first specific report of a root-knot nematode was that of Cornu (1879). He described *Anguillula marioni* = *Meloidogyne marioni* as the causal agent of galls in *Onobrychis sativa*.

Walker (1957) reported that in Wando pea, invasion of bacterial nodules by *Meloidogyne* spp. suppressed plant growth and that the presence of nematode within the nodule prevented *Rhizobium* from supplying the host plant with fixed nitrogen. Masefield (1958) was of the opinion that reduction in nodulation as a result of nematode infestation in pulses was due to a nutrient deficiency caused by the nematode itself. Chapman (1960) found that in red clover, the top dry weight was reduced by *M.incognita* and *M.hapla*. But the reduction was more rapid and pronounced with *M.incognita*. Epps and Chambers (1962) observed that in soybean (*Glycine max* (L.) Merr *Meloidogyne* spp. inhibited nodulation besides reducing the nitrogen fixing capacity of rhizobia. Nadakal (1963) was the first to report the incidence of *M.incognita* in Kerala in *Phaseolus multiflorus*. Taha and Raski (1969) found that in white clover (*Trifolium repens*) and soybean

(Glycine max (L.) Merr inoculated with Meloidogyne sp. severe nitrogen deficiency along with reduction in nodulation occurred. Many giant cells were formed without disrupting the nodular tissue. They suggested that the decrease in nodulation was due to an overall reduction in root system.

Mammen (1973) reported the occurrence of infestation by M.incognita in Kerala in a number of cultivated crops such as amaranthus, ginger, brinjal, bhindi, pepper, tomatoes and cowpea. Barker and Hussey (1976) observed that Meloidogyne spp. developed more readily within nodules of soybean than other legumes. Further, M.incognita was found inside vascular bundles of soybean nodules without altering their structural integrity. However, the bacteriodes did not develop adjacent to nematode invaded tissue. The infested nodules also deteriorated much rapidly. Singh (1976) also found that in soybean variety Jupiter, M.incognita produced significant reduction in top and root dry weight after ten weeks of inoculation.

Yeates et al.(1977) reported that in Trifolium repens, root-knot nematode infestation reduced the nitrogen fixing capacity of Rhizobium and the herbage dry matter yield. Castillo et al.(1978) found that in mung bean (Phaseolus aureus Roxb.) infestation by M.incognita acrita, resulted in chlorotic leaves, stunted growth and severely reduced and galled root system. Some of the plants were also killed prematurely. Besides, the nematode population increased by 24.4 times in pots and caused an yield loss of about 28 per cent under field conditions. Sharma *et al.*(1978), also found that groundnut (*Arachis hypogaea*), plants infested with *Meloidogyne* spp, especially *M.arenaria*, showed severe yellowing, reduced vigour and stunted growth with hard, small to medium size galls on root system.

Sasser *et al.*(1983) reported that four *Meloidogyne* species, *M.incognita*, *M.javanica*, *M.arenaria* and *M.hapla* were responsible for 95 per cent of all nematode infestations and caused an average crop loss of about 5 per cent on a world wide basis. Venkatesan (1984) found that out of the two *Meloidogyne* spp, *M. incognita* and *M.javanica*, commonly occurring in Kerala, *M.incognita* was more severe and widely distributed.

In cowpea, Goswami *et al.*(1975) observed that inoculation of *M.incognita* resulted in stunted root and shoot growth. Sharma and Sethi (1976) reported reduced plant growth, nodulation and shoot nitrogen content in cowpea infested with this nematode. A similar report was also made by Ali *et al.*(1981) who observed an inhibition of nitrogen fixation by about 63 per cent in nodular tissue. The plants inoculated with *M. incognita* and rhizobia showed more severe nitrogen deficiency and retarded growth than plants inoculated with nematode alone or uninoculated plants. Further, the infested nodules contained different developmental stages of the nematode without altering their structure. However, the nematode was found to prevent rhizobia from supplying the host plants with fixed nitrogen.

Screening of pulses for resistance to *Meloidogyne* species

Sasser and Taylor (1952) reported that although several soybean varieties appeared to have a high degree of resistance or tolerance to *Meloidogyne* spp, immunity from root-knot infestation was rather rare. Fifty varieties of soybean were tested in Delaware for resistance to *M. incognita* var *acrita* by Critbenden (1955) and out of these, 10 varieties showed high resistance to this nematode. However, when 5 of these varieties were further tested against *M. hapla*, all the 5 were found susceptible. In 1955 Reynolds screened 10 varieties of alfalfa (*Medicago sativa* L.) consisting of both northen (hardy) and southern (non-hardy) selections against *M. javanica* and *M. incognita acrita*. He observed that *M. javanica* was more damaging to most of the varieties. Douglas (1958) tested 13 white and 48 red clover introductions for resistance to M.incognita, M.incognita acrita, M.arenaria and M.javanica and found that with certain exceptions both red and white clovers were moderately to severely infested by all the four species of nematode. Stanford et al.(1958) first reported the transfer of resistance to root-knot nematode in young seedlings of alfalfa. Hartmann (1971) explained that inheritance of resistance to root-knot nematode, M.incognita in beans was due to atleast three pairs of genes which were all equal in their action.

Williams et al.(1973) found that out of 42 soybean varieties screened against a new race of M.incognita, only F63-4000 and F66-1080 had a lower root-knot index than Bragg. Calinga and Ballon (1974) found that 17 out of 21 soybean varieties screened against M.incognita showed moderate to high resistance. Baldwin et al.(1975) reported that in soybean cultivars like Lee and Forrest, after 50 and 135 days of inoculation with 15000 eggs of M.incognita per plant and Rhizobium japonicum, nematode population was 11 and 35 times more in Lee and Forrest respectively.

Minton and Hammons (1975) found that 512 cultivars, breeding lines and plant introductions of peanut were susceptible to M.arenaria. According to Singh (1975) among the three cultivars of *Cajanus cajan*, the University of West Indies Dwarf variety supported the largest population of *M.incognita*. Hunt *et al*.(1978) found that lucerne germ plasm NMP-9 was resistant to *M.hapla*. Lopez (1980) observed that when different cultivars of *Phaseolus vulgaris* L. were inoculated with *M.incognita*, there were significant differences among them in fresh weight of root, shoot and pods and in root-knot index. Verma *et al*.(1981) reported that out of 34 varieties of cluster bean screened for resistance to *M.incognita* none were immune or resistant. Hasan (1983) observed that out of 94 germ plasm accessions of chick pea (*Cicer arietinum*) screened, 38 were immune, 20 resistant and 15 moderately resistant to *M.incognita*. Sultan *et al*.(1984) found that only 7 out of 40 mung bean cultivars screened, showed some resistance to *M.incognita*.

Hare (1959) rated Iron cowpea and four breeding lines such as M-255, M-455, M-755 and M-855 as resistant to root-knot nematodes, *M.incognita*, *M. incognita acrita*, *M.javanica* and *M.arenaria*. Out of 72 cultivars and lines of cowpea tested for their susceptibility to *M.incognita* by Amosu (1974), 36 lines and cultivars were found resistant. The remaining cultivars and lines were moderately to highly susceptible. He further suggested that resistance to root-knot nematode in cowpea was controlled by a single dominant gene designated as Rk. Singh et al.(1975) reported that VITA-3 a tropical strain of V.unguiculata selected from the introduction VU5 from Kenya, had resistance to M.incognita. Caveness (1975), found that 48 cultivars of cowpea showed a mixed response to M.incognita which indicated the existence of heterogenity within cowpea lines for resistance to this nematode. Sharma and Sethi (1976) screened 30 varieties and lines of cowpea against infestation by M.incognita and found that 18 were highly resistant. Patel et al.(1977) found that cowpea variety C-152 was highly resistant to M.incognita. Obuji (1978) tested 103 cultivars of V.unguiculata for resistance to M.incognita and found that while 41 cultivars were resistant, 25 were consistently susceptible and 37 inconsistent in their reaction to M.incognita.

Raj and Patel (1978) reported that resistance to root-knot nematode in cowpea was associated with one dominant or one recessive gene depending on the source. They also found that resistance in the cultivar, Iron, was recessive rather than dominant. Thakar and Patel (1983) reported that cowpea variety V-16 was also resistant to M.incognita.

Effect of inoculation with different levels of M.incognita and age of host plant on nodulation and gall formation in pulses

Bergerson (1968) suggested that expression of resistance or tolerance to M.incognita was influenced by host age. Balasubramanian (1971) reported that in soybean, inoculated with 10, 100 and 1000 larvae of M.javanica per plant, 100 and 1000 larvae caused maximum reduction in nodulation. The reduction in nodulation was found either due to the nematode interfering directly with the establishment of nitrogen fixing bacteria or due to the rendering of infested roots physiologically incompatible to Rhizobium infection. Catibog and Castillo (1975) observed that seedlings of Phaseolus aureus inoculated with 5, 15, 25 and 50 eggs of M.javanica died one month after infection and that the extent of root galling was increased with higher nematode level. There was also a reduction in top weight and yield along with an increase in the initial inoculum. Hussaini and Seshadri (1975) reported that mung bean when inoculated with M.incognita at various levels such as 1000, 2000, 3000 and 4000 larvae per plant, separately, simultaneously, or in sequence with Rhizobium there was significant reduction in plant height, fresh and dry weight of shoot and root and the nitrogen content of shoot and root at all inoculum levels. Srivastava et al.(1975) observed a continuous reduction in length and weight of root and shoot in chick pea inoculated with an increasing level of *M. javanica* from 10 to 10,000 per 500 g soil.

Jamal (1976) found that in *Cicer arietinum*, *M. incognita* preferred the soft apical meristematic tissues of roots for its colonisation. Singh et al.(1977) reported that in *Phaseolus aureus* Roxb with an increase in the level of *M. incognita* from 100 to 10,000 larvae per plant, there was a corresponding decrease in chlorophyll content, nodule number and nitrogen content of shoot. They suggested that the decrease in chlorophyll content was due to an alteration in host nutrition and physiology and that the reduction in nitrogen fixation was the result of the nematode secreting certain hydrolytic or oxidative enzymes or growth regulators which played a detrimental role in nodule development and function.

Townshend and Potter (1978) observed that in *Trifolium pratense* and *T. repens* inoculation with *M. incognita* at 0, 4000 and 24,000 larvae per kg soil affected seedling establishment to the extent of 50 per cent of the control. The dry weight was reduced in proportion to increasing inoculum density. The plant height was also less in infested

Trifolium pratense. In chick pea, Gaur *et al*.(1979) found that 1000 and 10,000 larvae of *M.incognita* per kg soil was pathogenic in three varieties. Nath *et al*.(1979) reported that in Bengal gram, an increase in the inoculum level of *M.incognita* resulted in a proportional decrease in nodulation, shoot length, fresh weight of shoot, root length and seed production. Further, an inoculum of 100 larvae per 500 g soil was found to be the damaging threshold level. Plants inoculated with 1000 and 10,000 larvae per 500 g soil were stunted in growth.

Raut (1980) observed a significant reduction in plant height, fresh weight of shoot and root length in mung bean inoculated with 1000 larvae of *M.incognita* per plant. A similar observation was also made by Raut and Sethi (1980) in soybean inoculated with 10, 100, 1000 and 10,000 larvae of *M.incognita* per plant. Significant reduction in top growth, root length and bacterial nodulation was observed with an initial inoculum level of 1000 larvae per plant which was considered to be the damaging threshold level for this crop.

Dhruj and Vaishnav (1981) found that in groundnut variety GAUG-10, an inoculum level of 100 or more larvae of *M.incognita* per plant was the damaging threshold level. There was significant reduction in all plant characters studied at this inoculum level. Besides, plants inoculated with 1000 and 10,000 larvae showed varying degrees of chlorosis and stunting with a reduction in leaf size. Gall number and nematode population increased with an increase in inoculum level. In black gram (*Vigna mungo*), Mishra and Gaur (1981) observed a significant reduction in plant height, fresh weight of shoot, pod formation, root length and nodulation in plants inoculated with 1000 and 10,000 larvae. In moth bean (*Vigna aconitifolius* Jacq.), also, Mishra and Gaur (1981) observed, a significant correlation between inoculum levels of *M.incognita* and plant growth. There was significant reduction in shoot length with 10,000 larvae. The root length was also reduced as against an increase in fresh weight due to gall formation.

Sharma (1982) found that seedlings of *Phaseolus vulgaris* L. when inoculated with 10, 100, 1000 and 10,000 eggs of *M.javanica* there was significant reduction in dry weight with an inoculum level of 1000 or more eggs. In chick pea, Dhangar and Gupta (1983) reported that an initial inoculum of 1000 larvae of *M.javanica* per plant in smaller pots of

15 cm size and 1000 and 10,000 larvae per plant in larger pots of 25 cm size were pathogenic after two and five months respectively. A decrease in shoot length was also observed at 1000and 10,000 larvae per plant. Grewal et al.(1984) found that in groundnut inoculated with 10, 100, 1000, 10,000 larvae of M.arenaria there was reduction in plant growth, especially with the highest inoculum level. Mani and Sethi (1984) also observed a progressive reduction in plant growth in chick pea as the inoculum level of M.incognita was increased. However, nodulation was adversely affected at all nematode inoculum levels.

In cowpea, variety Pusa Barsali , Sharma and Sethi (1975) reported that the minimum threshold level of M.incognita required to produce any significant effect on plant growth was 100 larvae per 500 g soil. However Gupta (1979) found that in cowpea, CV HFC 42-1, 1000 or 10,000 larvae of M.javanica per plant were required for any significant reduction in plant height and fresh weight of shoot and root.

Control of root-knot nematodes in pulses

Minton et al.(1978) found that application of 1,2, dibromo-3-chloropropane (DBCP) and subsoiling under the row in Tifton sandy loam soil infested with M.incognita increased the yield of four soybean cultivars. Mankau (1980) suggested that *Bacillus penetrans* could be successfully used for the biological control of *M.incognita*.

Sharma *et al.* (1980) observed that a crop rotation with groundnut and wheat gave maximum yield for a subsequent crop of nematode susceptible mung bean in an infested soil. The larval population of *M.incognita* was also reduced considerably under groundnut-mustard rotation. Jain and Hasan (1984) reported that the aqueous extract of the leaf, seed and pod shell of Su-babool, (*Leucaena leucocephala*.L) killed *M.incognita* to a great extent. However, the seed extract was found more effective in causing immobility to the nematode. Further, the percentage mortality of the larvae in different extracts also increased with an increase in the exposure time, with a maximum after 24 hours. Reddy (1984) reported that in cowpea, french bean and pea infested with *M.incognita*, fenamiphos at one per cent concentration, gave the least root-knot index in all the crops studied followed by aldicarb sulfone and carbofuran at 10 per cent concentration.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation on the effect of root-knot nematode, *Meloidogyne incognita* on nodulation in cowpea was conducted at the College of Agriculture, Vellayani, Trivandrum, during 1982-1985. In all, three pot culture experiments were conducted to study the effect of host variety, inoculum level and age of host plant on infestation by *M.incognita* in cowpea.

I. 1. The nematode

A pure culture of *M.incognita* obtained from the Nematology section of Department of Entomology, College of Agriculture, Vellayani, Trivandrum, was used for the present investigation. The nematode was identified on the basis of its perineal pattern by the method described by Eisenback *et al* in 1981. Fresh roots of coleus (*Coleus blumei*) infested by *M.incognita* were used for this purpose. These were washed initially in tap water to remove the adhering soil particles and then fixed in boiling cotton blue lactophenol for 3 minutes.

Cotton blue lactophenol

Phenol	-	20 g
Lactic acid	-	20 g
Glycerine	-	40 ml
Cotton blue (1 % aqueous solution)	-	5 ml
Distilled water	-	20 ml

After fixation, the excess of stain was removed by washing in tap water and the root bits were transferred to fresh lactophenol for disecting out the nematode. The posterior part of each nematode was carefully cut and separated for observing its perineal pattern under a stereo microscope.

I.2. Maintenance of *M.incognita*

A pure culture of *M.incognita* was maintained in coleus (*Coleus blumei*) plants raised in pots containing sterilized soil. The availability of sufficient number of larvae for various experiments was ensured by maintaining sufficient number of those plants.

I.3. Preparation of nematode inoculum

The nematode inoculum was prepared by collecting egg masses from infested roots of coleus and allowing them to hatch in distilled water. In order to ensure good vigour of the inoculum, only those larvae hatched within 24 hours of collecting the egg masses were used. The number of larvae present in the inoculum was determined with the help of a stereomicroscope and a hand tally counter. This suspension was then diluted with fresh distilled water if necessary, for preparing the different levels of inoculum used during this investigation. Plant inoculation was done by pipetting

10 ml of such a suspension into four holes punched near the root zone of the host plant. Control plants were inoculated with 10 ml of distilled water alone.

2.1. The Rhizobium

A specific strain of Rhizobium, for cowpea, KAU-11 developed by the Microbiology section of Department of Plant Pathology, College of Agriculture, Vellayani, was used for seed inoculation. This culture was maintained aseptically on yeast extract mannitol agar medium of following composition.

Yeast extract mannitol agar (Allen, 1953)

Mannitol	10.0 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
$CaCo_3$	3.0 g
Yeast Extract	1.0 g
Congo Red (1% aqueous solution)	2.5 ml
Agar	15.0 g
Distilled water	1000 ml
pH	7

Seeds of cowpea were initially surface sterilized with 0.1 per cent mercuric chloride solution and washed repeatedly in sterilized tap water before inoculation with Rhizobium culture by known standard procedures. This inoculation was done uniformly for all the three experiments conducted during this investigation.

3.1. Host varietal variations in cowpea to infestation by M.incognita

A pot culture experiment was conducted using sterilized soil for screening different varieties of cowpea for resistance to infestation by M.incognita. The experiment was laid down in completely randomised design using the following ten varieties of cowpea.

No.	Variety	Source
1	New Era	Department of Agronomy, College of Agriculture, Vellayani.
2	Pathinettumaniyan	"
3	HG-22	"
4	PTB-1	"
5	PTB-2	"
6	C-152	"
7	Sundari	"
8	V-16	Department of Plant Breeding, Colleg of Agriculture, Vellayani.
9	V-37	"
10	V-240	"

The potting mixture consisted of soil, sand and cowdung in the ratio of 2:1:1 and this was steam sterilized in an autoclave at 121°C for 2 hours. NPK fertilizers were added uniformly prior to sowing at the rate of 20:30:10 kg/ha. Three replications were maintained for each variety with a single plant in every pot. These were inoculated with 1000 second stage larvae of M.incognita on 14th day by the method described earlier. Observations on gall number, gall fresh weight, nodule number, nodule fresh weight, leaf number, plant height, root length, fresh and dry weight of shoot, nematode population in infested root and soil were taken on 50th day of plant growth by routine standard procedures.

The gall and nodule numbers were determined after carefully removing each plant with its intact root system from the pots and cleaning them thoroughly in running tap water. The number of galls formed in each rootlet were counted. These were then carefully cut out for determining its fresh weight. The nodule number was also counted after carefully separating them from the root system. The fresh weight was determined by using a chemical balance. The plant height and root length were measured from the base of the shoot system to the maximum growing tip by using a meter scale. The fresh and dry weight of shoot for each variety were determined immediately after harvest and after drying the samples to a constant weight at 60°C respectively.

3.2. Estimation of nematode population in infested root

One gram of infested root was cut into small pieces and placed over a cotton wool filter supported on a metallic sieve. This in turn was transferred to an extraction disc with enough water to immerse the root bits. After 2 days, the filter with the sieve was removed and the number of nematode in the suspension was determined with the help of a stereomicroscope after making its volume to a constant level. The presence of nematode in infested nodule was also determined in a similar manner. However no quantitative estimation of larvae present in root nodules was done during this investigation.

3.3. Estimation of nematode population in infested soil

The population of the nematode in infested soil was determined by the modified Baermann Funnel technique of Christie and Perry (1951). For this 100 g of the inoculated soil was collected from each pot after mixing its contents throughly. The representative sample was transferred to a plastic basin and mixed with water. The coarse particles and foreign materials were allowed to settle and the supernatent was passed through a 60 mesh sieve. The filterate was then allowed to stand for few minutes before it was decanted and passed serially through 200 and 325 mesh sieves. The fine silt and nematodes collected in these sieves were washed into a beaker with minimum quantity of water. The nematode suspension thus obtained was poured gently into a tissue paper kept in a Baermann funnel supported over a piece of wire guaze. The funnel was filled with water upto the level of the tissue paper and kept undisturbed for 24 hours. Ten ml of water from this was drawn out into a specimen tube through a pinchcock. The nematodes present in the sample were allowed to settle and the volume of the suspension was reduced to 5 cc by pipetting the excess of water from the top. Five ml of boiling 10% formalin was added to this to kill the nematodes before estimating their number with the help of a stereomicroscope.

4. Effect of inoculation with different levels of *M.incognita* on nodulation, gall formation and other characters in cowpea

A pot culture experiment using sterilized soil was conducted to study the effect of inoculation with different levels of *M.incognita* on various characters in cowpea. New Era, a susceptible variety of cowpea to *M.incognita* was used to conduct this experiment which was laid out in completely randomised design with six replications each. NPK fertilizers were added uniformly prior to sowing at the rate of 20:30:10 kg/ha. The different treatments were inoculation with 0 (control), 10, 100, 1000 and 10,000 second stage larvae of M.incognita per plant on 15th day of plant growth by the method described earlier. Following observations on nodule number, nodule fresh weight, gall number, gall fresh weight, leaf number, plant height, root length, fresh and dry weight of shoot and nematode population per gram fresh weight of root and 100 g of inoculated soil were taken on 50th day of plant growth by the methods described earlier.

The percentage nitrogen content of shoot was estimated by the modified micro-kjeldahl method of Jackson, 1967. Hundred milligram of powdered plant sample along with 10 g of the digestion mixture consisting of Potassium sulphate, Cupric sulphate and Selenium metal powder in the ratio of 10:1:0.1 were taken in a 100 ml kjeldahl digestion flask. Three ml of concentrated sulphuric acid of specific gravity 1.84 was added slowly to the above mixture and heated for 5 hrs till the material was completely digested. The flasks were allowed to cool down to room temperature before adding 25 ml of distilled water to each flask. After cooling, the contents were transferred to 100 ml volumetric flask and the volume was made up with distilled water. Ten ml of this sample from each flask was then added to a kjeldahl flask along with 10 ml of 50 per cent sodium hydroxide solution and steam distilled till about 100 ml of the distillate was collected in the receiver flask containing initially 10 ml of 2 per cent boric acid solution with a drop of mixed indicator. The ammoniacal nitrogen content of the distillate was determined by titration against 0.01 N hydrochloric acid and from the titre value, the percentage N was determined by the following equation.

$$\frac{V \times N \times V_1 \times 0.014 \times 100}{V_2 \times W}$$

Where V = Titre value - the blank

V_1 = Total volume of plant sample made up

V_2 = Volume of plant sample distilled

N = Normality of HCl

W = Weight of powdered sample used for digestion

5. Effect of age of host plant and infestation by M.incognita on nodulation, gall formation and other characters in cowpea

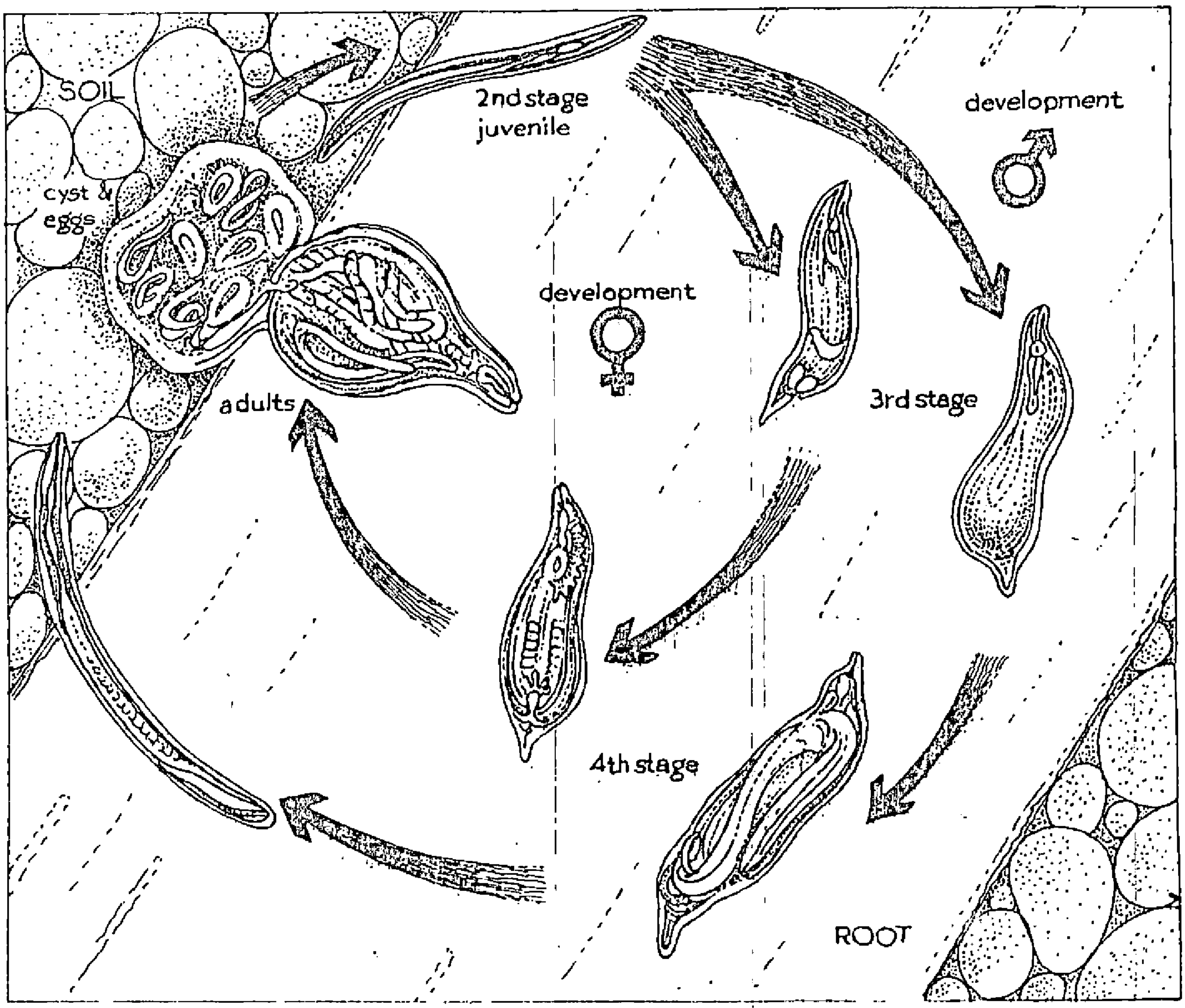
A pot culture experiment was conducted using sterilized soil to study the effect of age of plant and infestation by M.incognita on various plant characters in cowpea. New Era, a susceptible variety of cowpea to M.incognita was used to conduct this experiment which was laid out in completely randomised design with five replications each. N P K fertilizers were added uniformly prior to sowing at the rate of 20:30:10 kg/ha. The different treatments were inoculation with 1000 second stage larvae of M.incognita at the time of sowing and on the 7th, 14th, 21st and 28th day of plant growth by the method described earlier. Control plants were maintained without any nematode inoculation. Following observations on nodule number, nodule fresh weight, gall number, gall fresh weight, percentage nitrogen content of shoot, leaf number, plant height, root length, fresh and dry weight of shoot and nematode population per gram fresh weight of root and 100 g of inoculated soil were taken on 55th day of plant growth by the methods described earlier.

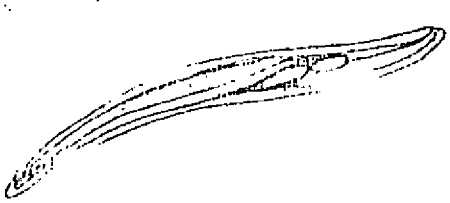
6. Statistical analysis

Data on various observations was analysed statistically by the methods described by Snedecor and Cochran 1967 for the analysis of variance of completely randomised design.

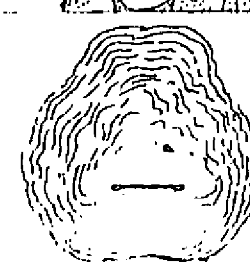
RESULTS

FIG-1. LIFE CYCLE OF *Meloidogyne incognita*





SECOND STAGE LARVA



PERINEAL PATTERN

RESULTS

1. The nematode

The nematode was identified as *Meloidogyne incognita* on the basis of its perineal pattern. A pure culture of the same was maintained in coleus (*Coleus blumei*) plants raised in sterilized soil. The life cycle of *M.incognita* along with an illustration of its typical perineal pattern is shown in Fig.1.

2. Host varietal variations in cowpea to infestation by *M.incognita*

The results of the pot culture experiment to study host varietal variations in cowpea to infestation by *M.incognita* are presented in Tables 1 to 4, Figure 2 and Plates I to V. The analysis of variance for the same are given in Appendices I(a) and I(b).

Among the ten varieties of cowpea screened for resistance to *M.incognita*, two varieties, New Era and PTB-2 were found susceptible to this nematode. In these varieties, 141.33 and 64.33 galls were formed per plant (Table 1 and Plate IV). However, in the remaining eight varieties such as Pathinettumaniyan, HG-22, PTB-1, C-152, Sundari, V-16, V-37 and V-240 there were no gall formation (Table 1 and Plates I, II, III and V).

Table 1. Effect** of inoculation with *Meloidogyne incognita* on gall formation and nodulation in different cowpea varieties.

Cowpea variety	Gall number	Gall fresh weight (g)	Nodule number	Nodule fresh weight (g)
New Era	141.33* (11.79)	1.41*	19.67* (4.39)	0.26*
Pathinettumaniyan	0 (1)	--	64.33 (6.55)	1.15
HG-22	0 (1)	--	60.67 (7.77)	1.37
PTB-1	0 (1)	--	55.33 (7.40)	1.15
PTB-2	64.33* (8.07)	0.22*	24.33* (4.79)	0.40*
C-152	0 (1)	--	72.00 (8.45)	1.49
Sundari	0 (1)	--	44.33 (6.63)	1.28
V-16	0 (1)	--	61.67 (7.84)	1.34
V-37	0 (1)	--	90.00 (9.48)	1.83
V-240	0 (1)	--	58.33 (7.59)	1.15
C.D.(5%)	1.24	0.29	0.45	0.61

** Mean of 3 replications.

Figures in paranthesis are $\sqrt{X+1}$ and $\sqrt{X}$ transformation values for gall number and nodule number respectively.

Plate I

Comparative effect of nematode infestation in different varieties of cowpea - 1.New Era
2. Pathinettumaniyan.

Plate. II

Comparative effect of nematode infestation in different varieties of cowpea - 1. New Era. 3.HG-22.

Plate - I

Plate III

Comparative effect of nematode infectation in different varieties of cowpea - 1. New Era 4.PTB-1.

Plate IV

Comparative effect of nematode infestation in different varieties of cowpea - 1.New Era 5.PTB-2.

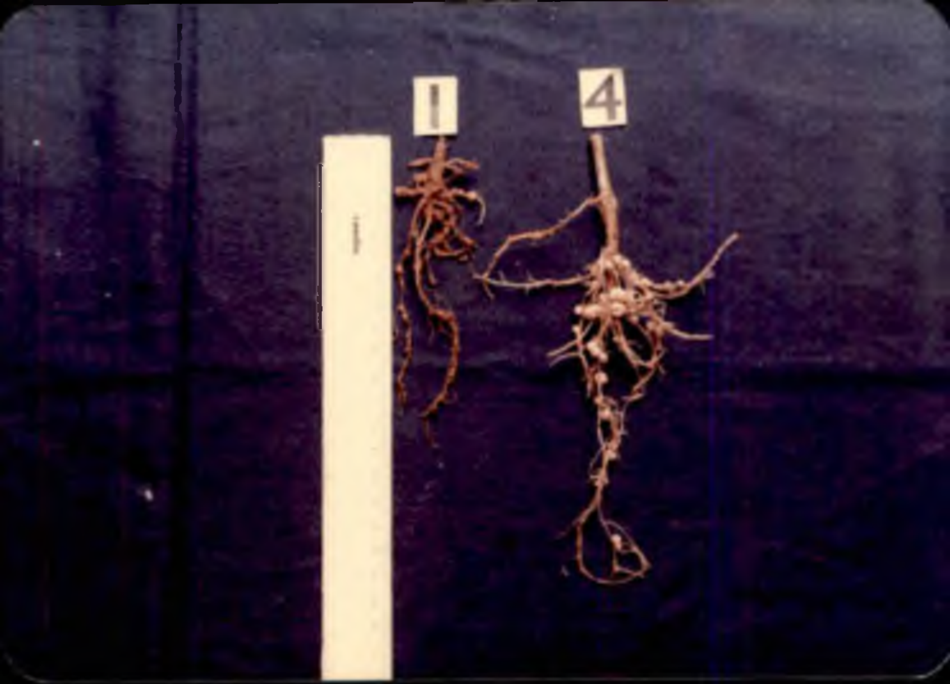


Plate-III

Plate V

Comparative effect of nematode infestation in different varieties of cowpea - 1.New Era 6.C-152.

Plate-V

FIG. 2. EFFECT OF INOCULATION WITH *Meloidogyne incognita* ON GALL FORMATION AND NODULATION IN DIFFERENT VARIETIES OF COWPEA.

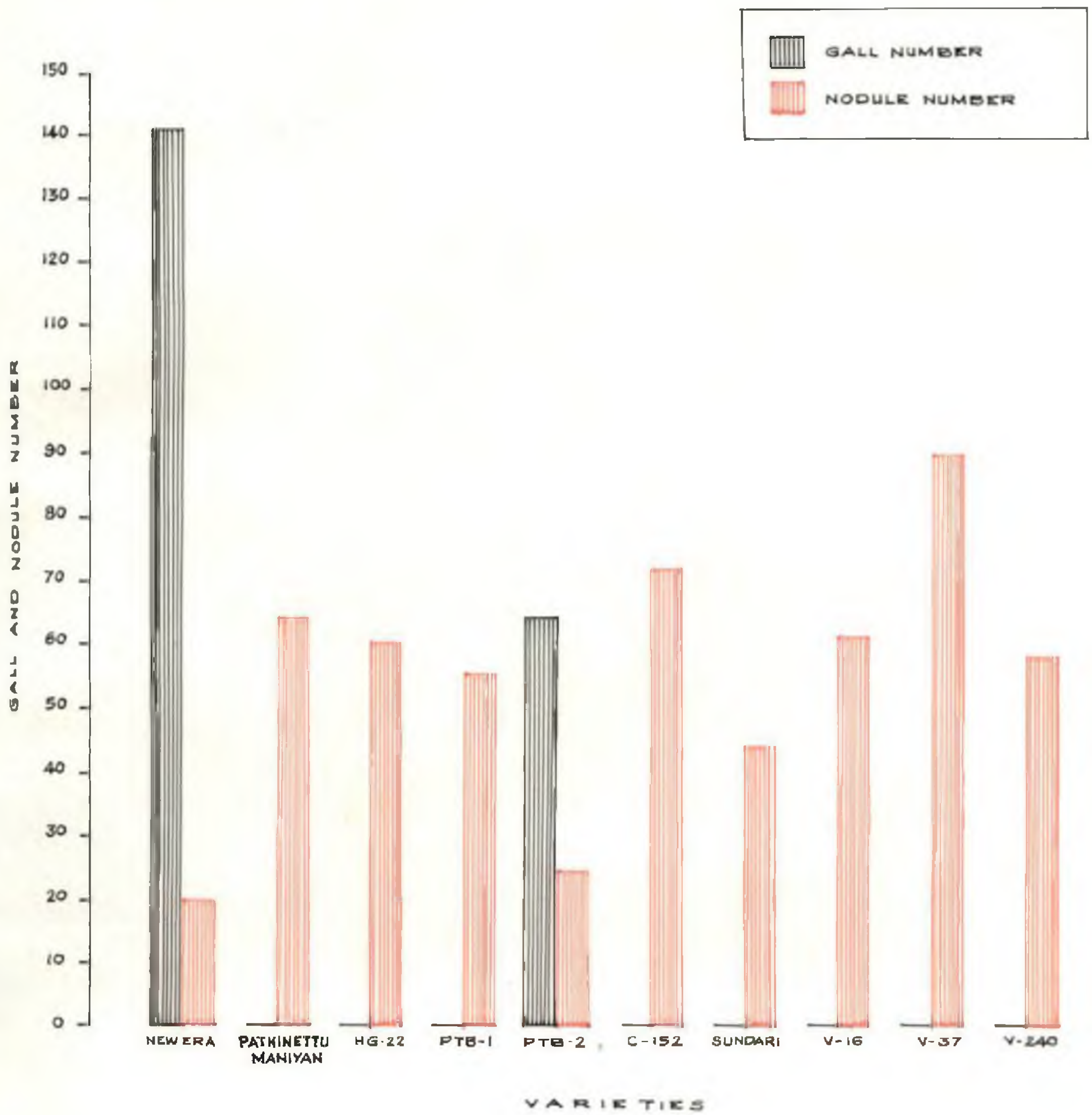


Table 2. Effect** of inoculation with *Meloidogyne incognita* on various plant characters in different cowpea varieties.

Cowpea variety	Leaf number	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
New Era	4.00* (1.95)	12.70*	11.07*	10.50*	0.71*
Pathinettumaniyan	11.33 (3.33)	37.00	21.23	23.73	1.79
HG-22	11.67 (3.41)	50.47	16.73	32.05	1.82
PTB-1	11.67 (3.40)	62.47	15.53	23.23	1.99
PTB-2	4.67* (2.16)	13.50*	15.13	12.31*	1.11*
C-152	10.33 (3.21)	28.70	17.37	22.90	1.52
Sundari	11.33 (3.35)	29.67	24.67	18.74	1.71
V-16	12.44 (3.46)	30.47	31.70	21.39	1.66
V-37	12.67 (3.55)	32.47	19.50	21.68	1.61
V-240	12.00 (3.46)	30.97	21.47	19.11	1.58
C.D.(5%)	0.58	14.18	4.16	6.13	0.20

** Mean of 3 replications

Figures in paranthesis are $\sqrt{X}$ transformation values for leaf number

The fresh weight of galls was in proportion to their number in susceptible varieties with a maximum of 1.41 g in New Era. Further, in these varieties there was a significant negative correlation between gall formation and nodule number (Table 4 and Fig.2). In New Era and PTB-2, only 19.67 and 24.33 nodules were formed. This was significantly lesser than the number of nodules in resistant varieties where a 'maximum of 90 nodules were formed in the variety V-37 followed by C-152 (Table 1). Further, the nodules in New Era and PTB-2 were infested by nematodes. These were soft and dark brown in colour when compared to the hard and flesh coloured nodules of resistant varieties. The fresh weight of nodules was also significantly low in susceptible varieties. These were only 0.26 and 0.40 g respectively for New Era and PTB-2 in comparison to 1.83 g for the resistant variety V-37 (Table 1).

The number of leaves formed per plant, plant height, fresh and dry weight of shoot were significantly low in New Era and PTB-2 (Table 2). The number of leaves formed in these varieties were only 4 and 4.67 respectively. Similarly, the plant height was significantly reduced in New Era and PTB-2 to the extent of 12.70 and 13.50 cm respectively (Table 2). However, a similar significant reduction in root length was not observed. The root length of 15.13 cm in PTB-2 was

Table 3. Nematode number in infested root and soil of different cowpea varieties**

Cowpea variety	Nematode number per gram of infested root	Nematode number in 100 g of soil
New Era	342.30* (2.53)	203.77* (2.31)
Pathinettumaniyan	2.33 (0.63)	2.00 (0.26)
HG-22	3.33 (0.49)	2.67 (0.36)
PEB-1	7.67 (0.88)	2.00 (0.26)
PEB-2	147.33* (2.17)	89.67* (1.95)
C-152	2.67 (0.40)	2.67 (0.42)
Sundari	7.33 (0.81)	3.67 (0.48)
V-16	4.00 (0.58)	3.00 (0.36)
V-37	5.33 (0.62)	4.33 (0.59)
V-240	4.67 (0.62)	2.33 (0.36)
C.D. (5%)	0.32	0.40

** Mean of 3 replications

Figures in paranthesis are logX transformation values for nematode number per gram of infested root and 100 g of soil.

Table 4. Correlation between the number of nematodes in infested root and soil and nodule number and gall formation in different cowpea varieties

	Gall number	Nematode number per gram of root.	Nematode number in 100 g of soil
Nodule number	-0.57716*	-0.58353*	-0.57319*
Gall number		+0.92126*	+0.93471*
Nematode number per gram of infested root			+0.99193*

statistically on par with that of resistant varieties such as HG-22, PTB-1 and C-152. The fresh and dry weight of shoot were also significantly low in both the susceptible varieties. These were only 10.50 and 12.31 g and 0.71 and 1.11 g respectively for New Era and PTB-2 (Table 2).

The nematode number per gram fresh weight of infested root and 100 g of inoculated soil were more in the case of susceptible varieties such as New Era and PTB-2. These were 342.30 and 147.33 and 203.77 and 89.67 respectively and were significantly higher than the population in resistant varieties (Table 3). The lowest number of nematodes in infested root and soil was seen in the case of the resistant variety, Pathinettumaniyan. There was also significant positive correlation between nematode number in one gram fresh weight of infested root and 100 g of inoculated soil and the number of galls formed in susceptible varieties (Table 4). A positive correlation also existed between nematode number in the infested root and soil.

3. Effect of inoculation with different levels of *M.incognita* on nodulation, gall formation and other characters in cowpea

The results of the pot culture experiment to study the effect of inoculation with different levels of *M.incognita* on nodulation, gall formation and other plant characters in

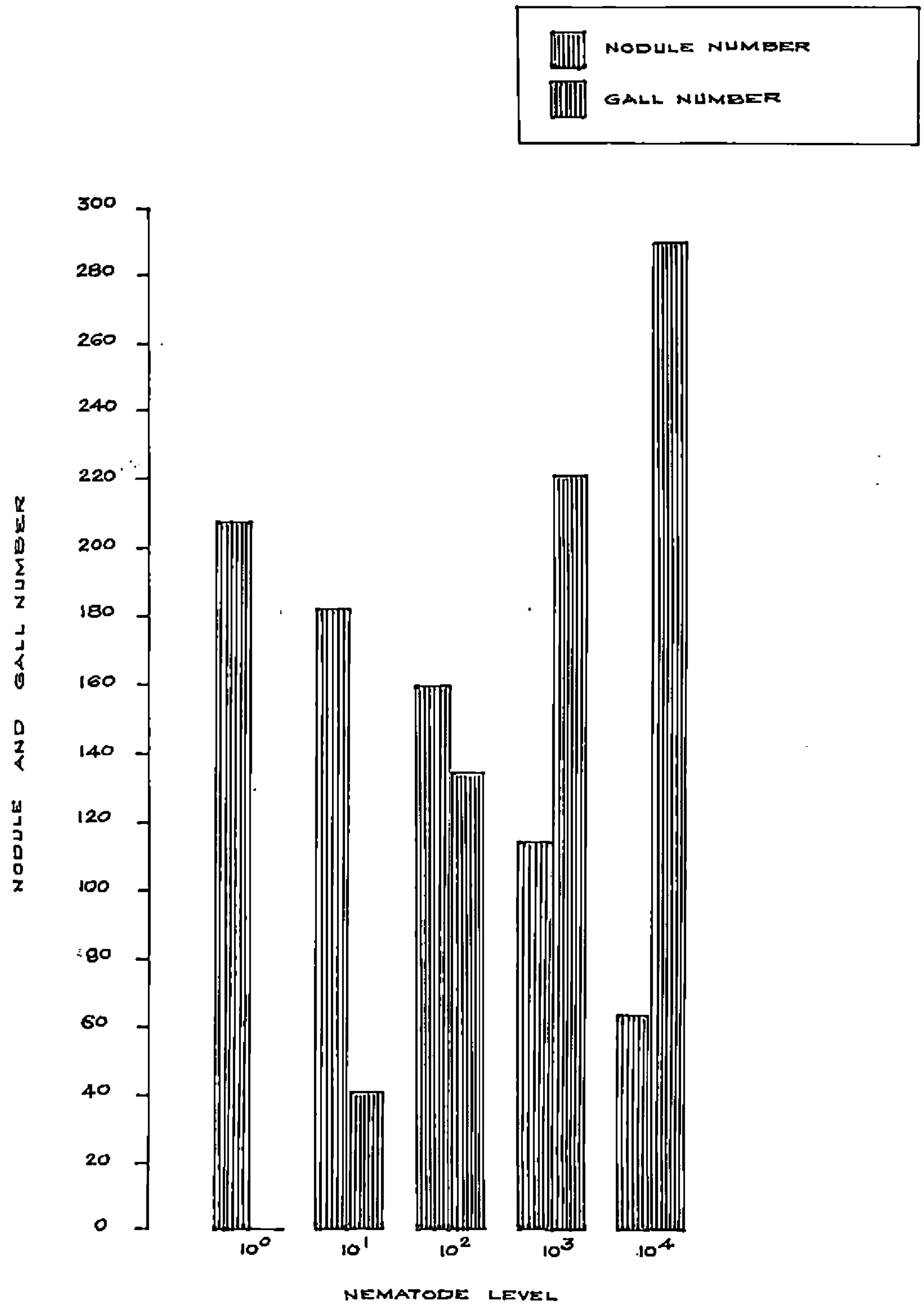
Table 5. Effect** of inoculation with different levels of *Meloidogyne incognita* on nodulation and gall formation in cowpea.

Nematode number	Nodule number	Nodule fresh weight (g)	Gall number	Gall fresh weight (g)	Percentage nitrogen content of shoot
0	209.33 (14.47)	5.43	0 (1)	0	3.34
10	183.50 (13.58)	4.83	41.33* (6.42)	0.55*	3.30
100	161.00* (12.69)	4.03*	135.67* (11.64)	1.53*	3.15
1000	115.33* (10.74)	2.87*	222.00* (14.90)	2.15*	2.60*
10,000	63.33* (7.96)	2.46*	290.67* (17.04)	3.37*	2.37*
C.D.(5%)	1.12	0.96	1.53	0.49	0.34

** Mean of 6 replications

Figures in parenthesis are $\sqrt{X}$ and $\sqrt{X+1}$ transformation values for nodule number and gall number respectively.

FIG. 3. EFFECT OF INOCULATION WITH DIFFERENT LEVELS OF *Meloidogyne incognita* ON NODULATION AND GALL-FORMATION IN COWPEA.



cowpea are presented in Tables 5 to 8 and Figures 3 to 5. The analysis of variance for the same are given in Appendices II(a) and II(b).

A maximum reduction in nodule number was observed in plants inoculated with 10,000 nematodes. Here only 63.33 nodules were formed in comparison to 209.33 nodules of uninoculated control plants (Table 5). Inoculation with 100 and 1000 nematodes also significantly reduced the nodule number (Fig.3). The nodule fresh weight was more or less in proportion to their number and was significantly low in treatments involving 100, 1000 and 10,000 nematodes. The fresh weight was only 2.46 g in plants inoculated with 10,000 nematodes were as in control plants this was as high as 5.43 g (Table 5). However, inoculation with 10 nematodes did not have any significant effect on nodule number and fresh weight (Table 5). Further, nodules in all the treatments, except the control were infested with the nematode.

There was significant increase in gall number and gall fresh weight in various treatments except the control. The maximum increase was observed in plants inoculated with 10,000 nematodes. Here 290.67 galls with a fresh weight of 3.37 g were formed (Table 5 and Fig.3). However, there was no gall formation in uninoculated control plants. The number of

FIG. 4. EFFECT OF INOCULATION WITH DIFFERENT LEVELS OF *Meloidogyne incognita* ON PERCENTAGE NITROGEN CONTENT OF SHOOT IN COW PEA.

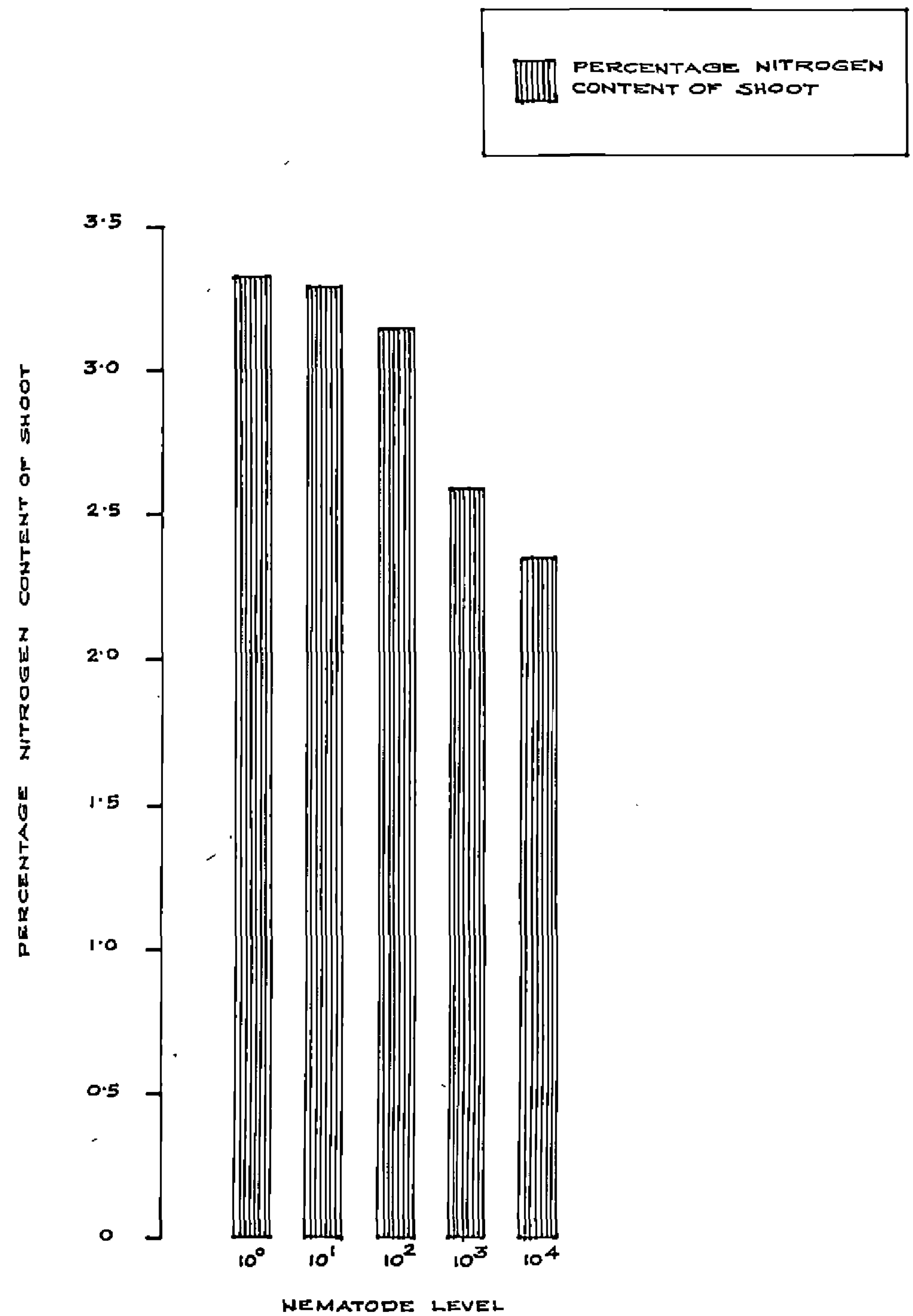


FIG. 5. CORRELATION BETWEEN NODULE NUMBER, GALL NUMBER AND PERCENTAGE NITROGEN CONTENT OF SHOOT IN COW PEA.

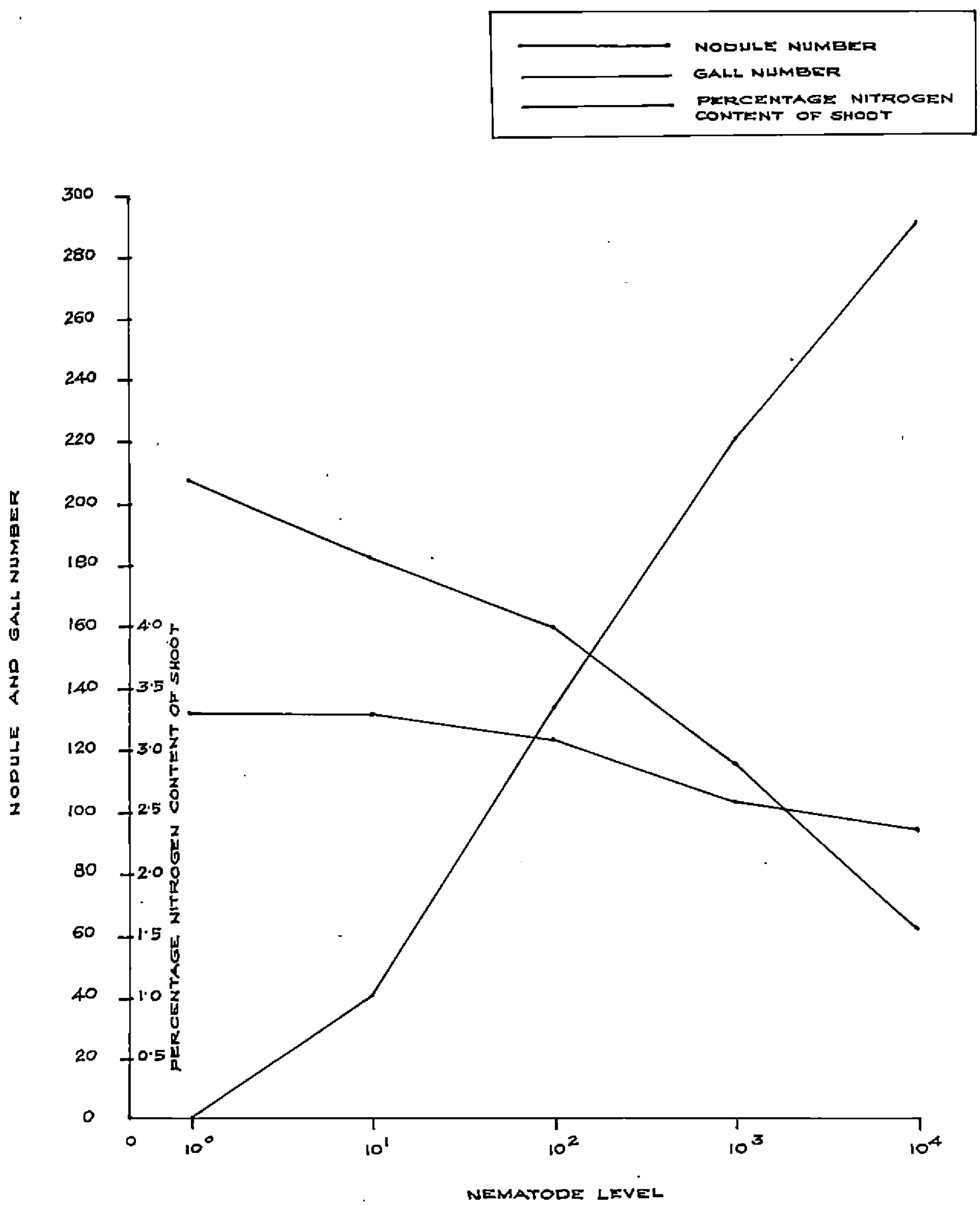


Table 6. Effect of inoculation with different levels of *Meloidogyne incognita* on various plant characters in cowpea.**

Nematode number	Leaf number	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
0	14.17 (3.76)	118.15	62.30	81.17	7.57
10	13.67 (3.69)	119.12	61.13	77.55	6.86
100	12.67 (3.55)	113.90	55.58	75.57	6.05
1000	8.33* (2.88)	78.55*	46.03*	63.05*	3.97*
10,000	5.50* (2.34)	49.63*	35.47*	46.72*	2.26*
C.D.(5%)	0.27	13.43	2.63	7.99	1.69

** Mean of 6 replications

Figures in parenthesis are $\sqrt{X}$ transformation values for leaf number.

galls formed and their fresh weight due to inoculation with 10, 100 and 1000 nematodes was also higher to the extent of 41.33, 135.67, and 222 and 0.55, 1.53 and 2.15 g respectively (Table 5). There was also significant negative correlation between gall formation and nodule number in different nematode inoculated treatments (Table 8 and Fig.3 and 5).

A significant reduction in percentage nitrogen content of shoot was observed as a result of inoculation with 1000 and 10,000 nematodes. The percentage nitrogen content of plants in these treatments were only 2.60 and 2.37 respectively in comparison to 3.34 per cent of control plants (Table 5 and Fig.4). But there was no significant reduction in the nitrogen content of plants inoculated with 10 and 100 nematodes (Table 5). A significant positive correlation between nodule number and percentage nitrogen content of shoot was observed (Table 8 and Fig.5). However, the correlation between gall number and percentage nitrogen content of shoot was significantly negative.

The number of leaves formed per plant, plant height, root length and fresh and dry weight of shoot were also significantly low due to nematode inoculation especially with 1000 and 10,000 larvae per plant. The maximum deleterious

Table 7. Effect of inoculation with different levels of <u>Meloidogyne incognita</u> on nematode number in infested root and soil of cowpea

Nematode number	Nematode number per gram of infested root	Nematode number in 100 g of soil
0	0 (0)	0 (0)
10	49.67* (1.70)	18.17 * (1.27)
100	113.33* (2.15)	56.67* (1.75)
1000	228.00* (2.37)	120.83* (2.08)
10000	290.50* (2.46)	284.67* (2.45)
C.D.(5%)	0.19	0.15

** Mean of 6 replications

Figures in paranthesis are log X+1 transformation values for nematode number per gram of infested root and 100 g of soil.

Table 8. Correlation between the number of nematodes in infested root and soil and nodule number, gall formation and percentage nitrogen content of shoot in cowpea

	Gall number	Percentage nitrogen content of shoot.	Nematode number per gram of root infested	Nematode number in 100 g of soil
Nodule number	-.86265*	.74782*	-.89265*	-.85766*
Gall number		-.79369*	.94150*	.88179*
Percentage nitrogen content of shoot.			-.83409*	-.79370*
Nematode number per gram of infested root.				.88242*

effects were observed as a result of inoculation with 10,000 larvae. There were only 5.50 leaves in these plants in comparison to 14.17 of control plants (Table 6). Similarly, the plant height and root length were only 49.63 and 35.47 cm respectively, in this treatment. The fresh and dry weight of shoot were 46.72 and 2.26 g respectively when compared to 81.17 and 7.57 g for uninoculated plants (Table 6). However, inoculation with 10 and 100 nematodes did not have any significant effect on these plant characters.

A significant increase in nematode number in one gram fresh weight of infested root and 100 g inoculated soil was observed. This increase was maximum in plants inoculated with 10,000 larvae. There were 290.50 and 284.67 nematodes respectively in infested root and soil of this treatment. The increase in nematode number was also higher in plants inoculated with 100 and 1000 nematodes (Table 7). There was a significant positive correlation between nematode number in one gram fresh weight of infested root and 100 g of inoculated soil and the number of galls formed in different treatments (Table 8). A positive correlation also existed between nematode number in infested root and soil.

4. Effect of age of host plant and infestation by *M.incognita* on nodulation, gall formation and other characters in cowpea

The results of the pot culture experiment to study the effect of age of host plant and infestation by *M.incognita* on nodulation, gall formation and other plant characters in cowpea are presented in Tables 9 to 12, Figures 6 to 8 and Plates VI to XXI. The analysis of variance for the same are given in Appendices III(a) and III (b).

The susceptible variety of cowpea, New Era, was inoculated with 1000 second stage larvae of *M.incognita* at the time of sowing and on 7th, 14th, 21st and 28th day of plant growth. There was significant reduction in nodule number and fresh weight in different treatments when compared to control. The maximum reduction was observed on 14th day. The number of nodules formed and their fresh weight in this treatment were only 23.80 and 0.43 g respectively in comparison to 234 and 5.31 g for control plants (Table 9, Fig. 6 and Plates XIII and XIV). The nodule number and fresh weight due to inoculation at the time of sowing and on the 7th day of plant growth were also low to the extent of 46.80 and 37.60 and 1.73 and 1.31 g respectively (Table 9 and Plates VII, VIII, X & XI). A similar reduction was seen as a result of nematode inoculation

Table 9. Effect** of age of host plant and nematode infestation on nodulation and gall formation in cowpea

Day of inoculation	Nodule number	Nodule fresh weight (g)	Gall number	Gall fresh weight (g)	Percentage nitrogen content of shoot
0	46.80* (6.77)	1.73*	115.80* (10.78)	2.04*	2.70*
7	37.60* (6.00)	1.31*	116.20* (10.81)	2.12*	2.54*
14	23.80* (4.85)	0.43*	127.00* (11.17)	2.26*	2.34*
21	76.40* (8.73)	2.38*	87.20* (9.37)	1.43*	2.69*
28	94.80* (9.73)	2.89*	77.20* (8.83)	1.19*	3.02*
Control	234.00 (15.26)	5.31	0 (1)	0	3.47
C.D.(5%)	1.00	0.77	1.32	0.50	0.16

** Mean of 5 replications

Figures in paranthesis are $\sqrt{X}$ and $\sqrt{X+1}$ transformation values for nodule number and gall number respectively.

FIG. 6. EFFECT OF AGE OF THE PLANT AND INFESTATION BY *Meloidogyne incognita* ON NODULATION AND GALL-FORMATION IN COWPEA.

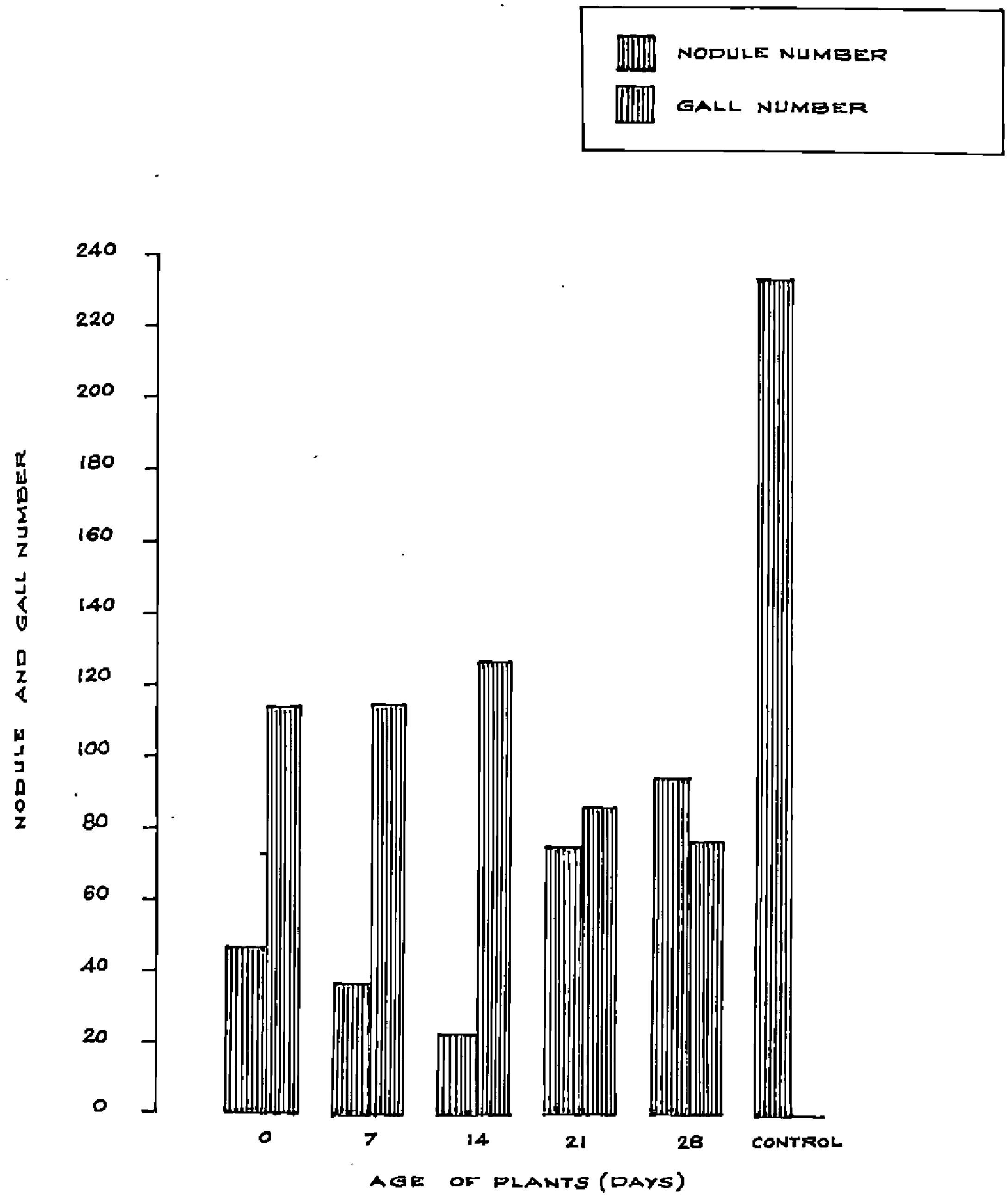


Plate VI

Effect of nematode inoculation at the time of sowing on plant height and leaf number of New Era

C-Control

1 - nematode inoculation at the time of sowing

Plate - VI

Plate VII

Effect of nematode inoculation at the time of sowing on nodulation and gall formation in New Era.

C . Control

1 . nematode inoculation at the time of sowing

Plate VIII

Effect of nematode inoculation at the time of sowing on nodulation and gall formation in New Era (enlarged view).

C . Control

1 . nematode inoculation at the time of sowing.


C
I

Plate IX

Effect of nematode inoculation on 7th day of plant growth on plant height and leaf number of New Era.

C. control

2. nematode inoculation on 7th day of plant growth.

Plate - IX

Plate X

Effect of nematode inoculation on 7th day of plant growth on nodulation and gall formation in New Era.

C. control

2. nematode inoculation on 7th day of plant growth.

Plate XI

Effect of nematode inoculation on 7th day of plant growth on nodulation and gall formation in New Era (enlarged view).

C. control

2. nematode inoculation on 7th day of plant growth.

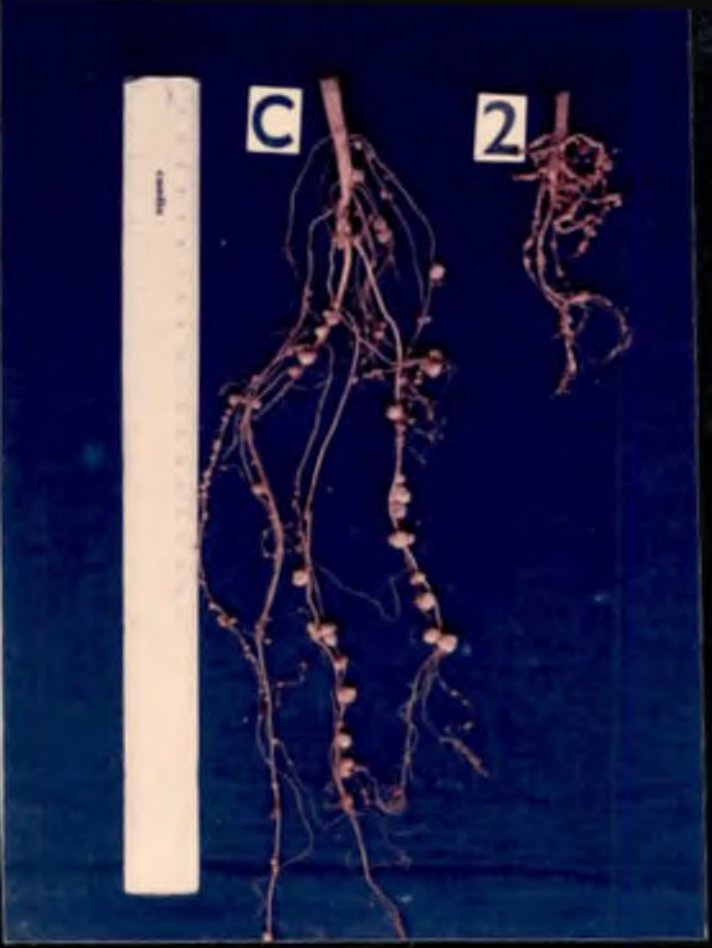


Plate - X

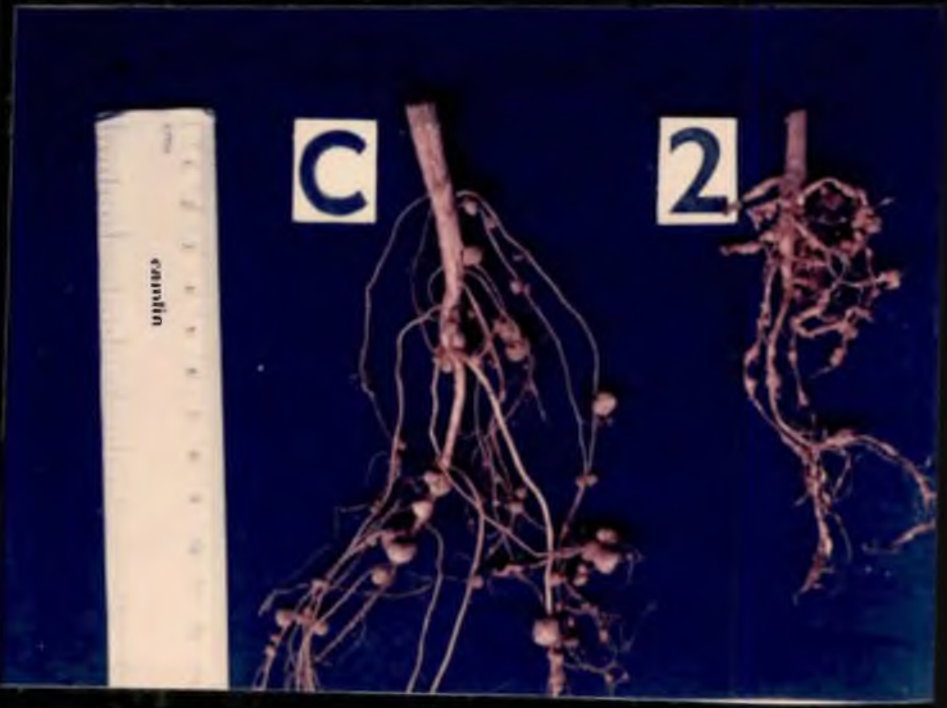


Plate-XI

Plate XII

Effect of nematode inoculation on 14th day of plant growth on plant height and leaf number of New Era.

C. control

3. nematode inoculation on 14th day of plant growth.

Plate - XII

Plate XIII

Effect of nematode inoculation on 14th day of plant growth on nodulation and gall formation in New Era.

C. control

3. nematode inoculation on 14th day of plant growth.

Plate XIV

Effect of nematode inoculation on 14th day of plant growth on nodulation and gall formation in New Era (enlarged view).

C. control.

3. nematode inoculation on 14th day of plant growth.


C
3

Plate XV

Effect of nematode inoculation on 21st day of plant growth on plant height and leaf number of New Era

C. control

4. nematode inoculation on 21st day of plant growth.


C
4

Plate XVI

Effect of nematode inoculation on 21st day of plant growth on nodulation and gall formation in New Era.

C. control

4. nematode inoculation on 21st day of plant growth.

Plate XVII

Effect of nematode inoculation on 21st day of plant growth on nodulation and gall formation in New Era (enlarged view).

C. control

4. nematode inoculation on 21st day of plant growth.

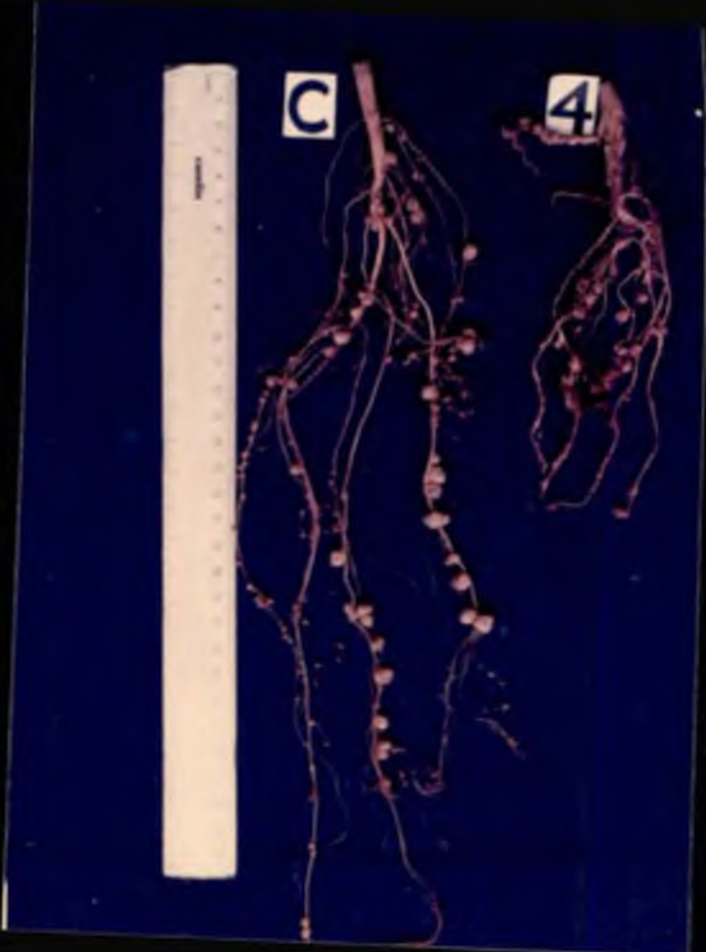

C
4

Plate XVIII

Effect of nematode inoculation on 28th day of plant growth on plant height and leaf number of New Era.

C. control

5. nematode inoculation on 28th day of plant growth.

Plate-XVIII

Plate XIX

Effect of nematode inoculation on 28th day of plant growth on nodulation and gall formation in New Era.

C. control

5. nematode inoculation on 28th day of plant growth.

Plate XX

Effect of nematode inoculation on 28th day of plant growth on nodulation and gall formation in New Era (enlarged view).

C. control

5. nematode inoculation on 28th day of plant growth.

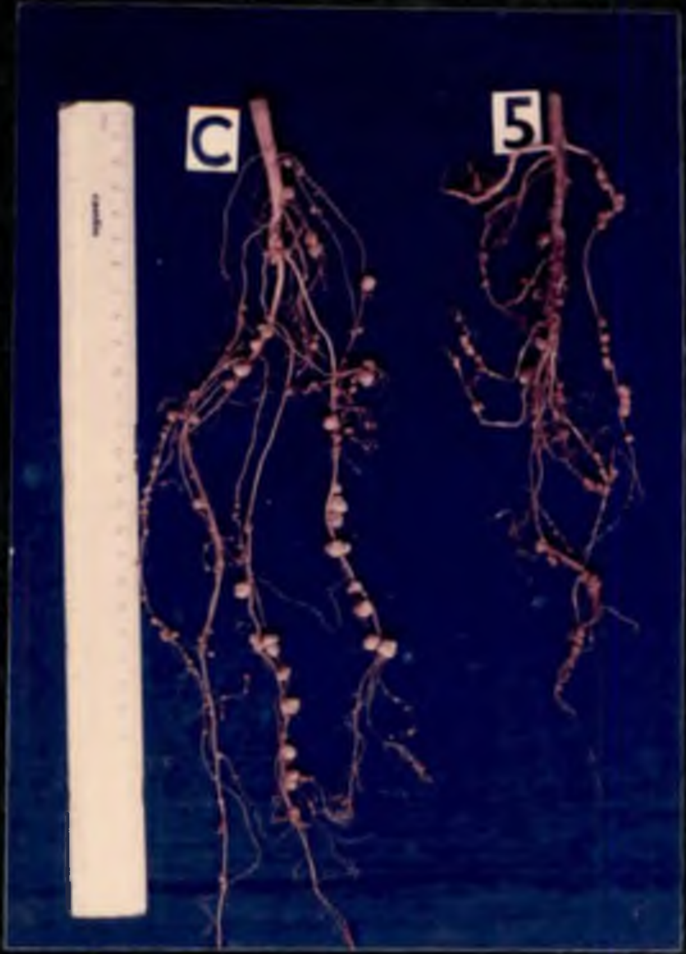


Plate - XIX

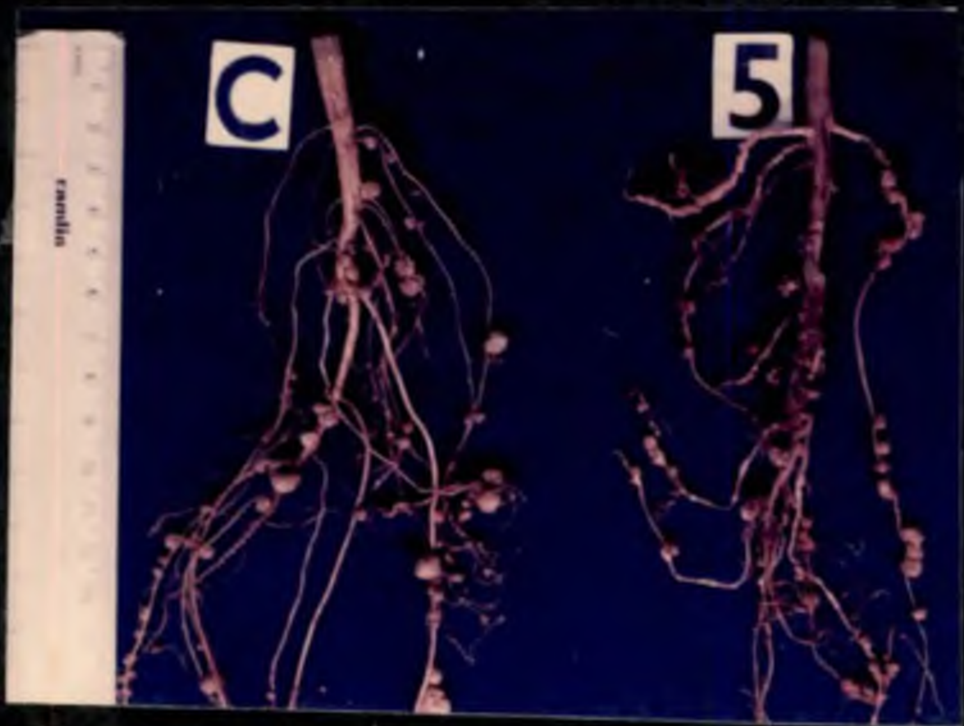


Plate-XX

Plate XXI

Effect of nematode infestation of cowpea root nodule.

1. healthy nodule

2. nematode infested nodule.

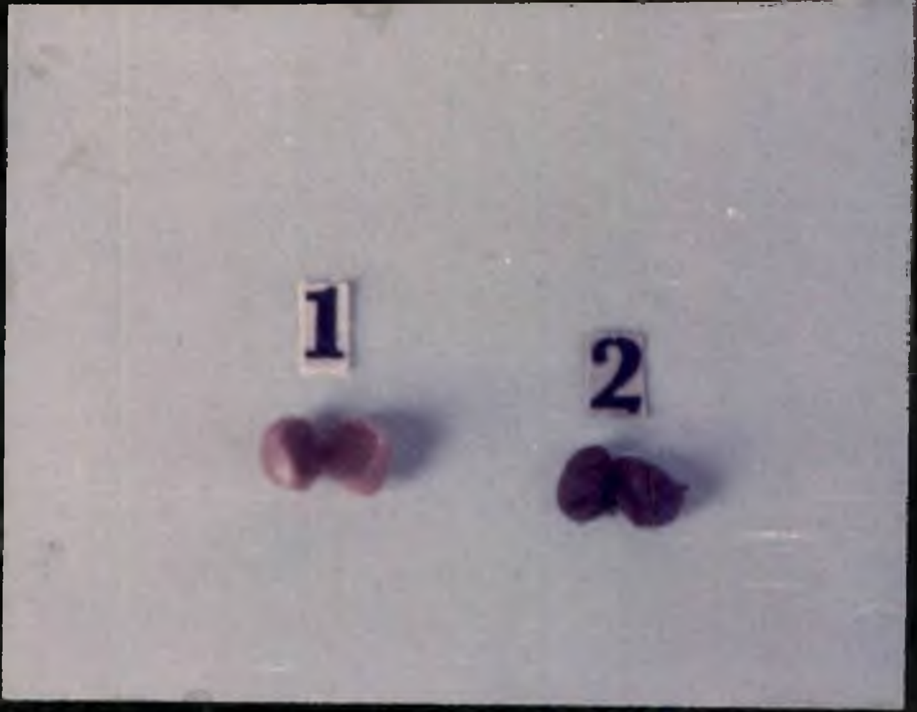


Plate - XXI

FIG. 7. EFFECT OF AGE OF THE PLANT AND INFESTATION BY *Meloidogyne incognita* ON PERCENTAGE NITROGEN CONTENT OF SHOOT IN COW PEA.

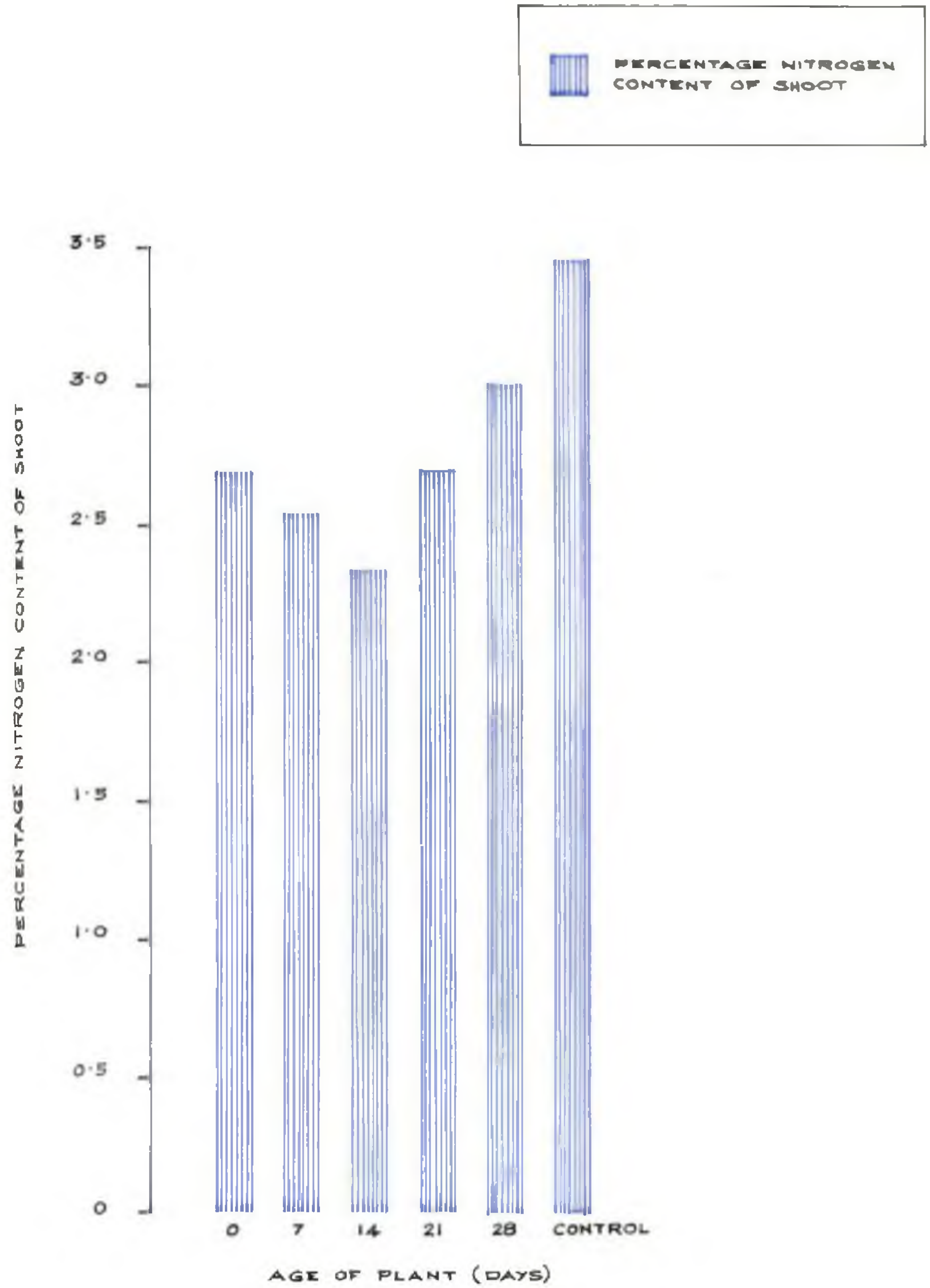
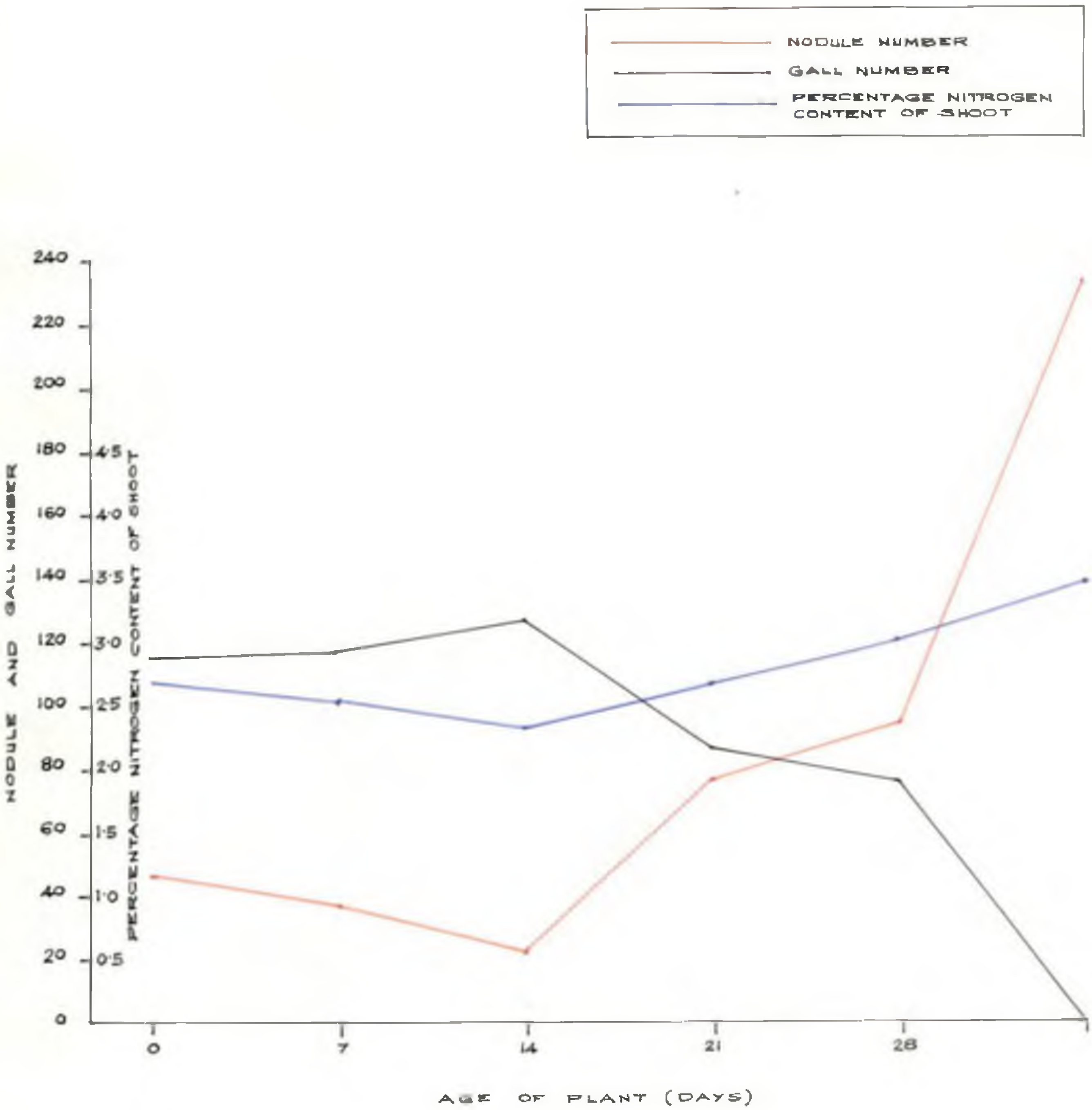


FIG. 8. CORRELATION BETWEEN NODULE NUMBER, GALL NUMBER AND PERCENTAGE NITROGEN CONTENT OF SHOOT IN COW PEA.



on 21st and 28th day as well (Table 9 and Plates XVI, XVII, XIX & XX). Further, the nodules in all the treatments, except the control were infested with the nematode (Plate XXI).

There was significant increase in gall number and their fresh weight in all the treatments except the control. The maximum increase was observed on 14th day. Here, 127 galls with a fresh weight of 2.26 g were formed (Table 9 Fig.6 and Plates XIII and XIV). However, there was no gall formation in uninoculated control plants. The number of galls formed and their fresh weight due to nematode inoculation at the time of sowing and on 7th day were also higher to the extent of 115.80 and 116.20 and 2.04 and 2.12 g respectively (Table 9 and Plates VII, VIII, X and XI). A similar increase was seen as a result of nematode inoculation on 21st and 28th day as well (Plates XVI, XVII, XIX and XX). There was also significant negative correlation between gall formation and nodule number in different nematode inoculated treatments (Table 12 and Fig.6 and 8).

A significant reduction in percentage nitrogen content of shoot was observed due to nematode inoculation at different stages of plant growth. The maximum reduction was seen on 14th day. The percentage nitrogen content of plants in this treatment was only 2.34 in comparison to 3.47 per cent

Table 10. Effect of age of host plant and nematode infestation on various plant characters in cowpea**

Day of inoculation	Leaf number	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
0	7.20* (2.66)	46.74*	18.66 *	24.06*	2.47 *
7	4.60* (2.12)	29.14*	11.62 *	16.26 *	1.97*
14	4.00 * (1.98)	15.10*	7.94*	10.93*	0.90*
21	7.80 * (2.79)	46.14*	19.62*	20.82*	2.45*
28	8.00* (2.82)	53.30*	27.72*	26.86*	3.83*
Control	15.00 (3.87)	141.02	43.34	47.74	6.37
C.D.(5%)	0.39	14.78	6.36	7.57	1.42

** Mean of 5 replications

Figures in paranthesis are $\sqrt{X}$ transformation values for leaf number for control plants (Table 9 and Fig.7). The nitrogen content of shoot was also low due to nematode inoculation at the time of sowing and on 7th and 21st day of plant growth. A significan positive correlation between nodule number and percentage nit content of shoot was observed (Table 12 and Fig.8). However, the correlation between gall number and percentage nitrogen content of the shoot was significantly negative.

The number of leaves formed per plant, plant height, root length, fresh and dry weight of shoot were also significantly low due to nematode inoculation. The maximum deleterious effects were observed on 14th day of plant growth followed, by inoculation at the time of sowing and on 7th, 21st and 28th day of plant growth (Table 10, Plates XII, VI, IX, XV and XVIII). There were only 4 leaves in plants inoculated on 14th day in comparison to 15 leaves in control plants (Table 10 and Plate XII). Similarly, the plant height and root length were only 15.10 and 7.94 cm respectively in this treatment (Plates XII and XIII). The fresh and dry weight of shoot were 10.93 and 0.90 g respectively when compared to 47.74 and 6.37 g for uninoculated control plants (Table 10). The inoculation with the nematode at other stages of plant growth also had a significant effect on these plant characters especially with inoculation at the time of sowing and on 7th and 21st day of plant growth.

Table 11. Effect of age of plant on nematode number** in infested root and soil of cowpea.

Day of inoculation	Nematode number per gram of infested root.	Nematode number in 100 g of soil
0	304.80* (2.48)	157.00 * (2.19)
7	344.00* (2.53)	175.80* (2.25)
14	377.00* (2.58)	191.80* (2.28)
21	307.00* (2.47)	153.40* (2.19)
28	242.00* (2.38)	94.20 * (1.97)
Control	0 (0)	0 (0)
C.D. (5%)	0.07	0.09

** Mean of 5 replications

Figures in paranthesis are log X + 1 transformation values for nematode number per g fresh weight of infested root and 100 g of soil respectively

Table 12. Correlation between the number of nematodes in infested root and soil and nodule number, gall formation and percentage nitrogen content of shoot in cowpea.

	Gall number	Percentage nitrogen content of shoot	Nematode number per gram of infested root	Nematode number in 100 g of soil
Nodule number	-.88344*	.91179*	-.92587*	-.94014*
Gall number		-.83018*	.89054*	.82003*
Percentage nitrogen content of shoot			-.87180*	-.92462*
Nematode number per gram of infested root				.89338*

A significant increase in nematode number in one gram fresh weight of infested root and 100 g of inoculated soil was observed. This increase was maximum in plants inoculated on 14th day. There were 377 and 191.80 nematodes respectively in the infested root and soil of this treatment (Table 11). The increase in nematode number was also higher in plants inoculated at the time of sowing and on 7th and 21st day of plant growth. There was a significant positive correlation between nematode number in one gram fresh weight of infested root and 100 g of inoculated soil and the number of galls formed in different treatments (Table 12). A positive correlation also existed between the nematode number in infested root and soil.

DISCUSSION

DISCUSSION

Root-knot nematodes infest a large number of crop plants especially fruits, vegetables and cereals. More than 50 *Meloidogyne* spp. are currently described. They account for nearly 95 per cent of all nematode infestations and cause an average crop loss of about 5 per cent on a world wide basis (Sasser *et al.* 1983). In Kerala, mainly two species of *Meloidogyne*, *M. incognita* and *M. javanica* are seen. Out of these two species, *M. incognita* is widely distributed in the state causing considerable economic loss for a large number of cultivated crops including several pulses and vegetables (Venketesan 1984). Among the pulses grown in Kerala, cowpea (*Vigna unguiculata* (L.) Walp) is cultivated both for vegetable and grain purpose. Infestation of this crop by *M. incognita* in Kerala was first reported by Mammen (1973). However, a detailed study on the effect of this nematode infestation on nodulation by *Rhizobium* is yet to be done. It was under this circumstance, the present investigation was carried out. Three separate pot culture experiments were conducted to find out the effect of host variety, inoculum level and age of host plant on infestation by *M. incognita* in cowpea.

The occurrence of resistant varieties against a particular pathogen is a well documented phenomenon among a large number of cultivated crops. Therefore, in the first part of this investigation 10 cowpea varieties, New Era, Pathinettumaniyan, HG-22, PTB-1, PTB-2, C-152, Sundari, V-16, V-37 and V-240 were screened for resistance to infestation by *M.incognita*. In this study, 8 varieties namely Pathinettumaniyan, HG-22, PTB-1, C-152, Sundari, V-16, V-37 and V-240 were found resistant to this nematode. In these varieties there was no gall formation by inoculation with 1000 second stage larvae of *M.incognita* on 14th day of plant growth (Table-1 and Plates I, II, III and V). However, in the remaining two varieties, New Era and PTB-2 there was gall formation. In these susceptible varieties, 141.33 and 64.33 galls were formed per plant with a maximum fresh weight of 1.41 g in New Era (Table 1 and Plate IV). Cowpea varieties resistant to *M.incognita* were reported earlier by a number of other workers such as Hare (1959) Amosu (1974) Singh *et al*.(1975) and Sharma and Sethi (1976). In fact, two of the varieties C-152 and V-16 found resistant to *M.incognita* during this investigation were also reported earlier to be resistant to this nematode by Patel *et al*.(1977) and Thakar and Patel (1983) respectively.

The formation of root-knots or galls in the susceptible varieties of cowpea had a significant effect on nodulation and other plant characters. There was significant negative correlation between gall formation and nodulation in both New Era and PTB-2 (Table 4). In these varieties 19.67 and 24.33 nodules were only formed when compared to a maximum of 90 nodules formed in the resistant variety, V-37 (Table 1). However, the fresh weight of nodules in all the varieties was in proportion to their number. There was also significant reduction in leaf number, plant height and fresh and dry weight of shoot, in both the susceptible varieties (Table 2). Such ill effects due to nematode infestation is reported earlier by Epps and Chambers (1962) in soybean, Srivastava et al.(1975) in chick pea, Castillo et al.(1978) in mung bean and Sharma et al.(1978) in groundnut.

An interesting observation made during this investigation was a preferential colonisation by M.incognita in the rhizosphere of the susceptible varieties of cowpea. A significant increase in nematode number was observed in the infested root and soil of both New Era and PTB-2, when compared to other resistant varieties (Table 3). This increase in nematode number had a significant effect on both nodulation and gall formation. Thus in New Era and PTB-2, there was significant positive correlation between nematode number in infested root and soil and the number of galls formed. However, the correlation between nematode number and nodulation was significantly negative for these varieties (Table 4). In New Era and PTB-2, there was also a decrease in nodule number with an increase in the number of galls formed. The probable reason for such a reduction in nodulation as a result of nematode infestation is discussed later in this chapter.

The presence of resistant genes in cowpea preventing infestation by M.incognita were reported by Amosu (1974) and later by Raj and Patel (1978). However, the actual mechanism of providing resistance to infestation by Meloidogyne spp. by these genes is not yet clear. The presence of certain inhibitory substances in the root exudates of resistant varieties as a product of these resistant genes cannot be ruled out. This appears to be true to a certain extent because a significant increase in nematode number in infested root and soil during this investigation was observed only in the case of the susceptible varieties, New Era and PTB-2, eventhough both the resistant and susceptible varieties were inoculated with an identical number of 1000 second stage larvae on 14th day of plant growth (Table 3). Therefore, it will be worthwhile to conduct further investigation on this line under controlled conditions in order to identify the exact nature of the resistant factor present in the root exudates of the resistant varieties.

The identification of cowpea varieties resistant to *M.incognita* is important from another point of view, in that it will be possible to cultivate such varieties throughout the state even in areas known to have an endemic population of *M.incognita*. This will avoid an unnecessary biomagnification of this nematode in areas suspected to have an suboptimal population in the soil. This will also be beneficial to the farmer because it will enable him to raise successfully a succeeding crop susceptible to this nematode which will not have been possible otherwise, if a susceptible variety of cowpea was cultivated in this area earlier. Some of the resistant varieties identified during this investigation namely Pathinettumaniyan, PTB-1 and C-152 are in fact very popular among the farmers of the state. Therefore all attempts should be made to popularise these varieties for cultivation in areas found to have an endemic or marginal population of *M.incognita*.

In the second part of the present investigation, a pot culture experiment was conducted to find out the minimum number of nematode required to produce a significant effect on nodulation, gall formation and other growth characters in cowpea. For this, one of the susceptible varieties of cowpea identified earlier, New Era was used. Five different inoculum levels, 0, 10, 100, 1000 and 10,000 second stage larvae per plant were used for inoculation on 15th day of plant growth. A significant reduction in nodulation was observed in all plants inoculated with 100, 1000 and 10,000 larvae with a maximum reduction in plants inoculated with 10,000 nematodes. Here, only 63.33 nodules were formed in comparison to 209.33 nodules formed in the uninoculated control plants (Table 5). The fresh weight of nodules was in proportion to the number in all the treatments. However, there was no significant reduction for these characters in plants inoculated with only 10 nematodes. Therefore, it appears that a minimum of 100 nematodes per plant is required to produce any significant effect on nodulation in cowpea. Balasubramanian (1971), Singh et al.(1977) and Nath et al.(1979) have all reported a minimum threshold level of 100 nematodes per plant to produce any significant effect on nodulation and other characters in soybean, mung bean and chick pea respectively.

The reduction in nodulation by Rhizobium when a minimum threshold level of nematode is present in the soil is believed to be due to the larvae interfering directly with the establishment of Rhizobium on root surface. Further, as reported by Balasubramonian (1971), the nematode infestation may also alter the affinity of the host plant towards the microsymbiont. The possibility of nematode feeding on Rhizobium as such or the soft nodule primordia can be another reason for the observed reduction in nodulation. This is because whenever there was an increase in nematode population in infested root and soil, there was also a significant reduction in nodule number (Table 8). Apart from these factors, the overall reduction in root system resulting from gall formation can also greatly reduce the available root surface area for the Rhizobium to enter and initiate nodule formation. It is in this context, the competition between Rhizobium and nematode for early root colonisation and host nutrients as suggested by Masefield (1958) becomes important. However, it is difficult to arrive at a definite conclusion by the present investigation whether there was an actual competition between the macro and the micro organism for root colonisation, since the visible effects of root infestation by both were seen more or less simultaneously by the third week of inoculation.

There was a progressive increase in gall number and fresh weight as the inoculum level increased from 10 to 10,000. The maximum number of galls were formed in plants inoculated with 10,000 nematodes. Here, 290.67 galls with a fresh weight of 3.37 g were formed (Table 5 and Fig.3). The number of galls formed were also higher in plants inoculated with 100 and 1000 nematodes per plant. They were 135.67 and 222 respectively. This increase in gall number as the inoculum level of nematode increased was in accordance with the expected behaviour of the pathogen in the presence of a susceptible host. It is also apparent that the number of galls formed as a result of inoculation with 1000 and 10,000 larvae are much higher than the number of galls formed by inoculating with only 10 nematodes per plant (Table 5 and Fig.3).

The effect of progressive increase in gall formation by an increasing level of the nematode inoculum also resulting in a consequential decrease in nodulation was seen in the percentage nitrogen content of shoot in cowpea. A significant decrease in the nitrogen content of shoot was observed in all plants inoculated with 1000 and 10,000 nematodes in comparison to other treatments (Table 5 and Fig. 4). The nitrogen content of plants in these treatments were only 2.60 and 2.37 per cent respectively when compared to 3.34 per cent for the uninoculated control plants. It is also interesting to note that there was no significant reduction in nitrogen content after inoculation with 10 and 100 nematodes (Table 5) since plant nitrogen content is an indication of its overall growth and seed production capacity, the above observation that a significant reduction in the percentage nitrogen content of shoot was obtained only with the inoculation of 1000 and 10,000 nematodes is important because it indicated that the minimum threshold level of M.incognita required to produce any adverse effect on plant growth in cowpea is 1000 larvae per plant.

What can be the reason for the reduction in nitrogen content of plants? The extent of gall formation and its consequent effect on root growth, nodulation and nutrient uptake by the host plant appears to be the main reason for the reduction in nitrogen content of plants especially due to inoculation with 1000 and 10,000 nematodes. This reduction in nitrogen content in all treatments except the control even when NPK fertilizers were added uniformly supports this hypothesis. An actual infestation of root nodules by M.incognita was also observed during the investigation. Such nodules were soft and dark in colour in comparison to the hard slightly pink centred nodules of uninoculated control plants. These nodules also deteriorated much rapidly than the normal healthy nodules. This can infact lead to a depletion in the amount of fixed nitrogen available to host plant. Two other reasons reported earlier to affect the efficiency of nitrogen fixation by Rhizobium in nematode infested plants were the findings that the nematode could interfere with the nitrogen fixing process of Rhizobium by producing certain hormones which could affect the metabolic activity of the microsymbiont (Singh et al. 1977). Secondly, the nematode would actually inhibit the transfer of fixed nitrogen by Rhizobium to host plant by some unknown mechanism (Walker 1957 and Ali et al. 1981). However, these hypotheses were not tested during this investigation since a more fundamental study of the nematode and Rhizobium interaction will be required to understand the real effect of nematode on the physiological activity of the diazotroph.

The above effects of increased gall formation and reduced nodulation and nitrogen fixation due to an increasing level of the pathogen were also observed in other plant characters studied during this investigation. The number of leaves formed per plant, plant height, root length, fresh and dry weight of shoot were all less with an increasing level of nematode. However, a significant reduction was obtained only with the inoculation of 1000 and 10,000 larvae per plant (Table 6). These observations further support the conclusion arrived earlier based on plant nitrogen content, that 1000 nematodes per plant is the minimum threshold level required to produce any significant effect on growth of cowpea.

There was nothing unusual in the residual nematode population of infested root and soil in different treatments. The number of nematodes in both the cases increased with an increase in the initial inoculum level from 10 to 10,000. The effect of the continuous presence of a higher nematode population in plant rhizosphere was also seen in the significant positive correlation that existed between nematode number and gall formation and the significantly negative correlation between nematode number and nodulation and percentage nitrogen content of shoot in cowpea.

In the last part of the present investigation, the actual effect of age of host plant on infestation by M.incognita was investigated. This experiment was also done in pots by inoculating the susceptible variety, New Era, with 1000 nematodes from the date of sowing to 28 days of plant growth. Observations on nodulation, gall formation, percentage nitrogen content of shoot and nematode population in infested root and soil were taken on 55th day of plant growth. It appears from the literature reviewed that, not much work is done to understand the effect of host age and infestation by M.incognita in pulses, especially in cowpea. Therefore, the present study was carried out with the hope that some useful information will be available with regard to the relative susceptibility of cowpea at different stages of plant growth to infestation by this nematode.

In general, the plants were found more susceptible to nematode during the early stages of growth upto 21 days. Fourteen day old plants were found most susceptible to this nematode infestation. Thus, the reduction in nodulation, nodule fresh weight and percentage nitrogen content of shoot were maximum in this treatment. The number of nodules formed and their fresh weight were only 23.80 and 0.43 g respectively in comparison to 234.0 and 5.31 g for control plants (Table 9, Fig.6, Plates XIII and XIV). The percentage nitrogen content of shoot was only 2.34 when compared to 3.47 per cent for the uninoculated control plants (Table 9 and Fig.7). The nitrogen content was also low due to nematode inoculation at the time of sowing and on 7th and 21st day of plant growth.

There was significant increase in gall number and their fresh weight in all treatments except the control. The maximum increase was however, in plants inoculated on 14th day. Here, 127 galls with a fresh weight of 2.26 g were formed (Table 9). The number of leaves formed per plant, plant height, root length, fresh and dry weight of shoot were all significantly low due to nematode inoculation at different stages of plant growth. However, these effects were again maximum in plants treated with M.incognita on 14th day followed by inoculation at the time of sowing and on 7th, 21st and 28th day of plant growth (Table 10).

The above study thus indicated that there was a progressive increase in the susceptibility of cowpea to infestation by M.incognita from the time of seed germination. It reached a peak around 14th day after which there was a decline in the extent of susceptibility to this nematode. The early increase in the susceptibility of cowpea to M.incognita may be due to the presence of certain factors in the host root exudates which attracted the pathogen towards its root system. It is a well established fact that there is considerable difference in the nature and composition of root exudates during the early and latter stages of plant growth. Thus in a susceptible variety of cowpea, there should be some factor present in adequate quantity in the root exudates during the early stages which increased the affinity of the nematode to its host plant. This rhizosphere factor may get modified or become less available during subsequent stages of plant growth. This is also evident from the gradual decline in the nematode population in infested root and soil samples of plants inoculated on 21st and 28th day. It is therefore essential to conduct a detailed study on the nature and composition of root exudates of both susceptible and resistant varieties in order to identify such factors which favour the growth and multiplication of the pathogen in the rhizosphere of a susceptible variety or inhibit its growth in the rhizosphere of a resistant variety. Another reason for the early increase in the infestation of cowpea by M.incognita may be the preference of the nematode for the soft meristematic root tissues for its colonisation. A similar result was reported earlier by Jamal (1976) in the case of chick pea.

Based on the present investigation following important observations are made with regard to infestation by M.incognita in cowpea.

1. There is a varietal variation in cowpea to infestation by M.incognita. Two of the varieties, New Era and PTB-2 cultivated at present in the state are highly susceptible to this nematode while three other popular varieties such as Pathinettumaniyan, PTB-1 and C-152 are resistant. Therefore, all attempts should be made to popularise these varieties for cultivation by the farmer throughout the state especially in areas suspected to have an endemic or marginal population of M.incognita.

2. A minimum inoculum level of 1000 second stage larvae of *M.incognita* is required to produce an uniform significant effect on various plant characters studied especially on nodulation, gall formation and percentage nitrogen content of shoot.

3. Cowpea is most susceptible to infestation by *M.incognita* during the early stages, especially around 14th day of plant growth.

SUMMARY

SUMMARY

An investigation was carried out at College of Agriculture, Vellayani, Kerala during 1982-85 to study the effect of infestation with the root-knot nematode *M.incognita* on nodulation in cowpea. Three separate pot culture experiments were conducted using sterilized soil to study the influence of host variety, inoculum level and age of host plant on infestation by *M.incognita* in cowpea.

Ten varieties of cowpea namely, New Era, Pathinettumaniyan HG-22, PTB-1, PTB-2, C-152, Sundari, V-16, V-37 and V-240 were screened for resistance to *M.incognita*. The experiment was laid down in completely randomised design with 3 replications each. The seedlings were inoculated with 1000 numbers of second stage larvae of the nematode on 14th day of plant growth. Following observations on gall number, gall fresh weight, nodule number, nodule fresh weight, leaf number, plant height, root length, fresh and dry weight of shoot and nematode population in infested root and soil were taken on 50th day of plant growth.

Among the 10 varieties of cowpea screened for resistance to the nematode, two varieties, New Era and PTB-2 were found susceptible. In these varieties 141.33 and 64.33 galls were

formed per plant. However, in the remaining eight varieties such as Pathinettumaniyan, HG-22, PTB-1, C-152, Sundari, V-16, V-37 and V-240 there were no gall formation. The fresh weight of galls was in proportion to their number in susceptible varieties with a maximum of 1.41 g in New Era. In these varieties there was a significant negative correlation between gall formation and nodule number. In New Era and PTB-2, only 19.67 and 24.33 nodules were formed. This was significantly lesser than the number of nodules in resistant varieties where a maximum of 90 nodules were formed in the variety V-37 followed by C-152. The nodules in New Era and PTB-2 were infested by the nematode and were soft and dark brown in colour when compared to the hard and flesh coloured nodules of resistant varieties. The nodule fresh weight was also significantly low in susceptible varieties.

The number of leaves formed per plant, plant height and fresh and dry weight of shoot were significantly low in New Era and PTB-2. However, a similar significant reduction in root length was not observed in PTB-2. The nematode number per gram fresh weight of infested root and 100 g of inoculated soil were more in susceptible varieties such as New Era and PTB-2. The lowest number of nematodes in infested root and soil was in variety Pathinettumaniyan.

However, there was significant positive correlation between the nematode number per gram fresh weight of infested root and 100 g of inoculated soil and the number of galls formed in susceptible varieties. Such a positive correlation also existed between nematode number in the infested root and soil.

In the second part of the present investigation, a pot culture experiment was conducted to find out the minimum number of nematodes required to produce a significant effect on nodulation, gall formation and other growth characters of cowpea. For this one of the susceptible varieties of cowpea identified earlier, New Era, was used. The experiment was laid down in completely randomised design with 6 replications each. Five different inoculum levels 0, 10, 100, 1000 and 10,000 second stage larvae per plant were used for inoculation on 15th day of plant growth. Observations on nodule number, nodule fresh weight, gall number, gall fresh weight, percentage nitrogen content of shoot, leaf number, plant height, root length fresh and dry weight of shoot and nematode population in infested root and soil were taken on the 50th day of plant growth.

A significant reduction in nodulation was observed in all plants inoculated with 100, 1000 and 10,000 larvae with a maximum reduction in plants inoculated with 10,000 nematodes. Here only 63.33 nodules were formed in comparison to 209.33 nodules formed in the uninoculated control plants. The nodule fresh weight was more or less in proportion to their number and was significantly low in treatments involving 100, 1000 and 10,000 nematodes. The fresh weight of nodules was only 2.46 g in plants inoculated with 10,000 nematodes where as in control plants this was as high as 5.43g. Further, nodules in all treatments except the control were infested with the nematode.

There was significant increase in gall number and gall fresh weight in all treatments except the control. The maximum increase was observed in plants inoculated with 10,000 nematodes. Here 290.67 galls with a fresh weight of 3.37 g were formed. However, there was no gall formation in uninoculated control plants. Further, there was a significant negative correlation between gall formation and nodule number in different nematode inoculated treatments.

A significant reduction in percentage nitrogen content of shoot was observed as a result of inoculation with 1000 and 10,000 nematodes. The percentage nitrogen content of plants in these treatments was only 2.60 and 2.37 respectively in comparison to 3.34 per cent for control plants. A significant positive correlation between nodule number and percentage nitrogen content of shoot was observed. But, the correlation between gall number and percentage nitrogen content of shoot was significantly negative.

The number of leaves formed per plant, plant height, root length and fresh and dry weight of shoot were also significantly low due to nematode inoculation especially with 1000 and 10,000 larvae per plant. The maximum deleterious effects were observed as a result of inoculation with 10,000 larvae. However, inoculation with 10 and 100 nematodes did not have any significant effect on these plant characters.

A significant increase in nematode number per gram fresh weight of infested root and 100 g of inoculated soil was observed with 10, 100, 1000 and 10,000 nematodes. This increase was maximum in plants inoculated with 10,000 larvae. There was a significant positive correlation between the nematode number per gram fresh weight of infested root and 100 g of inoculated soil and the number of galls formed in different treatments. Such a positive correlation also existed between the nematode number in infested root and soil.

In the last part of the present investigation, the actual effect of age of host plant on infestation by M. incognita was investigated. This experiment was also done in pots by inoculating the susceptible variety of cowpea, New Era, with 1000 nematodes from the date of sowing to 28 days of plant growth. The experiment was laid down in completely randomised design with 5 replications each. Observations were taken on nodule number, nodule fresh weight, gall number, gall fresh weight, percentage nitrogen content of shoot, leaf number, plant height, root length, fresh and dry weight of shoot and nematode population in infested root and soil were taken on 55th day of plant growth.

There was significant reduction in nodule number and fresh weight in different treatment when compared to control. The maximum reduction was observed on 14th day. The number of nodules formed and its fresh weight were only 23.80 and 0.43 g respectively in comparison to 2.34 and 5.31 g for control plants. Further, nodules in all treatments except the control were infested with the nematodes.

There was significant increase in gall number and their fresh weight in all the treatments except the control. The maximum increase was observed on 14th day. Here, 127 galls with a fresh weight of 2.26 g were formed. However, there was no gall formation in uninoculated control plants. Further, there was significant negative correlation between gall formation and nodule number in different nematode inoculated treatments.

A significant reduction in percentage nitrogen content of shoot was observed due to nematode inoculation at different stages of plant growth. The maximum reduction was seen on 14th day. The percentage nitrogen content of plants in this treatment was only 2.34 in comparison to 3.47 per cent for control plants. It was also low in plants inoculated at the time of sowing and on the 7th and 21st day of plant growth. Further, there was a significant positive correlation between nodule number and percentage nitrogen content of shoot. But, the correlation between gall number and percentage nitrogen content of shoot was significantly negative.

The number of leaves formed per plant, plant height, root length, fresh and dry weight of shoot were also significantly low due to nematode inoculation at different stages of plant growth compared to control plants. However, the maximum deleterious effects were observed on 14th day of plant growth followed by inoculation at the time of sowing and on the 7th, 21st and 28th day of plant growth.

A significant increase in nematode number per gram fresh weight of infested root and 100 g of inoculated soil was observed. This increase was maximum in plants inoculated on 14th day. The increase in nematode number was also higher in plants inoculated at the time of sowing and on the 7th and 21st day of plant growth. There was a significant positive correlation between nematode number per gram fresh weight of infested root and 100 g of the inoculated soil and the number of galls formed in different treatments. Such a positive correlation also existed between the nematode number in infested root and soil.

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* Original not seen

APPENDICES

APPENDIX - I (a)

Host varietal variations in cowpea to infestation by <u>Meloidogyne incognita</u>

Analysis of variance table

Source	df	Mean square			
		Gall number	Gall fresh weight (g)	Nodule number	Nodule fresh weight (g)
Total	29	..	..	..	..
Treatment	9	44.60*	0.59*	3.27*	0.70*
Error	20	0.51	0.03	0.07	0.13

* Significant at 5 per cent level of significance.

APPENDIX - I (b)

Host varietal variations in cowpea to infestation by _Meloidogyne incognita_

Analysis of variance table

Source	df	Mean square						
		Leaf number	Plant height cm	Root length cm	Fresh weight of shoot g	Dry weight of shoot g	Nematode population per gram fresh weight of root	Nematode population per 100 g soil.
Total	29	..	..	..	..	..	..	..
Treatment	9	0.99*	673.96*	99.01*	115.01*	0.41*	1.74*	1.67*
Error	20	0.12	69.32	5.98	12.97	0.06	0.03	0.06

* Significant at 5 per cent level of significance

APPENDIX - II (a)

Effect of inoculation with different levels of *Meloidogyne incognita* on nodulation and gall formation

Analysis of variance table

Source	df	Mean square				
		Nodule number	Nodule fresh weight g	Gall number	Gall fresh weight g	Percentage nitrogen content of shoot
Total	29	..	..	..	..	..
Treatment	4	40.30*	9.51*	256.74*	10.60*	0.92*
Error	25	0.39	0.66	1.65	0.17	0.08

* Significant at 5 per cent level of significance.

APPENDIX - II(b)

Effect of inoculation with different levels of *Meloidogyne incognita* on nematode population and different plant characters in cowpea

Analysis of variance table

Source	df	Mean square						
		Nematode population per gram fresh weight of root	Nematode population per 100 g soil	Leaf number	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
Total	29	..	..	..	..	..	..	..
Treatment	4	6.18*	5.40*	2.26*	5699.55*	768.40*	1195.41*	28.72*
Error	25	0.03	0.02	0.05	127.45	65.52	45.11	2.02

* Significant at 5 per cent level of significance

APPENDIX - III (a)

Effect of age of plant and infestation by *Meloidogyne incognita* on nodulation and gall formation in cowpea

Analysis of variance table

Source	df	Mean square				
		Nodule number	Nodule fresh weight (g)	Gall number	Gall fresh weight (g)	Percentage nitrogen content of shoot
Total	29	..	..	..	..	..
Treatment	5	69.29*	14.22*	73.93*	3.60*	0.80*
Error	24	0.59	0.35	1.03	0.15	0.02

* Significant at 5 per cent level of significance

APPENDIX-III (b)

--- Effect of age of plant and infestation by *Meloidogyne incognita* on nematode population and different plant characters in cowpea

Analysis of variance table

Source	df	Mean square						
		Nematode population per gram fresh weight of root	Nematode population per 100 g soil	Leaf number	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
Total	29	..	..	..	..	..	..	..
Treatment	5	5.19*	3.96*	2.25*	49047.31*	767.85*	972.86*	13.67*
Error	24	0.003	0.004	0.09	9309.46	23.72	33.60	1.19

* Significant at 5 per cent level

EFFECT OF ROOT-KNOT NEMATODE *Meloidogyne incognita* ON NODULATION IN COWPEA

By

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ABSTRACT OF A THESIS
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ABSTRACT

An investigation was carried out at College of Agriculture, Vellayani, Kerala, to study the effect of infestation with Meloidogyne incognita on nodulation and gall formation in cowpea. Ten varieties of cowpea, New Era, Pathinettumaniyan, HG-22, PTB-1, PTB-2, C-152, Sundari, V-16, V-37 and V-240 were initially screened for resistance to this nematode. The plants were inoculated with 1000 second stage larvae of M.incognita on 14th day and various observations on gall number, gall fresh weight, nodule number, nodule fresh weight, leaf number, plant height, root length, fresh and dry weight of shoot and nematode population in infested root and soil were taken on 50th day of plant growth. Eight varieties namely Pathinettumaniyan, HG-22, PTB-1, C-152, Sundari, V-16, V-37 and V-240 were found resistant while two varieties New Era and PTB-2 were highly susceptible to this nematode.

The effect of inoculation with different levels of M.incognita on nodulation and gall formation was also studied. For this, the cowpea variety found most susceptible to this nematode, New Era, and five different levels of nematode such as 0, 10, 100, 1000 and 10,000 were used. A progressive reduction in various plant characters studied was observed with an increase in the level of nematode inoculum. The number of

nodules formed per plant and their fresh weight were reduced significantly with 100, 1000 and 10,000 nematodes per plant. However, the number of galls formed and their fresh weight increased with an increase in nematode number. The percentage nitrogen content of shoot was reduced significantly as a result of inoculation with 1000 and 10,000 nematodes. A significant reduction in leaf number, plant height, root length and fresh and dry weight of shoot was also observed with 1000 and 10,000 nematodes. Further, the nematode population in infested root and soil increased with an increase in the nematode inoculum.

An experiment to study the effect of age of host plant and infestation by M.incognita on nodulation and gall formation was also conducted. In this experiment cowpea was inoculated with 1000 second stage larvae at the time of sowing and on 7th, 14th, 21st and 28th day of plant growth. Various observations were taken on 55th day. The lowest number of nodules were formed in plants inoculated on 14th day followed by 0, 7th, 21st and 28th day of plant growth. The fresh weight of nodules was correspondingly low in different treatments. Maximum number of galls were formed in plants treated with M.incognita on 14th day. The number of galls formed on 0 and 7th day of plant growth were also high. The fresh weight of galls was in proportion to their number in different treatments. The percentage nitrogen content of shoot was significantly low in different treatments except the control. A maximum reduction was seen in plants inoculated on 14th day. A significant reduction in other plant characters studied, such as leaf number, plant height, root length and fresh and dry weight of shoot was also observed.

Based on the present investigation following important observations are made with regard to infestation by _M.incognita_ in cowpea.

1. There is a varietal variation in cowpea to infestation by _M.incognita_. In fact, two of the varieties New Era and PTB-2 at present cultivated in the State are highly susceptible, while three other popular varieties such as Pathinettumaniyan, PTB-1 and C-152 are resistant to this nematode.

2. A minimum inoculum level of 1000 second stage larvae of _M.incognita_ is required to produce an uniform significant effect on various plant characters studied especially on nodulation, gall formation and percentage nitrogen content of shoot.

3. Cowpea is most susceptible to infestation by <u>M. incognita</u> during the early stages especially around 14th day of plant growth.