

**Pathogenicity and avoidable yield loss due to
Meloidogyne incognita in French bean**

**A Thesis submitted to the
Orissa University of Agriculture and
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in Partial fulfilment of the Requirement
for the degree of
Master of Science in Agriculture
(Nematology)**

By

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CERTIFICATE- I

This to certify that the thesis entitled “**Pathogenicity and avoidable yield loss due to *Meloidogyne incognita* in French bean**” submitted in partial fulfilment of the requirements for the award of degree of **Master of Science in Agriculture (Nematology)** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Sagarika Mohapatra** under my guidance and supervision.

No part of this thesis has been submitted for any other degree or diploma

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

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CERTIFICATE-II

This is to certify that the thesis entitled “**Pathogenicity and avoidable yield loss due to *Meloidogyne incognita* in French bean**” submitted by **Sagarika Mohapatra** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment for the degree of Master of Sciences (Agriculture) in the subject of **Nematology** has been approved by the students’ advisory committee and the external examiner.

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ABSTRACT

The study was conducted to determine potential level of damage by root knot nematode, *Meloidogyne incognita* in French bean c.v. contender. Fifteen days old seedlings grown in sterilized earthen pots each containing one kg steam sterilized soil were inoculated with 10,100,1000 and 10000 freshly hatched second stage Juveniles (J_2) of *Meloidogyne incognita*. The experiment was terminated after 60 days of inoculation and observations were recorded on plant growth characters and nematode multiplication. Findings revealed that an inoculum level of 1000 and above J_2 / plant significantly decreased the plant growth parameters. Nematode reproduction rate decreased with an increase in inoculum levels. It was maximum (107.4 times) at inoculum level of 10 J_2 / plant and minimum (2.05 times) at 10000 J_2 / plant. Nematode population in soil and root increased progressively with an increase in nematode inoculum level from 10 to 10000 J_2 / plant. Maximum population was recorded at inoculum level of 10000 J_2 / plant followed by 1000 J_2 / plant which was statistically at par with that of 10000 J_2 / plant. This clearly indicated that the threshold inoculum density of *M.incognita* causing significant reduction of various growth parameters in French bean was 1000 J_2 per plant. The field experiment conducted in a root knot nematode sick plot by growing French bean c.v. contender in pair plot design showed 18.77 % avoidable yield loss due to *M.incognita*. Significant differences were observed in plant heights, fresh and dry weights of plants between nematicidal treated and untreated plots. The treatment with nematicide resulted reduction in final nematode population i.e., 69.55% in soil, 73.27% on root and 45.00% decrease in root knot index.

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CHAPTER-1

INTRODUCTION

1.INTRODUCTION

Vegetables are a rich source of vitamins, minerals and the much needed roughage in our daily diet. India is the second largest producer of vegetables in the world next only to China with an estimated production of about 50.09 million tonnes from an area of 4.5 million hectares at an average yield of 11.3 tonnes per hectare. India shares about 12% of the world output of vegetables from about 2.0% of cropped area in the country.

Phaseolus vulgaris L. also known as snap bean, kidney bean, garden bean or string bean, is one of the most important leguminous vegetables grown for its tender fleshy green pods, shelled green seeds and also dry beans. It has anti-diabetic property and is good for natural cure of bladder burns and cardiac problems, diarrhoea and sciatica. It is a nutritious vegetable, rich in protein (1.7g), calcium (132mg), thiamin (0.08 mg) and vitamin C (24 mg per 100 g of edible pods). It is valued for its protein rich (23%) seeds. The fresh pods and green leaves are used as vegetable. The antimetabolites of dry beans need removal by cooking and soaking in water.

It is grown in the world in an area of 0.83 m ha with annual production of 5.64 m t with productivity of 6.76 t per ha. In India, it is mainly grown in Himachal Pradesh Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and Odisha. It accounts grown in an area of 0.15 m ha with production of 0.42 m t and productivity of 2.8 t per ha (FAO STAT, 2002) in India. Improvement made in crop varieties is mainly concentrated on increasing yield and yield attributing characters.

Adoption of latest management practices like use of improved, high yielding & hybrid varieties with the recommended dose of fertilizer could increase the yield of the crop. But the desired level of production has not reached due to the influence of abiotic and biotic factors. Among the major biotic factors that affected the French bean production root-knot nematode, *Meloidogyne incognita* is most serious in causing considerably low yield. Thus nematode has been repeatedly parasitized on the roots, cause galls on roots and being associated with other secondary pathogen. The

nematode gets its name because its feeding causes galls (swellings or “knots”) to form on the roots of infected plants. Plants become stunted, leaves show chlorotic symptoms, fruit size and production affected because the damaged root reduces the plant ability to extract available salt, water and nutrients.

Crop losses due to *Meloidogyne incognita* have been estimated by various workers varying 19.38 to 43.48% loss in pod yield. (Reddy, 2008). Bhatti and Jain (1977) reported losses as high as 90.9% in various crops due to *M.incognita*. However no systematic work was carried out regarding the pathogenicity and percent loss in terms of plant vigour and yield. Therefore present investigation is designed to determine the potential damaging level and extent of yield loss due to root knot nematode, *M.incognita* in French bean.

CHAPTER-2

REVIEW OF LITERATURES

2. RIVIEW OF LITERATURES

2.1 Pathogenicity of root knot nematode, *Meloidogyne incognita*

Jain & Bhatti (1978) indicated the effect of *M. incognita* on yield of brinjal and tomato in a pot experiment. *M. incognita* significant reduce yield of tomato in brinjal at all the inoculums levels (10, 100,1000,10000 larvae /pot) tested. Yield loss increases with increasing population level.

Effects of *Meloidogyne incognita* and *Macrophomina phaseolina* on root-rot of two cultivars of French bean were examined in a greenhouse experiment. Severity of *Macrophomina* root-rot increased by 54.5, 94.6, and 9.6% when both pathogens were introduced simultaneously, the nematode first by 2 weeks, and the fungus first by 2 weeks, respectively. Nematode infection and reproduction was adversely affected when the fungus was introduced first. Cultivar “Harvester” was more tolerant to both pathogens and less susceptible to the nematode than “Romano Italian”. (AlHazmi, 1985)

Vito (1986) studied the relationship between initial population densities of *M. incognita* and growth of susceptible (Yolo wonder) and resistant (Line 85558) *C. annuum* in the glass house at 24-28⁰ C. Final population densities of *M. incognita* increased in pots with low initial densities and planted with Yolo wonder but declined in those with high inoculums levels planted with Yolo wonder and at all initial population densities with line 85558.

There was a negative correlation between numbers of galls and fresh weight of *Phaseolus vulgaris*, and a positive correlation between number of galls and densities of final populations of *Meloidogyne* spp. (Marwoto & Rohana, 1987)

In pot experiments, onion (*Allium cepa* var. *aggregatum*) cv. CO 2 was inoculated with *Meloidogyne incognita*. Similarly Bellary onion (*A. cepa*) was inoculated with *M. arenaria*. Population levels of 100, 1000 and 10,000 *M. incognita* or 1000 and 10 000 *M. arenaria* / pot were pathogenic. (Babu & Vadivelu, 1989)

In a pot experiment, *C. arietinum* were inoculated with 10, 100, 1000, 2000, 4000 or 8000 2nd-stage juveniles of *M. incognita*/ plant. The number of galls/ plant was maximum at the 2000 inoculum level and minimum at the 10 inoculum level. An initial inoculum level of 100 juveniles/ plant was sufficient to cause reduction in the plant growth parameters. (Bhatti & Bhatti, 1989)

There was a progressive decrease in plant growth of *V. unguiculata* with increasing inoculum levels of *R. reniformis* and *M. incognita* from 10 to 10 000 nematodes/plant. A significant reduction in plant growth and nodulation in comparison to uninoculated plants was observed at an initial level of 1000 nematodes or above per kg soil which is considered here as the damage threshold. Development of nodules on galls and galls on nodules was observed infrequently. The rate of multiplication of each nematode species was highest at the lowest and lowest at the highest initial inoculum level. Plant growth was reduced to a greater extent by *M. incognita* than by *R. reniformis*. (Khan & Husain, 1989)

Pea var. Boneville were inoculated with 10, 100, 1000 or 10000 *M. incognita* larvae in a pot experiment. There was a progressive decrease in all plant growth characters with increasing inoculum level, a significant reduction occurring at or above 1000 larvae/plant. Plants showed chlorosis, rugose leaves and shedding of a few basal leaves at the highest inoculum level. (Bhagawati & Phukan, 1991)

Sunflower, safflower and mustard seedlings were inoculated in a pot experiment with 10, 100, 1000 or 10 000 *M. incognita* juveniles/pot. After one month the reduction in shoot length at the 10 000 level was 8.3, 30.8 and 49.8% in sunflower, safflower and mustard, resp. Flower weight was also reduced at increasing inoculum densities, no flower set occurring at the highest level. The number of root galls increased with increasing inoculum density. (Prasad & Chawla, 1992)

Soyabean cv. Pusa-24 seedlings were inoculated in a pot experiment with 0, 10, 100, 1000 or 10 000 J2 of *M. incognita*/pot. All plant growth parameters were reduced with increasing inoculum levels, maximum reduction occurring at the 10 000 level, followed by 1000 nematodes/pot. At these levels plants showed stunting, yellowing, wilting and a sickly appearance. The number of bacterial nodules was also

reduced with increasing inoculum level. Soyabean was a good host for *M. incognita*. (Meena & Mishra, 1993)

Carrots c.v Early Nantes, were inoculated in pot trials with 0, 10, 100, 1000, 5000 or 10 000 *M. Incognita* J₂/pot. Significant reductions in shoot length, fresh and dry weight of shoot and tap root were recorded at or above 100 nematodes/plant. There was a significant increase in forking in all treatments and tap roots became dwarfed and knotty with circular constrictions in plants inoculated with 100 nematodes/ plant or above. (Devi & Das, 1994)

Aubergines var. JC-1 were inoculated with 0, 10, 100, 1000, 5000 or 10 000 *M. incognita* J₂/plant in pot trials. There was a significant reduction in plant height, root and shoot weights in plants inoculated with 1000 nematodes/plant or above. (Hazarika & Phukan, 1994)

Pathogenicity studies of *M. incognita* on *P. vulgaris* indicated that there was a progressive decrease in the plant growth characters as the nematode inoculum levels increased from 10 to 10 000 J₂ per plant. Significant reduction of all the plant growth characters was recorded with an initial inoculum level of 10 J₂ per plant over control. Maximum number of galls, eggmasses and final nematode population was recorded at 1000 J₂ per plant. The ratio of male to female nematode was maximum at the highest inoculum level. (Hussain & Bora, 1995)

Survey conducted in different chickpea growing areas of Karnataka, India, indicated a variable occurrence of root-knot nematode with a variation in population levels in the districts. The mean inoculum level was 0.12 larvae/g soil under field conditions. An inoculum level of 2 larvae/g soil was found as the optimum damaging threshold level on cv. Annegiri-1 under greenhouse conditions. An increase in the level of larval inoculum resulted in a proportional decrease in plant growth and an increase in root-knot disease on chickpea. (Rao & Krishnappa, 1995)

Pathogenicity tests gave conclusive evidence of the destructive potential of *Meloidogyne incognita* on cowpea. A progressive decrease in plant growth and rate of reproduction were observed with increased initial inoculum levels (10, 100, 1000 and 10,000 J₂/plant). An initial population of 1000 J₂/500 g of soil caused significant

reduction in shoot length, root weight, root length and number of leaves and proved to be pathogenic to cowpea.(Sarmah & Sinha, 1995)

The effect of *M. incognita* on soya bean var. 49 was studied under greenhouse conditions at inoculum levels of 0, 10, 100, 1000, 5000 and 10 000/ pot. A significant reduction in almost all the growth parameters was observed in soya bean with the increase in the level of nematode inoculum. Maximum reduction in all the growth characters was noted at the 10 000 larvae per pot level. At levels of 1000 and above, plants exhibited dwarfing, yellowing and wilting; Bacterial nodules were also significantly reduced with increasing inoculum levels. (Ahmad & Srivastav, 1996)

Screen house experiments were conducted in 2000-3000 cm superscript 3 plastic bags to investigate the relationships between a range of population densities (0, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 eggs and J₂/cm superscript 3 soil of *M. incognita* race 2 and yield of the susceptible cultivars Tenerife, Montalban and Manuare of *P. vulgaris* and Metro of *V. unguiculata*. Results demonstrated that all cultivars were very susceptible to *M. incognita*. Seinhorst's curves fitted the data well, and tolerance limits to *M. Incognita* of 0.02 J₂ + egg/cm superscript 3 soils for the cultivars Tenerife and Montalban and 0.03 J₂ + egg/cm superscript 3 soils for cultivars Manuare and Metro were derived. Minimum yields at larger nematode densities were 36.5, 43, 53 and 28%, respectively. Populations of *M. incognita* increased in soil infested with up to 8 J₂ + eggs/cm superscript 3 soils, but generally remained at same level at larger initial population densities except for the cultivar Manuare on which a sharp decline was observed. Nematode reproduction rates were higher the lower the initial densities. Seinhorst's equation for population growth also fit data for initial and final nematode densities, assuming maximum reproduction rates of 20, 16, 36 and 5, equilibrium densities of 25, 12, 28 and 9 J₂ + eggs/cm superscript 3 soil, and maximum potential nematode populations of 64, 64, 42 and 35 J₂ + eggs/cm superscript 3 soil for the cultivars Tenerife, Montalban, Manuare and Metro, respectively. (Crozzoli *et al.* 1997)

The pathogenicity of *M. incognita* race 1 on chickpea cultivars Pusa-209, Pusa-212, Pusa-244, Pusa-256, Pusa-267 and Pusa-436 was studied under inoculum levels of 0, 10, 100, 1000 and 10 000 J₂/pot under artificial inoculations. There was a progressive decrease in plant growth as the inoculum levels of the

nematode increased. An inoculum level of 1000 J₂ per pot was found to be a damaging threshold level for Pusa-212 and Pusa-267 whereas 10 000 J₂ per pot caused significant reduction in growth parameters of all cultivars. Rhizobial nodulation was adversely affected at all inoculum levels and this effect was significant at 1000 J₂ and above. The number of functional nodules per root system decreased as the level of inoculum increased and this was maximum at 10 000 J₂ per pot. (Nejad and Khan, 1997)

Experiments on the pathogenicity of *M. incognita* on soyabean and the screening of some genotypes and cultivars of chickpea, pea and soyabean were carried out under glasshouse conditions. There was a highly significant reduction in growth with respect to root length, and fresh and dry weight recorded at 2500 second stage larvae (L₂)/500 cc soil. However there was no significant reduction in growth at the highest inoculum level of 10 000 L₂/500 cc soil. The results of screening tests showed that 3 lines (BG-369, GL 90033 and JCP-40) of chickpea out of 115 lines; 1 line of pea (LPF-48) out of 39 lines and 2 lines of soyabean (JS 7981 and MSCS-1) out of 20 lines were found highly resistant. (Bhatt *et al.*, 1998)

The pathogenicity of *Meloidogyne incognita* (race-3) to turmeric (*Curcuma longa*) cultivars BSR-1 and PTS-10 was studied under the glasshouse by inoculation experiments. The nematode was pathogenic at 5000 and 10,000 J₂/plant, inoculated 15 days after planting. The nematode was pathogenic to both the cultivars studied in the experiment. The leaves of the inoculated plants were pale in colour and shoot weight reduced by 6-10% in both the varieties. Rhizome yield reduction in cv. BSR -1 was 25 and 18.6% at 5000 and 10000 nematodes/plant, respectively while it was 48.2 and 41.3% at these inoculum levels for PTS- 10. Levels of protein, carbohydrate, chlorophyll a, b, and total and rhizome curcumin levels were all lower in plants inoculated with 10, 0000 juveniles when compared to healthy ones, in both the turmeric cultivars. It was concluded that the nematode caused loss in yield as well as quality of the produce in turmeric. (Poornima & Vadivelu, 1998)

Screenhouse experiments were conducted in 3 litre pots to investigate the relationship between a range of population densities (0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 eggs and second stage juveniles/cm superscript 3 soil) of *M. incognita* race 1 and yield of a resistant cultivar (Ojito Negro) of *V. unguiculata*. Seinhorst's yield-loss

model was fitted to yield data, and confirmed that this cowpea cultivar is only slightly affected by *M. incognita*. Cultivar Ojito Negro tolerates up to 0.74 J₂ + egg of *M. incognita*/cm³ soil and at the highest population density, seed weight and dry aerial weight, were reduced only 20% and 10%, respectively. Numbers of *M. incognita* increased in soil infested with initial population densities up to 6 J₂ + eggs/cm³ soil, and remained at same level at larger initial population densities. Nematode reproduction rates were inversely related to the initial population densities. Data for initial and final nematode densities were well fit by Seinhorst's model of population growth, assuming a maximum reproduction rate of 1.6, equilibrium density of 3 J₂ + eggs/cm³ soil, and maximum potential nematode populations of 6 J₂ + eggs/cm³ soil. (Crozzoli *et al.*, 1999)

A significant reduction in shoot and root weight of *T. dioica* was observed at an initial inoculum density of 1000 J₂ of *M. incognita* per kg soil. This was considered as the damaging threshold level. The rate of nematode reproduction was density dependent, the maximum being at the lowest inoculum density, and the minimum at the highest density. Bare-root dip of 10-day-old rooted seedlings of pointed gourd in 0.05% concentration of carbosulfan 25 EC for 8 h effectively reduced nematode multiplication and root galling. Root dip in 0.1% carbosulfan for 6 h exhibited lethal toxicity in seedlings. (Mahapatra *et al.* 1999)

The effects of inoculating *M. incognita* (10, 100, 1000, and 10 000 nematodes/50g of soil) on cowpea cv. Pusa Komal seedlings were studied under greenhouse conditions. Observations after 45 days of inoculation were recorded. Plant vigour and rhizobial nodulation decreased with the increase in the inoculation rate. Root galls, which were sometimes big and irregular, were distributed in the root system. Plant growth was significantly reduced at an initial population of 1000 nematodes per 500 g of soil (potential pathogenic level). The nematode population increased due to the inoculation of up to 1000 nematodes per 500 g of soil; from an inoculation level of 10 000, the population started to decrease to a level lower than the initial population. (Singh & Goswami, 2000)

Seeds of sunflower cv. MSFH-8 were sown in 15 cm diameter earthen pots containing 1 kg autoclaved soil per pot. After seven days of seed germination, a single plant per pot was inoculated with freshly hatched second stage larvae of the root-knot

nematode *Meloidogyne incognita* at different concentrations (0, 10, 100, 1000 and 10 000 larvae per pot). In a separate experiment, seven-day-old sunflower plants grown in autoclaved soil were inoculated with the root rot fungus *Macrophomina phaseolina* at 0.25, 0.50, 1.0 and 2.0 g/kg soil. Observations were recorded 45 days after inoculation (DAI) with the nematode, and 30 and 60 DAI with the fungus. Treatment with 1000 larvae per pot and above reduced the shoot and root lengths, and fresh and dry weights. Increasing the nematode inoculum level increased the root-knot index and nematode population build up in the soil and roots. Pathogenicity studies of the fungus revealed that there was no reduction in any of the growth parameters of sunflower plants at 30 DAI, whereas all growth parameters were significantly affected 60 DAI with 1.0 and 2.0 g fungus/kg soil. (Singh *et al.*, 2000)

A greenhouse experiment was conducted to evaluate the effect of increasing population densities of *M.incognita* race 1 on the yield of parsley cv. Double Curled. The initial nematode densities were: 0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0 and 256.0 eggs + second-stage juveniles (J2) cm⁻³ soil. Fitting of the Seinhorst model to average plant top and total fresh weight and top dry weight gave tolerance limits (*T*) of 0.17, 0.02 and 0.025 eggs + J2 cm⁻³ soil, respectively. The minimum relative yields (*m*) were 0.5 at $P_i \geq 16$ eggs + J2 cm⁻³ soil, 0.55 at $P_i \geq 2$ eggs + J2 cm⁻³ soil and 0.34 at $P_i \geq 4$ eggs + J2 cm⁻³ soil for top fresh weight, total fresh weight and top dry weight, respectively. The maximum nematode reproduction rate was 37-fold at $P_i = 0.25$ eggs + J2 cm⁻³ soil and an equilibrium density were estimated at 5 eggs + J2 cm⁻³ soil. Anatomical modifications observed in galled roots were similar to those induced by this root-knot nematode species in other hosts. (Aguirre *et al.* 2003)

An initial inoculum level of 100 juveniles of *Meloidogyne incognita* per plant caused significant reduction in growth characters of pulse crops and proved to be pathogenic. Reduction in growth characters varied from 1.13 to 69.06 per cent at different inoculum levels. Maximum decrease was in lathyrus while minimum in french bean at 10,000 level. The multiplication rate of nematode was maximum at 10 inoculum level and minimum at the highest level. Gradual reduction in number of bacterial nodules was observed with the increase of inoculum levels in all the test crops maximum was at 10,000 levels. (Haider *et al.*, 2003)

The response of 5 pulse crops, namely green gram (*Vigna radiata*), black gram (*Vigna mungo*), horse gram (*Dolichos biflorus* [*Macrotyloma uniflorum*]), soyabean and French bean (*Phaseolus vulgaris*), to root-knot nematode (*M. incognita*) infestation at 3 inoculum levels (100, 500 and 1000 second stage juveniles) was evaluated under glasshouse conditions. Although, in all the crops a growth-promoting effect was observed at inoculum level of 500 (in comparison to the lowest and the highest inoculum levels), the variations in growth parameters (root weight, shoot weight and shoot length) were not always statistically significant. On the basis of root gall index and nematode multiplication potential, soyabean and horse gram were graded as highly resistant and resistant, respectively, while black gram, green gram and French bean were found nematode-susceptible crops, in descending order. (Sharma, 2003)

Under greenhouse conditions, faba bean (*Vicia faba* L.) plants inoculated with 0, 10, 100, 1000 and 10000 larvae of *Meloidogyne incognita* root-knot nematode per pot showed significant reduction in plant growth and yield under the highest inoculum ($p \leq 0.05$ and/or 0.01). Number of nematode galls was greatly affected by the initial nematode population. Cellular alterations of faba bean root infected with the *Meloidogyne* nematode root knot nematode showed that 2 stage nematode larvae penetrated the roots of faba bean by puncturing action of the stylet and inter-intra cellular migration into the cortex and endodermis layers causing damage to the cells. Mature females and thick-walled multinucleate giant cells with dense and granular cytoplasm were formed in the cortex, endodermis and stele regions. (Youssef & El-Nagdi, 2004)

A study was conducted to determine whether and to what extent the root-knot nematode, *M. Incognita*, would interfere with nodulation and the damaging potential of this nematode on green gram. One-week-old seedlings of green gram were inoculated with freshly hatched second stage juveniles of *M. incognita* at 0, 500, 1000, 2000 or 4000/pot. *M. incognita* decreased all the growth characteristics (dry weights of shoots and roots, and number of pods) of green gram, irrespective of initial inoculum level, with maximum damage being at 4000 juveniles per pot. The highest number of galls per plant was recorded at 4000 juveniles per pot. The number of galls per plant and multiplication of nematode varied considerably

with the initial inoculum level. The maximum and minimum reproductive rates of nematode were recorded in the lowest inoculum (500) and highest inoculum level (4000), respectively. (Ansari & Azam, 2005)

Studies were made to determine the effect of initial inoculum levels (P_i) of *Meloidogyne* on *Mentha arvensis* cv. Gomti. There was a positive relationship between the initial inoculum levels of *M. incognita* and reduction in shoot-root/sucker fresh and dry weights, oil yield, total chlorophyll, total phenol and total sugar content of fresh leaves. The maximum reduction in corresponding parameters was recorded at the highest initial inoculum level (25,000 $J_2/5$ kg soil) as compared to uninoculated control. There was a negative relationship between initial inoculum densities and rate of nematode multiplication. Maximum final nematode population P_f (1, 33,430) and root-knot index (3.00) were observed at the highest P_i (25,000 $J_2/5$ kg soil), whereas, maximum reproduction-factor was observed at minimum P_i (500 $J_2/5$ kg soil). (Perveen *et al.* 2006)

In the screenhouse, tomato (cv. DT 69/257) seedlings grown in steam-sterilized soil were inoculated with graded inoculum of 5000, 10 000, 15 000, 20 000 and 25 000 eggs of *Meloidogyne incognita*. At inoculum levels of 15 000, 20 000 and 25 000 eggs of *M. incognita*, the number of leaves per plant, plant height, fruit yield and root-galls were significantly reduced. In the field planted with tomato seedlings, aqueous extracts from the roots of marigold (*Tagetes erecta*), nitta (*Hyptis suaveolens*) and basil (*Ocimum gratissimum*) plants were applied to root-knot nematode infected soil at four concentrations, viz. 25 000, 500 000, 750 000 and 1 million/ppm per tomato plant. All the aqueous plant root extracts reduced the root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield over the untreated control treatment. (Olabiyi TI, 2006)

Different inoculum levels (10, 100, 1000 and 10 000 infective second juvenile/pot) of *Meloidogyne incognita* were evaluated for their effect on various growth parameter, i.e. number of leaves and fruits, and weight of fruits of okra cv. Pusa Sawani. The results showed the significant reduction in the growth parameters of okra when the nematode infestation was high. (Mishra & Awasthi, 2007)

An on-farm survey of ten randomly selected soybean fields for root knot nematodes (*Meloidogyne* spp.), their population and pathogenicity under natural infestation in the Aiyeye Osun state of Nigeria was undertaken. The experiment was conducted during the 1992 growing season and was planted out in a randomized complete block design on each field with sixteen varieties of soybeans assessed for pathogenicity by the nematode. Possible alternate host crops and weeds were uprooted and roots examined for the presence or absence of galls. All the fields sampled were infested with the root-knot nematode species *M. incognita* race 2. The pre-plant population density of this nematode was low in all the fields sampled. Nematode population significantly increased in mid season and decreased at post harvest season. Responses of the soybean showed that varieties 1614-E and 984-2E were highly resistant while 1458-4E, 1627-5F, 1636-8F, 1627-14F, 1596-1E and M351 were resistant and 1455-1E, 1455-3E and 1458-1E proved moderately resistant. Moderately susceptible varieties included 1460-1E, 1178-1E, 1642-1E, 1113-3D and 1499-1DRE. None rated susceptible or highly susceptible to the pest. There is need to confirm the resistance response in fields with higher population density levels of this nematode. The weeds that harboured the pest constitute possible alternate hosts and reservoirs of this nematode that enables it to re-establish infection of susceptible crops each planting season. (Iheukwumere et al., 2008)

Pathogenic level of *Meloidogyne incognita* was calculated to be 2 J₂/g soil as indicated by the reproduction of the nematode. On the basis of wilt index on pea, 1 g of mycelium mat could produce significantly high index value. The presence of root knot nematode irrespective of the presence of other organism showed reduction in shoot length to a significant manner. However, maximum reduction in shoot length was observed in nematode inoculated one week prior to fungus. Both the organisms suppressed the plant growth characters in general and shoot length in particular. Maximum reduction in nematode population was found to be in nematode alone and nematode followed by fungus inoculation. The wilt index on pea after 50 days was recorded to be 3.5, 4.0 and 4.2 in F+N, F -> N and N->F, respectively. (Kumar & Kamalwanshi, 2009)

A study was carried out to determine the effects of different inoculum levels (10, 100, 500, 1000 and 10 000 J₂) of *M. incognita* on vegetative growth of soybean

and its root system. The inoculum levels of *M. incognita* exhibited suppressive effects on fresh and dry weights of roots and shoots of soyabean. Their impacts were more pronounced on the roots than on the shoots when compared with the uninoculated (control) plants. At the highest inoculum level, all the parameters were drastically reduced compared with the control. The seed weight from the nematode-inoculated plants was lower than the control. The seed weight at 10 and 100 J₂ inoculum level was no significant but at 10 000 J₂ it was significantly lower than the control. There was a significant increase in the number and size of galls per plant at inoculum levels 1000 and 10 000 J₂. The number of mature females per gram root and number of egg masses per plant were low at the lower inoculum levels; however, they increased with an increase in inoculum level. The highest number of egg masses was collected at the highest inoculum level. The number of eggs per egg mass decreased with an increase in inoculum level. The reproduction factors decreased with an increase in inoculum level. (Robab *et al.*, 2009)

Pot culture experiments were conducted under greenhouse conditions by growing *Lageneria ciceraria*, *Cucumis sativa*, *Momordica charantia* and *Cucurbita pepo*. All the four cucurbits were found to be highly or moderately susceptible to infection with *M. incognita* and population growth of the nematode was found to be a determining factor in the pathogenesis of the infection. A general response was a disturbance in the plant growth pattern due to limitation in water and nutrient translocation from infected roots to above-ground plant tissues. An inverse relationship was found to exist between population density, population growth and severity of root galls. The onset of galls has been considered to be a protective measure of the plant for restriction of parasitic movement and maintenance of stable parasite population. The root knot nematodes have been considered to use both r and k strategies for their efficient survival and perpetuation of species depending on inter and intra-specific competition prevailing. (Chandra *et al.* 2010)

Lycopersicon esculentum (tomato) plants, grown in sterilised clay pots, were inoculated with 50, 500, 1000, and 3000 second-stage juveniles (J₂) of the root-knot nematode (*Meloidogyne incognita*) and were kept in a greenhouse. A non-significant reduction in plant growth and yield was noticed in T₁ plants. Significant reductions in plant growth and yield were found in T₂, T₃, and T₄ plants. Highest reductions, in

growth and yield, were observed in T₅ plants. Transverse and longitudinal sections revealed that *M.incognita* traversed through the cortical tissues of the root, caused infection in the differentiating vascular tissues and successfully established in the infected roots. The post-infection changes in the affected parts were hypertrophy and hyperplasia, around the head of the nematodes. Five to 10, among the hypertrophied cells, developed into very large, multinucleate, prominent, and highly specialised giant cells. The nuclei in each giant cell enclosed one or more nucleoli. Xylem and the phloem strands were found to be disoriented. Abnormal xylem and phloem comprised a substantial portion near the giant cells. The metabolic changes in the affected part led to the formation of galls, characteristic of the root-knot infection. (Azam *et al.* 2011)

Cow pea, broad bean and french bean are important vegetable crops widely grown in North East part of India including Manipur. Root-knot nematode (*Meloidogyne incognita*) causes heavy losses in these crops. Experiments were carried out to study the effect of *M. incognita* on germination by inoculating in different inoculum level i.e., 0 (control), 10, 100, 1000 and 10,000 second stage juveniles per 200 gm. of soil. With the increase of inoculum levels of *M. incognita*, there was a progressive decrease in the rate of germination and in growth parameters of the crops. Significant reduction in the length of short, root and weight of whole germinated seed were found in 10,000 inoculum level. The number of larvae penetrated into root and soil were significantly increased as the inoculum level increased. (Devi *et al.* 2011)

An investigation was carried out to study the pathogenicity of root knot nematode *Meloidogyne incognita* on okra and its management through various organic amendments. The inoculum level of 1000 juveniles per plant showed significant reduction in various plant growth parameters, which reveals that *M. incognita* is a potential pathogen of okra. With the increase in inoculums level of *M. incognita* (J₂), there was a progressive decrease in various plant growth parameters. The maximum reduction in plant growth parameters was observed at an inoculum level of 4000 juveniles per plant. (Ganaie *et al.* 2011)

The seedlings of *Vigna unguiculata* were inoculated with 10,000, 1000 and 10000 freshly hatched J₂ of *M.incognita* Race-1/ pot. Significant reduction in growth parameters were recorded at 1000 and 10000 juveniles/ plant. Number of galls/ plant,

eggmass/ plant, eggs/ eggmass and soil population of *M.incognita* were observed highest in maximum inoculum level. However, multiplication rate of total population was roughly 124 times in case of 10 J₂ and 2.99 times in case of 10,000 J₂. The damaging threshold in cowpea was found J₂/g in the present investigation. (Kumar *et al.* 2011)

The comparative studies on the pathogenic potential of *M.arenaria*, *M.incognita* and *M. javanica* on mung bean were carried out. The results clearly indicated that the significant reduction in plant growth parameters (Plant length, fresh and dry weight of plant, seed weight, and number of nodules/root system) were recorded at an initial inoculum level of 1000 J₂ of *M. incognita*, 2000 J₂ of *M. javanica* and 4000 J₂ *M. arenaria*/kg soil. The mung bean plants at these levels were poor in vigour and showed stunted growth. Affected plants also exhibited yellowing premature drying and shedding of leaves. It was also observed that with the increase in the level of inoculum there was a progressive increase in host infestation as indicated by the number of galls as well as nematode multiplication. Moreover, the trend in nematode multiplication seems to be negatively correlated with the inoculum of root-knot nematodes. It can be concluded from these results that the pathogenic potential of *M. arenaria*, *M. incognita* and *M. javanica* on mung bean was 4000, 2000 and 1000 J₂/kg soil, respectively. (Khan *et al.* 2012)

Survey of okra fields were conducted for the occurrence of economically important plant parasitic nematodes of some localities of Jammu and Samba district of J&K. Among the different plant parasitic nematodes *Meloidogyne* spp. was more frequently encountered in maximum localities of Jammu and Samba districts. Experiment was conducted to find out the effect of different inoculum levels of nematode and effect of nematicides on the plant growth and nematode multiplication in pot condition on okra plant. Significant reduction occurred in plant growth parameters viz., shoot length, root length, shoot and root mass, dry shoot mass and number of leaves of plant inoculated with 500, 1000 and 2000 juveniles/pot. The highest gall index was recorded at inoculum levels of 1000 and 2000, respectively. Total nematode population/pot significantly increased progressively with an increase in nematode population from 20-2000 J₂/pot. Reduction in multiplication of the nematode was observed with increase inoculum levels. Application of nematicides

significantly increased plant growth and decreased the host infestation by root-knot nematode, *Meloidogyne incognita* over control. Maximum plant height was recorded in carbofuran @ 1.5 kg a.i/ha treated soil followed by carbofuran 1.0, phorate 1.5, and phorate 1.0 kg a.i/ha respectively. However, no more differences between carbofuran 1.5 kg a.i/ha and phorate 1.0 kg a.i/ha at plant growth and nematode multiplication levels. The minimum population was recorded in carbofuran at 1.5 kg a.i/ha and maximum population in phorate at 1.0 kg a.i/ha treated soil. The both nematicides are effective as a form of chemical control measures. The maximum reduction emergence of juveniles from *M.incognita* was noted at 40 ppm concentration of carbofuran. (Singh *et al.* 2012)

Damaging threshold levels of root-knot nematode *Meloidogyne incognita* and root-rot fungus *Fusarium solani* on plant growth parameters, viz., plant length, fresh and dry weight of chilli were determined by conducting their pathogenicity trials in pot experiments. The results revealed a significant reduction in the plant growth parameters at and above the inoculum level of about 1000 J₂ per plant of *M. Incognita* and the highest reduction were recorded at 8000 J₂ per plant. Significant reduction in plant growth parameters was recorded at 1.00 g mycelia mat of *F. solani* per plant, while the highest reduction was observed at 8.00 g mycelia mat per plant. The damaging threshold level was 1000 J₂ per plant of *M.incognita* and 1.00 g mycelia mat of *F. solani*. (Ahmed *et al.* 2013)

The pathogenic potential of *Meloidogyne incognita* on okra (*Abelmoschus esculentus*) was determined at initial population densities of 0, 1000, 2000 and 4000 second stage juveniles per kg of soil in pots in the glasshouse inoculated after 2nd, 3rd, 4th and 5th week of emergence. Significant reductions in plant height and fresh shoot weight and increases in root weight, number of galls and egg masses were observed at all inoculum densities. With an increase in inoculum level, there was a progressive increase in height and shoot weight reductions, root weight, number of galls and egg masses. Plants inoculated after 2nd week of emergence were heavily damaged. However, with the increase in plant age at the time of inoculation, the damaging effects lowered significantly. Reductions in height and shoot weight and increase in root weight, number of galls and egg masses were found to be directly proportional to inoculum densities. On the other hand, with an increase

in the initial inoculum density and plant age there was a corresponding decrease in the reproduction factor being inversely proportional to inoculum densities and plant ages. (Mukhtar et al. 2013)

The experiment was carried out to study the effect of *Meloidogyne incognita* on growth and biochemical of a leguminous plant viz., *Vigna radiata* cv. PDM 139 under green house conditions by inoculating with different inoculum levels i.e., 0 (control), 200, 400, 800 and 1,600 second stage juveniles per 1.5 kg of soil/pot. With the increase in inoculum level of *M. incognita*, there was a progressive decrease in growth and biochemical parameters of the crop. Significant ($p \leq 0.05$) reduction in plant length, fresh and dry weight, leaf area, chlorophyll, seed protein, nitrogenase and leghaemoglobin contents in the root nodules at 400 J₂, while at higher inoculum levels i.e., 800 J₂ and 1,600 J₂, the reduction was more pronounced and significant at $p \leq 0.01$, level. (Abbasi & Hisamuddin, 2014)

Seedlings of the hybrid tomato 'Roma king' cultivar (cv.) grown in a steam sterilized soil were inoculated with Root knot nematode (*Meloidogyne incognita*) eggs, under screen house temperature 27⁺or-2 degrees C. Serially graded inocula of 2, 000, 4, 000, 6, 000, 8, 000 and 10, 000 eggs that hatched from the second stage juvenile (J₂) of the root-knot nematodes, *Meloidogyne incognita*, extracted from infested roots of a hybrid okra (*Abelmoschus esculentus*) were applied around the bases of 12 day-old tomato cv. seedlings. At high inoculums levels of 6000, 8000 and 10000 eggs, flowering, number of leaf, plant height and fruit yield were significantly ($p > 0.05$) reduced. 100% loss occurred on number of fruit and fruit weight of tomato plants inoculated with 10, 000 eggs. Stunted stems with darker linings, poor flowering, small and chlorotic leaves, reduced size and weight of fruits and root galling all increased with the initial nematode population. (Bawa et al. 2014)

The root knot nematodes (*Meloidogyne spp.*) are important group of plant parasitic nematode which poses threat to tomato (*solanum lycopersicum L.*) production. The study was conducted in shade net house located at the Agronomy Department, Faculty of Agriculture, University for Development Studies, Nyankpala from September to December, 2011 to investigate the effect of inoculum densities of root knot nematodes on the growth of tomato cv. Pectomec and the inoculum level that will cause the highest nematode infestations. The experiment was laid out in a

completely randomized design with four treatments and replicated four times. The inoculum levels were: 0, 500, 1000, and 2000 freshly hatched second stage juveniles (J₂) of root knot nematodes /1kg soil/pot. All pots were inoculated with root knot nematode J₂ 2 a week after transplanting of the tomato seedlings. Data were taken on plant girth, plant height, number of leaves, root galls, nematode eggs population density and root weights (fresh and dry weights). From the results obtained, it was observed that all the inoculum levels reduced the stem girth, plant height, number of leaves, and fresh and dry root weights. Increasing the nematode inoculums level resulted in corresponding increased in number of galls and nematode population build up. The reduction in growth parameters and nematode infestations were found to be proportional to the inoculum level. (Kankam & Adomako, 2014)

Investigation was carried out to find the pathogenic level of root-knot nematode, *Meloidogyne incognita* in green gram. Result of the pathogenicity test revealed that plant growth parameter was inversely proportional to the inoculum level of root-knot nematode, except fresh and dry weight of root. An initial inoculum level of 100 nematodes per kg of pot soil caused significant reduction in plant growth parameters and proved to be pathogenic to the green gram plants. Maximum galls and egg masses per root system were recorded in 100 and 1000 J₂ per kg of soil, which ultimately decreases at 10000 inoculum levels. The nematode population in soil increases with increase in inoculum levels as well as nematode reproduction rate was inversely proportional to the nematode inoculum level. (Sumita, 2014)

2.2 Yield loss due to *Meloidogyne incognita*

Bhatti and Jain (1977) made an experiment where field plots infested with *Meloidogyne incognita* and planted with tomato, brinjal (*Solanum melongena*) or okra (*Abelmoschus esculentus*), the nematode population at harvest had increased by 69% under okra, 40% under tomato and 32% under brinjal. Yield losses in the 3 crops 46%, 27% and 91%, respectively.

Ogunfowora (1977) studied the effect of different population levels of *M. incognita* on yield of tomato (*S. lycopersca*) in south western Nigeria. The marketable

yield of seven tomato cultivars were considerably reduce at all the inoculums levels. Pre plant population levels likely to cause economic loss (10% yield loss) were between 2222 and 6666 number /lit for cv. Rossol and 741 nematodes/lit for other varieties.

Gaur & Prasad (1980) studied population of *M. incognita* on eggplant (*S. melongena*) and its effect on host. Larvae were inoculated at 0, 250, 500, 1000, 4000/3liter of soil around the base of 40 days of old eggplant. After 15 days, number of leaves was significantly less at 1000 and 4000 inoculums levels, where as 45 days after, the control plants had more leaves than all inoculums levels. This effect increases with time. Similar trends occurred for growth parameter. e.g. no. of branches no. of flowers, shoot and root length, fresh and dry weight and root knot indices. Fruit yield was negatively co related with initial and final population. Yield Loss was 80.71% at 4000 larvae inoculum level.

A field experiment was conducted to assess the crop loss due to root-knot nematode, *Meloidogne incognita* in brinjal at the main Research Station, Hebbal, and Bangalore. Randomized complete block strip trial was adopted with paired treatments in a nematode sick plot. Plants were protected with aldicrab (Temik 10G) at the rate of 8 kg a.i. /ha applied a day prior to transplanting in five replications. Another set of unprotected plants served as check. Significant differences in plant growth and vigour were observed in shoot height, fresh weights of shoot and root and length of roots between protected and unprotected plants. The root-knot index in the scale of 1–5 was 0.7 in protected plots compared to 4.71 in unprotected plots. There was a significant increase in fruit yield per plant in protected plots. The per hectare fruit yield was 20,500 kgs in treated plots as against 11,300 kgs in unprotected check plots. Thus, 44.87 per cent yield loss could be accounted to be due to root-knot nematode alone in a heavily infested field. (Krishnappa *et al.*1981)

Reddy (1981) reported at higher level of inoculums of *Meloidogyne* the damage of crop may be around 40%.

Fery & Dukes (1984) demonstrated that *Capsicum annum* cultivars exhibited a wide range of galling reactions to *M. incognita* in greenhouse and field studies. Egg

mass production was closely associated with galling. A homozygous susceptible selection from Carolina hot gave only 12.5% of the marketable fruit compared to homozygous resistant plants supported less egg production than did roots of susceptible plants. Resistant plants contained fewer larvae than susceptible plant.

A study was made of the effect of a single generation of the root-knot nematode *Meloidogyne incognita* on the growth of potted French bean plants (*Phaseolus vulgaris*) inoculated at different stages of plant maturity. In separate experiments. 3-, 11- and 13-day-old plants were inoculated before primary leaf expansion (BPLE). At the appearance of trifoliolate leaves (TRIF) and at the flower bud (BDS) stages respectively, with 0, 2000, 4000 or 8000 second-stage juvenile nematodes and maintained in a growth chamber under controlled conditions. The photosynthetic rate of the plants inoculated at the TRIF and BDS stages decreased significantly with increasing inoculum level 7 days after inoculation. Although the respiration rate did not significantly change throughout the experimental period, the ratio of photosynthetic to respiration rate decreased significantly with increasing nematode inoculum level and duration of infection. Chlorophyll content, plant dry weight and the numbers of buds, flowers, pods and seeds were significantly lower in infected plants than in the controls; this effect increased with increasing levels of nematode inoculum for all three plant stages. The leaf area was significantly smaller only when nematode infection occurred at the BPLE stage. The plants which were youngest at the time of nematode infection produced the lowest yield; this appeared to result from the effect of nematodes on photosynthesis and related physiological processes. (Melakeberhan *et al.*1986)

Darker & Mhase (1988) reveals serious yield losses in brinjal and other Solanaceae vegetable crop due to Root-knot nematodes²⁻⁴ that is around \$ 77 billion.

The average loss in yield due to *M. incognita* in chickpea [*Cicer arietinum*] var. G-1 was 28.35% in field experiments in India. Treatment with carbofuran (3 G) at 6 kg a.i. /ha was effective in minimizing the nematode population in soil and the gall index. (Darekar & Jagdale, 1989)

Field studies were done in Jaipur, India between 1986-88 to assess the losses caused by *Meloidogyne javanica* on *Vigna radiata* and available genotypes were evaluated for resistance and yield potential under naturally infested field conditions. Available seed losses averaged 39% for the 3 years of study. The variety DMG 1052-2 gave the highest yields of 12.51 g/ha. However, DMG 1052-1 and DMG 1050-1 which were green and bold seeded were the most promising varieties for *M. javanica* and *M. Incognita* infested fields. (Handa & Mishra, 1989)

Application of carbofuran at 1, 2 or 4 kg/ha to *V. radiata* infested with *M. javanica* in the field in Haryana, India, gave estimated avoidable losses of between 4.21 and 93.4%. None of the 34 *V. radiata* genotypes screened in a pot experiment was resistant to *M. javanica*. Seed treatment with carbofuran at 1% + soil application with phorate at 0.5 kg/ha gave best control of *M. javanica* on *V. radiata* in field trials, while yields were highest with carbofuran + phorate at 1 kg. (Gupta & Verma, 1990)

In field trials in Gujarat, application of carbofuran at 2 kg/ha increased grain yield of *Phaseolus aureus* (*Vigna radiata*) by 14.6% and decreased *Meloidogyne incognita* and *M. javanica* infection by 23.3%. In cowpeas there was a 14.0% increase in grain yield and 29.2% reduction in *Meloidogyne infection*. (Patel *et al.*, 1990)

Soyabeans were inoculated in a pot experiment 5, 25 and 45 days after germination with *Meloidogyne incognita* or *M. javanica*. *M. javanica* decreased soyabean yield 36, 31 and 7% and *M. javanica* reduced yield by 30, 23 and 21% when inoculated 5, 25 or 45 days after germination respectively. (Valiente *et al.* 1990)

Yield loss due to root-knot nematodes (*M. incognita* and *M. javanica*) was assessed by a field-infestation technique, and comparable plots of the commercial bean cultivar Calima and the CIAT advanced bean line PVA 916 were planted in infested plots and in uninfested plots 15 m from the infested ones. The nematodes induced yield losses of 45-63% to Calima and 26-32% to PVA 916, as determined by dry seed weight. Yield loss due to nematodes was greater with concurrent leafhopper (*Empoasca kraemeri*) damage. (Mullin *et al.* 1991)

Fields naturally infested with *Meloidogyne incognita* and *M. javanica* in Rajasthan, India and growing peas, okras, tomatoes or *Lagenaria siceraria* were either treated with 1.5 kg/ha carbofuran or left unprotected. Avoidable losses due to *Meloidogyne* ranged between 46 and 56%. (Sharma & Baheti, 1992)

Carbofuran at 3 kg/ha was applied to microplots of carrot var. Early nantes in Assam to estimate losses due to *M. incognita*. There were significant reductions in plant height (10.53%), tap root length (27.52%) and yield (40.81%) in untreated plots compared with treated plots. (Devi & Das, 1994)

Jain *et al.* (1994) reveals the avoidable yield losses in tomato due to *Meloidogyne incognita* were 71.9% by using carbofuran @ 3kg a.i. /ha. However, at other site having *Meloidogyne javanica* infestation, the per cent avoidable losses in yields of tomato, brinjal and okra were 47.3, 41.8 and 29.9 per cent, respectively.

Meloidogyne javanica (pathotype 3) is a serious pest of fennel (*Foeniculum vulgare*) in Gujarat, India. Application of carbofuran (Furadan 3 G) at 2 kg/ha increased seed yield by 39.1% and reduced the root-knot index by 66.4%. (Patel *et al.* 1995)

Soil application of carbofuran @ 3 kg a.i./ha, 3 weeks after planting, decreased avoidable yield losses due to *Meloidogyne incognita*, to the extent of 33.61% in turmeric (*Curcuma longa*) and 26.30% in ginger (*Zingiber officinale*). Gall index values were correspondingly high in control plots compared with treated ones in both ginger and turmeric. (Ray *et al.* 1995)

Midha (1996) reported yield loss of 34-83% in vegetables due to root knot nematode, *Meloidogyne spp.*

The avoidable yield losses in okra due to *M. incognita* (initial nematode population 300 and 340 juveniles/250 cc soil in 1995 and 1996, respectively) were assessed to be 15.05% in 1995 and 21.58% in 1996, in experimental field plots. All the growth parameters recorded in untreated plots were significantly reduced

compared to those of the plots treated with carbofuran at 3 kg a.i./ha. (Deka & Rahman, 1997)

Carbofuran at 2 kg a.i. /ha significantly improved the plant growth of sunflower cv. Mordan and controlled numbers of infecting *M. incognita*. The nematode was calculated to cause 16.44% yield loss in infected plants. (Devappa et al., 1998)

Field experiments were conducted for 2 years at 2 sites in Jaipur, Rajasthan, India, infested with *M. incognita* (>2 larvae/g soil), to determine the percentage avoidable yield loss of green chillies (*Capsicum annuum* cv. Jaipur local) by spot application of carbofuran/phorate into the soil at a rate of 2 kg/ha. The data indicated an average percentage avoidable loss of 53.36 and 39.76 with carbofuran and phorate treatments, respectively. A general reduction in plant height and fruit length following nematode infection was observed in both the years. (Yadav & Mathur, 1999)

Field experiments were conducted in Udaipur, Rajasthan, India to assess yield losses caused by the root-knot nematode *M. Incognita*. Data revealed that the avoidable loss in chilli (cv. RCH-1) ranged between 11 and 33% with an average of 23% with the application of carbofuran at 2 kg a.i. /ha. In monetary terms, an additional benefit of Rs. 15 240/ha was obtained with chemical treatment. In the susceptible tomato cultivar (Pusa Early Dwarf), avoidable yield losses ranged from 19 to 29% with an average of 26% with the application of carbofuran at 2 kg a.i./ha. In the resistant tomato cultivar (PNR-7), avoidable yield losses ranged between 13 and 18% with an average of 25% with the application of carbofuran at 2 kg a.i. /ha. In monetary terms, benefits of Rs. 7548/ha and Rs. 8776/ha were obtained with chemical treatment. (Bhargava & Sharma, 2001)

Experiments were carried out to assess the avoidable yield losses in bitter melon (*Momordica charantia*) due to root-knot nematode (*Meloidogyne incognita*) for 5 consecutive years in the nematode-infested field in Solan, Himachal Pradesh, India. A significant increase in yield was observed in the plots receiving pre-sowing carbofuran application compared to the untreated control plots. The maximum

increase in yield was achieved during the second year when 5825 kg of fruits were harvested from nematicide-treated beds compared to 3333.3 kg/ha from the untreated control beds. Incidentally, the initial nematode count was also highest in that year. Yield losses ranging between 22.9 and 42.8% were recorded during different years. Initial nematode population seemed to play a major role as the initial number of below 200 (count of less than one nematode per gram) in the third year, though caused yield losses of 22.9%, their non-significant 't' value revealed the yields in treated plot to be at par with the untreated control. However, nematode count beyond this limit caused significant damage to the crop. (Khanna & Kumar, 2003)

Fennel is severely affected by rot-knot nematode, *Meloidogyne javanica* pathotype 2 especially in the Kapadwanj area of the Kheda District in Central Gujarat, India. Three years' (1999-02) pooled data of field experiments on loss assessment in the crop due to *M.javanica* pathotype 2 revealed that the application of carbofuran at 2 kg/ha in two equal splits (one at the time of transplanting in furrows and another at 2.5 months after as side dressing in fennel (PF 35) field) reduced root-knot index by 48.82% and resulted in a 26.69% increase in grain yield, indicating an overall 21.07% loss in grain yield of fennel due to *M. javanica* pathotype 2. (Patel, *et.al* 2003).

The yield losses recorded in 4 crops with different sowing dates ranged from 6.5 to 26.6%, however, *H. dihystra* was more damaging (yield losses 22.48-26.60%) than *M. incognita* (yield losses 6.5-17.82%). In addition, lower population of *H. dihystra* (423) was more damaging than high population of *M. incognita* (4176). French bean sown in May and June was more prone to infestation by *H. dihystra* than in July and August where it was more prone to *M. incognita* populations. (Sharma & Bhatia, 2004)

Kumar *et al.* (2005) describe the efficacy of different intercrops, marigold, mustard and sweet potato against root-knot nematode, *Meloidogyne incognita* infesting vegetables like tomato, okra and brinjal were evaluated by growing intercrops in vegetable-based cropping systems. Among the different intercrops evaluated, marigold intercropped with different vegetables reduced the nematode population in soil, number of galls, egg masses per root system, number

of eggs per egg mass and root-knot index as compared to growing of vegetables alone continuously.

Yield losses caused by the root-knot nematode, *Meloidogyne incognita*, were assessed in gherkin [cucumber] fields in Kolar and Bagepalli, Karnataka, India. Comparison was made between phenamiphos [fenamiphos]-treated (2 kg a.i./ha) and untreated fields. Root-knot nematode incidence and severity were high at both locations. Root-knot index ranged from 3.2 to 4.5 on a 0-5 scale with number of egg masses ranging from 69 to 98/g root. Infected primary and secondary roots were transformed into galls with very few feeder roots. Nematode incidence was observed in 25-50% of the fields surveyed, with severity in individual fields ranging from 25 to 50%. Phenamiphos treatment resulted in 38 and 43% higher yield in Kolar and Bagepalli, respectively, compared with the untreated control. (Nagesh *et al.* 2005)

Studies were conducted during May 1984 on estimation of losses in okra under field conditions naturally infested with *Meloidogyne javanica*. The yield of okra was reduced at population levels of 296 ± 5 *Meloidogyne javanica* larvae per 250 g soil. Percentage avoidable losses in yield ranged from 20.2 to 41.2% by using carbofuran and aldicarb at 2.0 and 4.0 kg. a.i /ha respectively. Aldicarb gave better results than carbofuran at both the doses. Cost-benefit ratio was found maximum (1:11.7) with aldicarb at 2.0 kg a.i. /ha whereas it was minimum (1:1-8) in carbofuran treated plots at 4.0 kg a.i. /ha. (Jain *et al.* 2008)

Fieldpea trials were laid down to estimate unavoidable yield losses in chickpea, fieldpea and lentil crops due to infestation of root-knot nematode, *Meloidogyne incognita* at Indian Institute of Pulses Research Kanpur. Paired plot treatment method was employed to know the extent of yield losses of these crops infested with root-knot nematode in sandy loam soils. Carbofuran and phorate @ 2 kg a.i./ha were used to check the root-knot nematode population in the infested fields of chickpea, fieldpea and lentil respectively. Results indicated that root-knot nematode had incurred unavoidable yield losses to the tune of 25.6% in chickpea and 15% each in pea and lentil. (Ali, 2009)

Avoidable yield losses due to infestation of *Meloidogyne incognita* and other plant parasitic nematodes on fieldpea cv. Bonneville was studied under field condition

for three consecutive years. Paired plot technique was employed using carbofuran @ 2 kg/a.i./ha along with untreated control, replicated six times. Yield of fieldpea was declined 18.32% in untreated plots as compared to treated plots. There was an increase of 52.06 % in total population of plant parasitic nematodes in untreated plots while a decrease of 60.5 % over initial nematode population was recorded in treated plots. There was a significant reduction in *rhizobium* nodulation in nematode infested plots than to treated plots. (Haider *et al.* 2009)

Anwar & Javid, (2010) reported root knot nematodes are obligate, sedentary parasites of vascular the tissue of plant roots. Infected plants usually exhibit root galling. Additional symptoms induced by nematode feeding include reduction in plant vigour and root lesion, rooting and deformation. The plants with nematode damaged roots. Nematode root damage reduces the plant ability to extract available salt water and nutrients the results being lack of vigour & yield loss.

Kaur and Dhatt (2010) reported that root knot nematode (*Meloidogyne incognita*) caused 30-50% yield losses in brinjal crop. Growing of resistance cultivars is regard as soil and ecofriendly practice to manage these nematodes among the 57 brinjal cultivars none of the line among cultivars showed immune and complete resistance reaction to root knot nematode. However 3 lines viz.; KG-219, IC-96630G-1, and IC-909007G-20 were some found resistance to root knot nematode with root gall index between 1.1-2.0 and 6 entries viz. PV-70-1, PBG-11 and HABR-6, Arkamidhi, 9249, SR-230 showed moderately susceptible with root gall index ranging from 2.1-3.0 among others 16 lines showed susceptible and 33 entries showed highly susceptible to reaction to root knot nematode.

The micro plot experiment was conducted to assess the avoidable yield loss in okra due to root-knot nematode, *M. incognita*. The results indicated that the loss in yield of okra was recorded to the extent of 27.02 per cent, when the crop was treated with carbofuran 3G @ 2 kg a.i. /ha. (Shendge *et al.*, 2010)

Field studies were conducted in 2008 and 2009 to determine yield loss of cowpea (cv. ART98-12) due to natural infestation by *M. incognita* using Carbofuran 3G at 2 kg a.i. /ha and untreated as check. The yield of cowpea was found to be higher with the application of nematicide-Carbofuran 3G at 2 kg a.i. /ha. The percentage

increase over control was 39.0 and 33.0% in the years 2008 and 2009, respectively. A significant reduction in the yield of cowpea in untreated plots was mainly attributed to direct damage of the root system by the feeding of root-knot nematode, *M. incognita*. The root knot nematode population in carbofuran treated plots was significantly lower than in check in the two years, also at harvest. In the check the nematodes reproduced many folds during the cropping season. Higher nematode population in the untreated check decreased plant growth and consequently reduced the number of harvested seeds and other agronomic parameters. (Adegbite, 2011)

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Among the most damaging root-knot nematode species, *Meloidogyne incognita* is one of the major constraints to okra (*Abelmoschus esculentus* L.) in vegetable-growing areas in Pakistan and other countries of the world. The relationship between a geometric series of six initial population densities (Pi) of *M. incognita* per Kg of soil and growth parameters and nematodes infestations was investigated on okra. Observations were recorded 45 days after inoculation with the nematode. It was found that all the inoculum levels reduced the shoot and root lengths, and fresh and dry weights. Increasing the nematode inoculum level increased the number of galls, egg masses and nematode population build up. The reductions in growth parameters and nematode infestations were found to be directly proportional to the inoculum level. On the other hand an inverse relationship was observed between the nematode build up and inoculum levels. (Hussain *et al.* 2011)

The root-knot nematodes (*Meloidogyne* species) are economically important pathogens, especially infesting vegetable crops. In the tropics and subtropics, *Meloidogyne incognita* causes an estimated yield loss of 5 to 43% in vegetable crops. (Gautam *et al.* 2014)

CHAPTER- 3

MATERIALS & METHODS

3. MATERIALS AND METHODS

Studies were conducted under pot culture condition in net house and experimental field of Department of Nematology, College of Agriculture, O.U.A.T., Bhubaneswar. Various materials and methods used in the present investigation have been categorized and detailed below.

3.1 Materials Used

Glasswares and Equipments

The following laboratory appliances and other materials required to conduct the experiment during the investigation were as follows:

- a) Aluminum Pans
- b) Beakers (250ml)
- c) Aluminum wire gauge
- d) Measuring Cylinder (100ml)
- e) Petridish
- f) Tissue paper
- g) Counting dish
- h) Capillary micro pipette
- i) Hand tally counter
- j) A set of sieves
- k) Wet collection bottles
- l) Wooden scales
- m) Lab wash
- n) Binocular stereoscopic microscope
- o) Hot air oven
- p) Electronic balance
- q) Earthen pots of 15cm diameter
- r) Phowarh
- s) Needle
- t) Rose can
- u) Bucket
- v) Butter paper

Chemicals used

- a) Carbofuran
- b) Glycerol
- c) Acid fuchsin
- d) Formalin
- e) Mercurochrome (0.1%)
- f) Sodium hypochlorite

The test nematode species

The test nematode species for the experiment was *Meloidogyne incognita*.

3.2 METHODS

Preparation of Soil and Pots

For experimental purpose well pulverized sandy loam soil, free from plant debris and pebbles, was collected from the University central farm. The soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1 which was autoclaved at 15 lb pressure/ sq inch for 20 minutes. Earthen pots of 15 cm diameter were cleaned and surface sterilized with 4% formaldehyde solution. Pots were air dried and then filled with aerated autoclaved soil.

Maintenance and culturing of nematode

The experimental pure culture of root-knot nematode, *Meloidogyne incognita* was originally obtained from a single egg mass progeny maintained and multiplied on brinjal seedlings in 30 cm diameter pots containing autoclaved soil. The population of nematode was sub-cultured periodically at 50-60 days intervals by inoculating new transplants with infective second stage juvenile suspension of *Meloidogyne incognita* obtained from old culture pots. Other intercultural operations were attended to as and when necessary.

Isolation of egg masses and juveniles of *M. incognita*

Galled roots of French bean plants were collected from the culture pots. The roots were washed free from soil under a tap with gentle stream of water. After partial air drying egg masses are picked up with the help of tweezers and needles and kept over the wire gauge tissue paper assembly rested on the Petridish containing clean tap

water just touching the bottom surface of the wire gauge. Petridishes were also covered to avoid evaporation loss. Freshly hatched second stage Juveniles (j_2) were collected in beakers at 24h intervals and fresh water was added to the petridish at each change. Collection of juveniles was continued for 7 to 8 days and used subsequently for experimental purpose.

One pot culture experiment to study the pathogenicity of root knot nematode infecting French bean was conducted in the net house and another field experiment was conducted by growing French bean in the field of Nematology Department to estimate the yield loss of French bean due to root knot nematode.

3.2.1 EXPERIMENT NO 1:

Pot culture Experiment

Pathogenicity of root-knot nematode, *Meloidogyne incognita* infecting French bean:

Pathogenicity experiment was conducted under net house condition to test the pathogenic potential of root-knot nematode, *M.incognita* on French bean (var.contender) plants. Five to six numbers of surface sterilized, (0.1% HgCl₂ solution for two minutes followed by repeated washing in distilled water) healthy seeds of French bean were sown in each pot at size 15cm diameter containing autoclave sterilized soil mixture. Seven days after germination, the French bean plants in each pot were thinned out keeping one healthy plant per pot. Regular care was taken for maintenance of the plants in the net house.

Inoculation of nematodes

Freshly hatched j_2 of *M.incognita* were isolated from pure culture pot and sterilized by treating with aqueous solution of mercurochrome (0.1%) for 30 minutes in order to get rid of any bacterial or fungal contaminants. Then the juveniles were washed 4-5 times in sterilized distilled water. Nematode population was estimated by counting replicated aliquots of the suspension. A measured volume of hatched juveniles suspension at different levels (10, 100, 1000, 10000 per pot) was pipetted and put around the root zone by making small holes according to necessity. Holes were then filled with soil followed by light watering. There were all together 5

treatments with 4 replications including an uninoculated check arranged in completely Randomized Design. The treatments were as follows.

T₁= Check (No nematodes)

T₂= 10 nematodes (J₂) / pot

T₃= 100 nematodes (J₂) / pot

T₄= 1000 nematodes (J₂) / pot

T₅= 10000 nematodes (J₂) / pot

At 60 days after inoculation, the experiment was terminated and observations were recorded on plant growth parameters as well as on the nematode population development.

Recording of observations:

At 60 days after inoculation, each plant was removed from the pot soil carefully. Roots were washed free of soil and adhering particles under slow stream of water and observation were recorded on different plant growth characters, number of galls and nematode population in soil as well as in root.

Shoot length

Shoot length of each plant was measured from the plant up to the top most portions in meter scale.

Root length

Root portion of each plant was cut, labelled and knots if any, were opened followed by straightening of roots. The root length of each plant was measured up to the tip in the meter scale.

Fresh weight of shoot and root

Fresh weight of shoot and root of individual plant were recorded in grams. These were further labelled and kept for recording of dry weights.

Dry weight of shoot and root

Shoot and root of individual plant were air dried and kept in separate paper packets. Such packets were then placed in hot air oven at 70⁰ C temperature for 48 hours after which, the dry weight were recorded in gram.

Number of galls

Total number of galls on roots of each plant was counted with the help of hand tally counter before drying and recorded.

Number of egg masses/plant

Number of egg masses in roots were counted under stereoscopic microscope and recorded.

No. of Eggs/ Larvae per egg mass

Randomly, 5 egg masses were picked from each plant and were shaken well in 0.5% Naocl for 3 min. for separation of eggs/ larvae and counted.

Nematode population in soil

Soil from each pot was mixed thoroughly and 200 ml sample from each pot was collected and screened by Cobbs' sieving technique (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1961) for estimation of nematode population in different treatments.

The ratio of nematode multiplication in different treatment was calculated by using the formula.

$$R = \frac{P_f}{P_i}$$

Where,

R= Rate of nematode multiplication

P_f = Final nematode population

P_i = Inoculated (initial) nematode population

Statistical analysis

Various observation recorded during the course of investigation were subjected to statistical analysis in a Completely Randomized Design. Data on number of root galls, egg masses and final nematode population in soil as well as in root were analyzed after necessary transformation wherever necessary. Fisher's methods of analysis of variance at 5% level of significance were followed. Further, the comparison of the treatment means was done by calculating standard error of mean S.E. (M) and least significant difference (L.S.D) in the following manner.

$$\text{S.E (M) (standard Error of Means)} = \sqrt{\frac{2 \times \text{Error meansquare}}{\text{Number of replication}}}$$

L.S.D. (Least Significant Difference) or C.D. (critical Difference) = t at 5% for error d.f. \times S.E (M)

The difference between the two treatment means if greater than the LSD value, it indicated the significant difference between the treatments.

3.2.2 EXPERIMENT NO 2:

Assessment of yield loss due to root-knot nematode in French bean

The experiment was conducted in a field of Department of Nematology, College of Agriculture, OUAT naturally infested with root-knot nematode using pair plot technique to assess the avoidable yield loss of French bean c.v. contender due to root-knot nematode *Meloidogyne incognita*.

The field was thoroughly cultivated and pulverised Soil samples (200cc) were drawn with the help of a hoe up to a depth of 15cm. Five such samples from each plot of one treatment were collected and mix thoroughly. Out of this composite sample, one sample of 200 cc taken and processed for nematode extraction by Cobb's sieving and decanting method (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1961) to know initial nematode population. The field was divided into two blocks i.e. treated and untreated each having 10 sub plots of size 3m x 2m serving as replication. For treated plots, carbofuran (Furadon 3G) @ 3kg ai/ha was applied before planting of French bean seeds followed by light watering. The crop was grown following standard recommended package and practice with row to and plant to distance of 40cm x 20cm. The observations on final nematode population, (200 cc soil and 5g root) root gall indices (1-5 point scale), fresh weights of shoot and root, Dry weight of shoot and root and yield per plot were recorded at the time of termination of experiment. Data obtained were analysed according to 't' test for paired comparison at 5% level of probability.

$$\text{'t' at error degree of freedom} = \frac{\bar{X}_1 - \bar{X}_2}{S_d}$$

Where \bar{X}_1 = mean of treated plot

\bar{X}_2 = mean of untreated plot

$$S_d = S/\sqrt{n} \text{ where } S = \text{standard deviation} = \sqrt{\frac{\sum d^2 - \frac{(\sum d)^2}{n}}{n-1}}$$

n= number of paired plots.

d= differential value between two paired plots.

The avoidable yield loss and percent increase in yield over control (untreated) by following formulae. (Pradhan1964).

$$\text{Avoidable yield loss (\%)} = \frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{mean yield of treated plot}} \times 100$$

$$\text{Increase in yield (\%)} = \frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{mean yield of untreated plot}} \times 100$$

Fig. 2. Yield loss of French bean due to *M.incognita*



Figure. 1. Effect of initial levels of *Meloidogyne incognita* on shoot & root growth of French bean



T1= No J₂/pot, T2= 10 J₂/pot, T3= 100 J₂/pot, T4=1000 J₂/pot,T5= 10000 J₂/pot

4. RESULTS

Experiment no 1:

Pathogenicity of *Meloidogyne incognita* on french bean:

Experiment on pathogenicity of *M.incognita* infecting French bean was conducted during rabi, 2014 and observations were recorded.

Plant growth characters

Shoot height, shoot fresh and dry weight

The height, fresh and weight of shoots of French bean plants recorded and presented in Table 1 and illustrated in Fig 3. Progressive decrease in these parameters with the corresponding increase in the initial inoculum levels of nematode was exhibited. Although significant differences was noticed among treatment means of these parameters the data revealed that there were no significant differences in the shoot growth parameters with initial inoculum level of 10 & 100 J₂ per pot in comparison to uninoculated treatment (T₁). The reduction in shoot length, fresh and dry shoot weights ranged from 4.00 to 25.14, 7.77 to 36.06 and 4.97 to 36.77%, respectively. The reduction of these parameters started at the lowest inoculum level. But significant reduction could be observed at and above 1000J₂/ kg of soil. At 1000J₂ / kg soil inoculum level there were 22.00, 25.40 and 29.42% reduction in shoot length, fresh and dry weight of shoots respectively.

Root length, root fresh and dry weight

The data on length, fresh and dry weight of roots have been presented in Table 2 and illustrated in Fig.4. The data also revealed a progressive decrease from lowest inoculum level with the corresponding increase in initial inoculum levels of nematodes. It ranged from 4.46 to 38.92, 14.00 to 36.00 and 4.4 to 40.55% with respect to length, fresh and dry weight of roots respectively. Significant reduction of these root parameters was observed at and above 1000J₂ per kg soil.

Number of galls, egg masses and Final nematode population in soil

Observations on number of galls, egg masses per plant and nematode population in soil have been recorded and presented to Table 3 and Fig.5. This showed a corresponding increase in host infectivity with the increase the inoculum

level of nematodes. Statistical analysis of the data indicated significant difference among the treatments. Increase in the level of nematode inoculum from 10 to 1000 Juveniles resulted in a significant increase in host infestation as indicated by number of root knot galls. Further increase to 10,000 juvenile in the inoculum resulted in more increase in root knot galls but not significant with 1000 level. The number of egg masses / plant varied from 2.25 to 88.0 with the increase of inoculum level from 10 to 10,000 /juveniles per kg of soil. The trend of these number of egg masses per plant under inoculum level was similar to the number of galls.

As the inoculum level increased soil population per 200 cc of soil and also total nematode population both in soil and root increased significantly. The nematode population per 200cc of soil was minimum (80.5) at 10 juveniles level and maximum (272.0) at 10,000 Juveniles level, when inoculated initially.

Total population of nematodes both in soil and root varied from 1074.00 to 20588.00 due to the increase of inoculum level from 10 to 10,000 Juveniles. However, no significant difference was recorded between the total nematode populations of pots with initial inoculum level of 1000 Juveniles and 10,000 Juveniles.

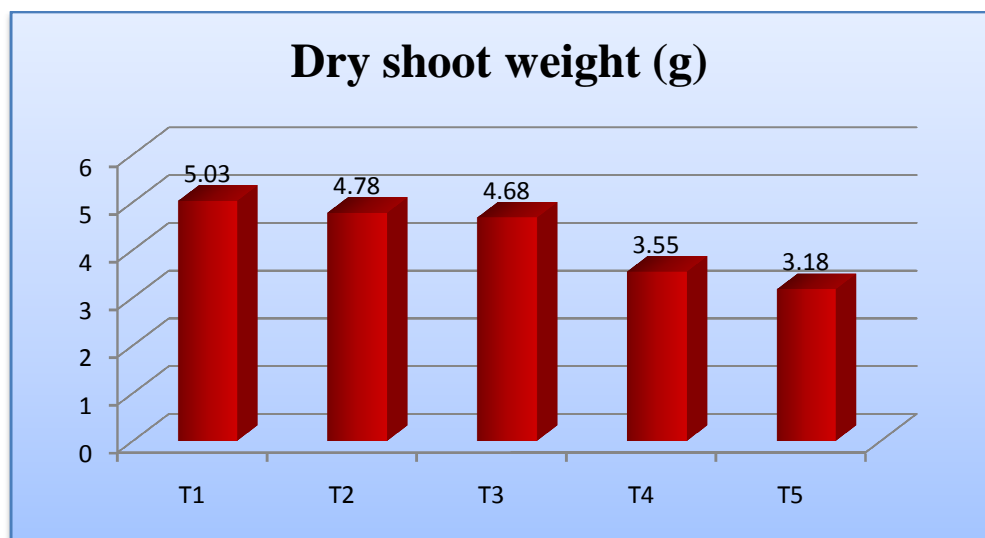
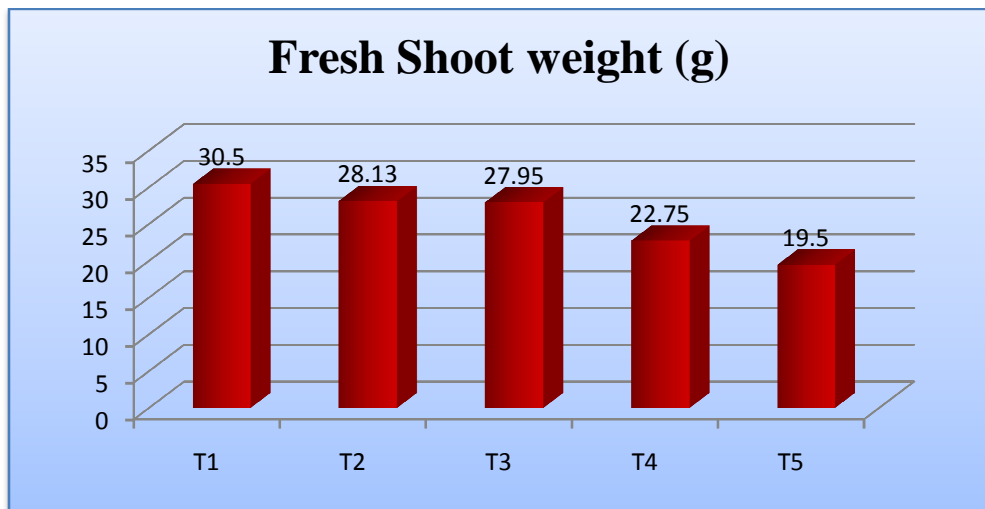
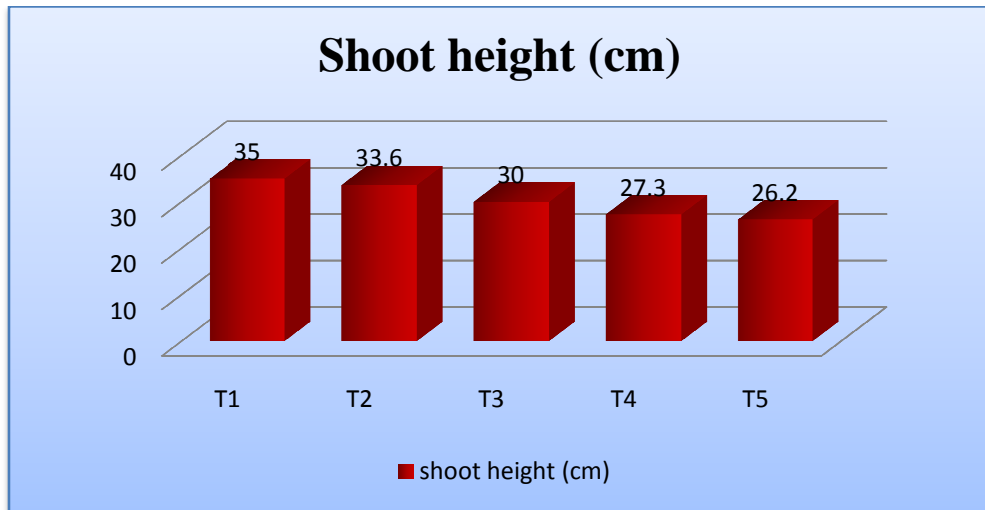
Although, final nematode population increased from the lowest inoculum level to the highest, multiplication rate, however, was recorded to be in reverse order, maximum multiplication (107.40) was recorded in treatment with 10J₂ per pot as against the minimum (2.05) in 10,000 J₂/pot. The multiplication rate was 19.25 in 1000 J₂ per pot.

Table: 1. **Effect of different initial inoculums of *M.incognita* on shoot growth parameters of French bean**

(Average of 4 replications)

Treatments	Shoot height (cm)	% decrease	Fresh shoot weight (g)	% decrease	Dry shoot weight (g)	% decrease
T ₁ = Un-inoculated	35.0	–	30.5	–	5.03	–
T ₂ = 10 J ₂ / kg soil	33.6	4.0	28.13	7.77	4.78	4.97
T ₃ = 100 J ₃ / kg soil	30.0	14.28	27.95	8.36	4.68	6.95
T ₄ = 1000 J ₂ / kg soil	27.3	22.00	22.75	25.40	3.55	29.42
T ₅ = 10000 J ₂ / kg soil	26.2	25.14	19.50	36.06	3.18	36.77
SE(M) (0.05)	(2.13)	–	(0.87)	–	(0.17)	–
CD (0.05)	(6.55)	–	(2.69)	–	(0.53)	–

Figure.3.Effect of Different initial inoculums of *M.incognita* on shoot growth parameters of French bean



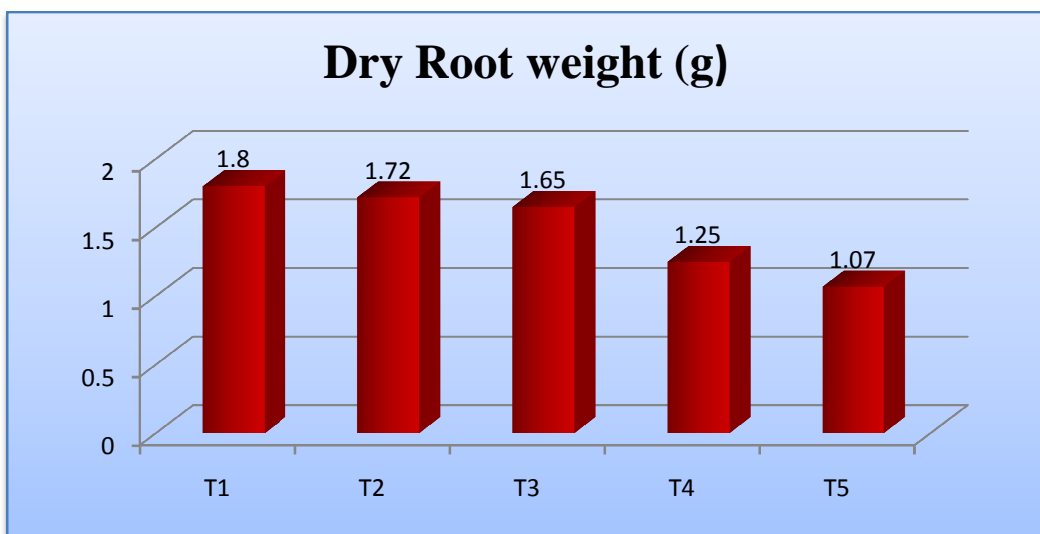
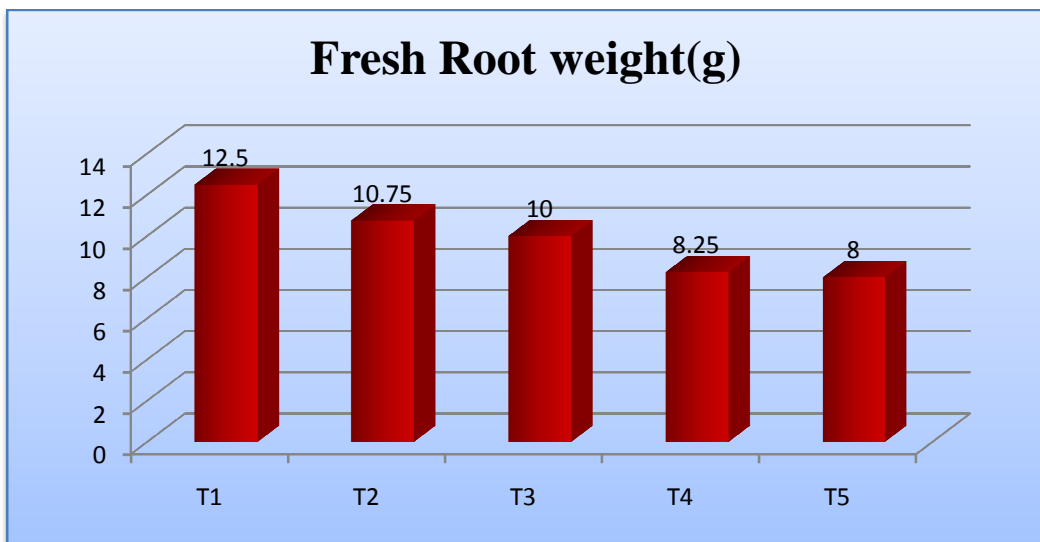
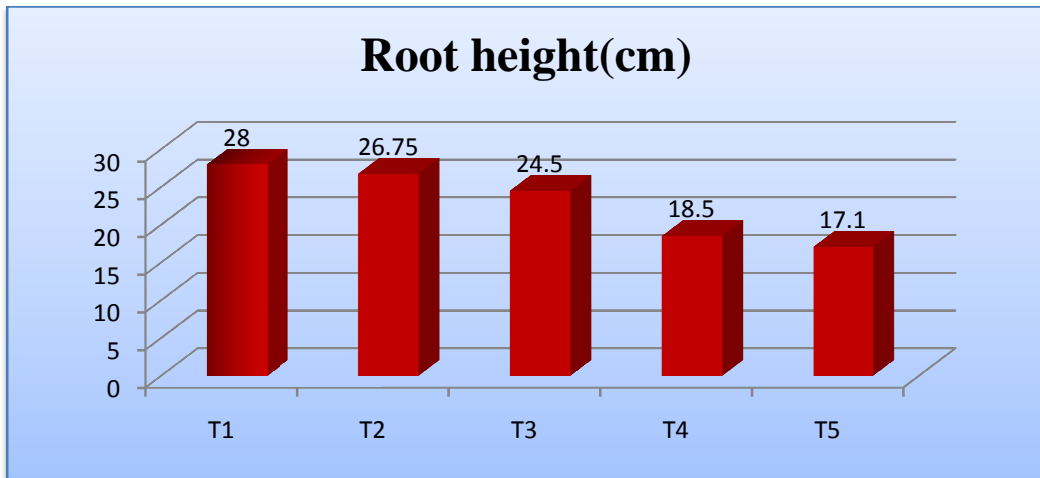
T1= No J_2 /pot, T2= 10 J_2 /pot, T3= 100 J_2 /pot, T4=1000 J_2 /pot, T5= 10000 J_2 /pot

Table: 2. Effect of different initial inoculums of *M. Incognita* on root growth parameters on French bean

(Average of 4 replications)

Treatments	Root length (cm)	% Decrease	Fresh root weight (g)	% decrease	Dry root weight (g)	% Decrease
T ₁ = Un-inoculated	28.0	–	12.50	–	1.8	–
T ₂ = 10 J ₂ / kg soil	26.75	4.46	10.75	14.00	1.72	4.4
T ₃ = 100 J ₃ / kg soil	24.50	12.50	10.00	20.00	1.65	8.33
T ₄ = 1000 J ₂ / kg soil	18.50	33.92	8.25	34.00	1.25	30.55
T ₅ = 10000 J ₂ / kg soil	17.10	38.92	8.00	36.00	1.07	40.55
SE(M) (0.05)	(1.22)	–	(0.90)	–	(0.10)	–
CD (0.05)	(3.75)		(2.77)	–	(0.30)	–

Figure.4. Effect of Different initial inoculums of *M.incognita* on root growth parameters of French bean



T1= No J_2 /pot, T2= 10 J_2 /pot, T3= 100 J_2 /pot, T4=1000 J_2 /pot, T5= 10000 J_2 /pot

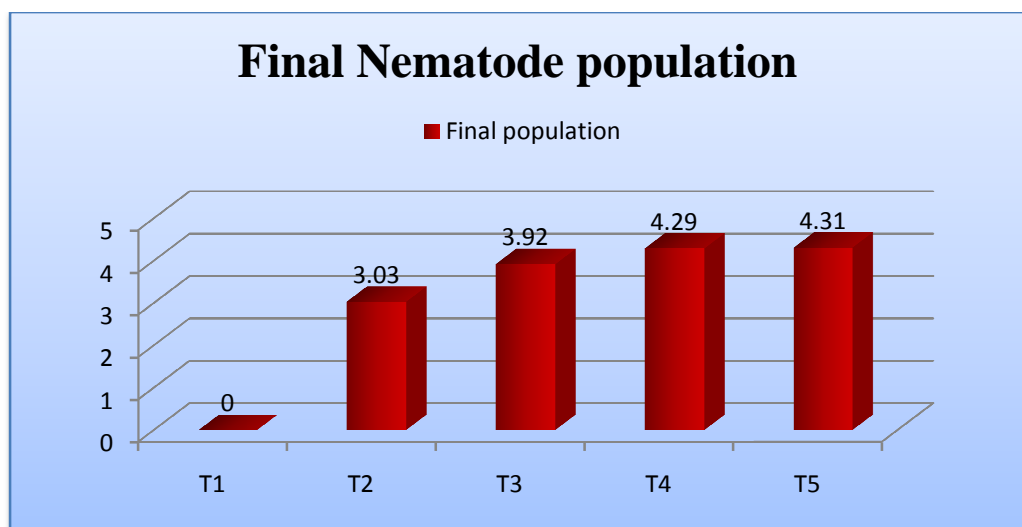
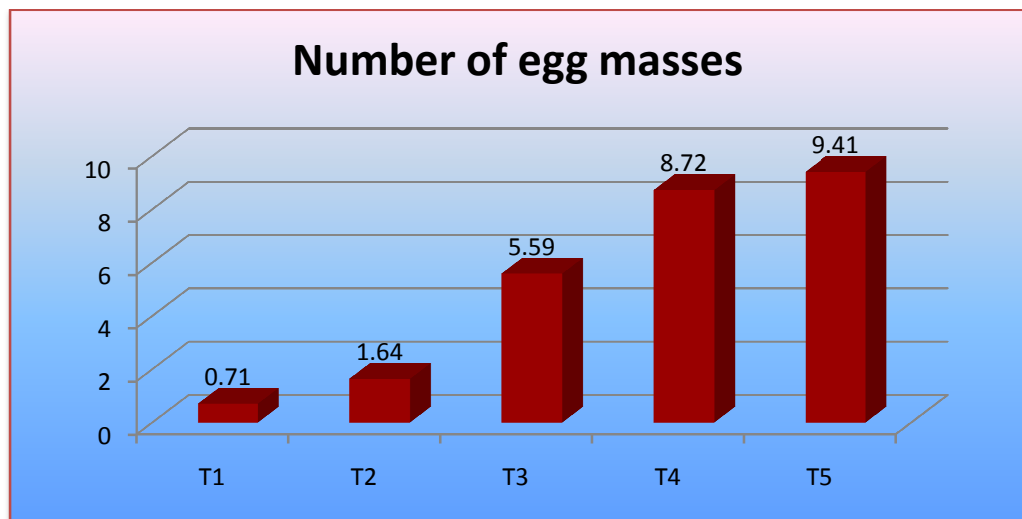
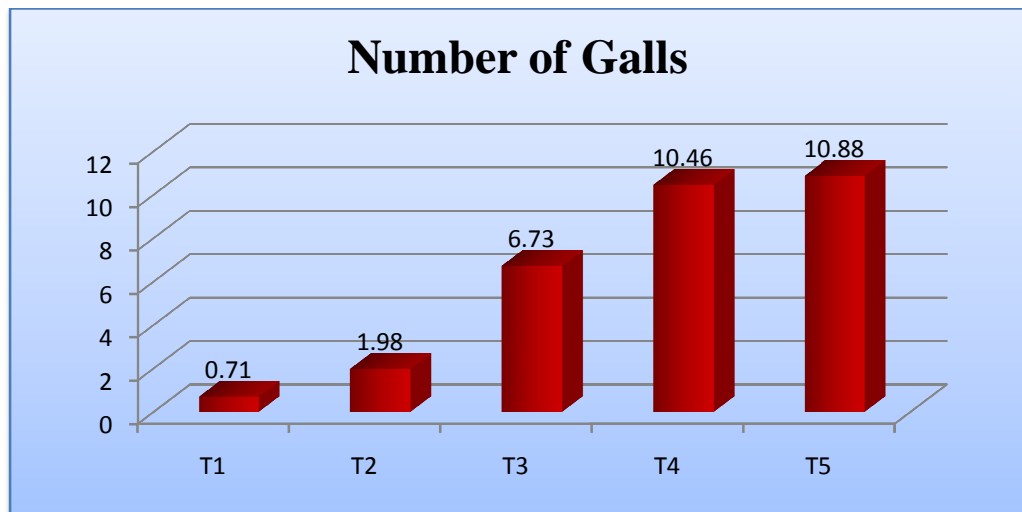
Table: 3. Effect of different initial inoculums of *M incognita* on nematode multiplication in French bean

Treatments	* Number of galls per plant	*Number of egg masses per plant	**Nematode population (200cc soil)	**Final population	Multiplication factor $R=P_f/P_i$
T ₁ = Un-inoculated	0 (0.71)	0 (0.71)	0 (0)	0	0
T ₂ = 10 J ₂ / kg soil	3.5 (1.98)	2.25 (1.64)	80.50 (1.91)	1074.0 (3.03)	107.40
T ₃ = 100 J ₂ / kg soil	44.75 (6.73)	30.75 (5.59)	120.0 (2.08)	8257.50 (3.92)	82.57
T ₄ = 1000 J ₂ / kg soil	109.0 (10.46)	75.5 (8.72)	250.75 (2.40)	19253.75 (4.29)	19.25
T ₅ = 10000 J ₂ / kg soil	118.0 (10.88)	88.0 (9.41)	272.0 (2.44)	20588.00 (4.31)	2.05
SEM= (0.05)	(0.16)	(0.10)	(0.02)	(0.01)	
CD = (0.05)	(0.49)	(0.31)	(0.06)	(0.03)	

* Figure in parentheses are $\sqrt{n+0.5}$ values

**Figure in parentheses are log (n+1) values

Figure:5. Effect of initial levels of *Meloidogyne incognita* on final nematode multiplication in French bean



T1= No J₂/pot, T2= 10 J₂/pot, T3= 100 J₂/pot, T4=1000 J₂/pot, T5= 10000 J₂/pot

YIELD LOSS

Results of yield loss assessment in French bean due to root knot nematode depicted in Table No.4 and Fig.6 showed that yield per plant in treated plot (3.09kg) was significantly higher than untreated plot (2.51kg), thereby leading to 18.77% avoidable loss in French bean yield due to *M.incognita* Root knot index at harvest in treated plot (2.2) was lower than untreated plot (4.00) (Table 7). The differences in yield and root knot index when compared by applying paired 't' test revealed significant differences (P=0.5) in treated plots thereby reflecting upon the efficacy of carbofuran in controlling root knot nematode and also for its utility as an effective nematicides for loss estimation studies.

Significant differences were also noticed between the treated plot and untreated plot with respect to root knot nematode population in 5g of root and 200cc of soil, Plant growth character like plant height, Fresh and dry weights of shoot, and roots. Root knot nematode population of 312.6 per 200cc soil and 53.5 in 5g root was recorded from untreated plot as against 95.2 and 14.3, respectively from treated plot. (Table No.7 Fig.10). Mean plant height of 42.70cm along with fresh shoot weight 64.70g and fresh root weight of 14.6g were observed from the treated plot where as the untreated plots had produced the plants of 31.1 height having 45.0 g weight of shoot and 9.3 g fresh weight of roots. (Table No. 5 &6, Fig.7, 8 & 9).

Table 4:- Effect of *M.incognita* on yield of French bean

(Mean of ten replications)

Treatments	Yield (Kg/Plot)	%loss in yield over treatment	% increase in yield over untreated
Treated (Carbofuran@3kg a.i/ha)	3.09		23.10
Untreated	2.51	18.77	
Cal. 't' value	3.05		
Table 't' value	2.26		

Fig.6. Effect of *M.incognita* on yield of French bean

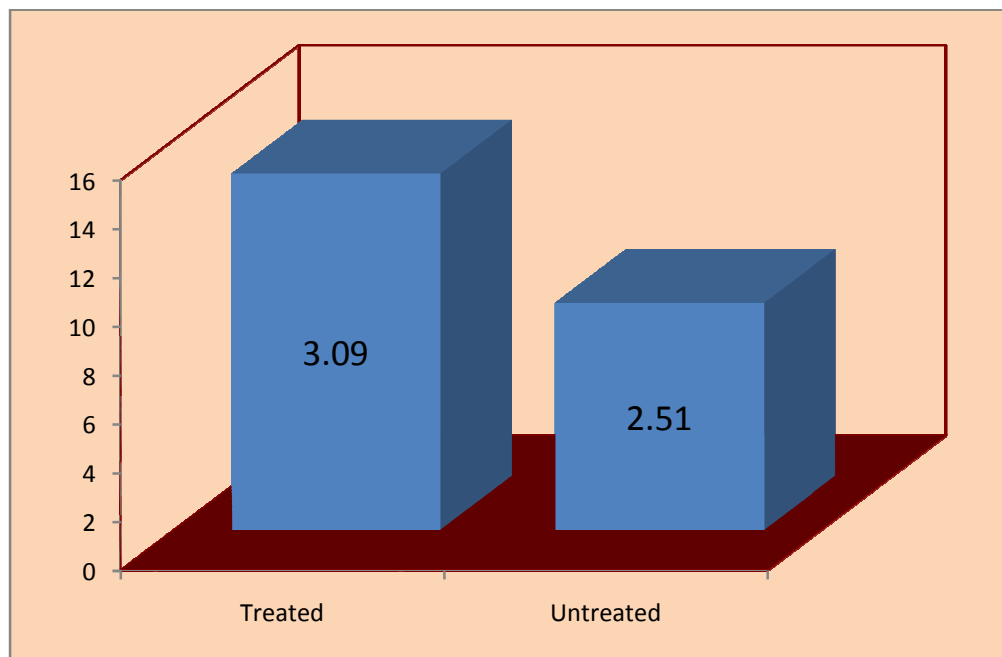


Fig. 7.Effect of *M.incognita* on plant height of French bean

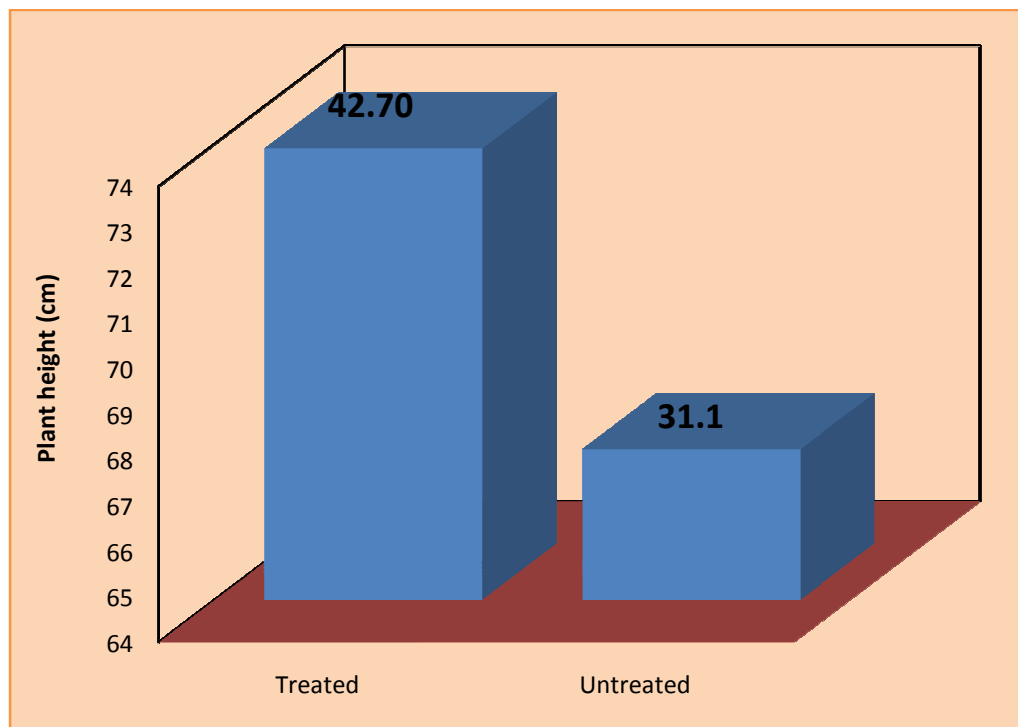


Table: - 5. Effect of *M.incognita* on vegetative growth of French bean**(Mean of ten replications)**

Treatments	Plant Height (cm)	% Decreased over treated	% increased over untreated	Fresh Weight of Shoot (g)	% Decreased over treated	% increased over untreated	Dry Weight of Shoot (g)	% Decreased over treated	% increase over untreated
Treated(Carbofuran@3Kg a.i/ha)	42.70		36.73	64.7		43.77	11.54		54.24
Untreated	31.10	27.17		45.0	30.45		7.45	35.44	
Cal. 't' Value	17.58			25.58			8.02		
Table 't' Value	2.26			2.26			2.26		

Table: - 6. Effect of *M.incognita* on roots of French bean**(Mean of ten replications)**

Treatments	Fresh Weight of root (g)	% Decreased over treated	% increased over untreated	Dry Weight of root (g)	% Decreased over Treated	% increased over untreated
Treated(Carbofuran@3Kg a.i/ha)	14.6		50.53	2.99		106.20
Untreated	9.3	36.30		1.45	51.84	
Cal. 't' Value	9.14			6.74		
Table 't' Value	2.26			2.26		

Table: - 7. Final Population of *M.incognita*& root knot index in French bean**(Mean of ten replications)**

Treatments	Root knot nematode population						Root gall index	% Increased over treated	% decreased over untreated
	200cc soil	% Increased over treated	% decreased over untreated	5g root	% Increased over treated	% decreased over untreated			
Treated (Carbofuran@3kg a.i/ha)	95.2		69.55	14.3		73.27	2.20		45.00
Untreated	312.6	228.36		53.5	274.12		4.00	81.81	
Cal. 't' Value	73.95			48.4			6.0		
Table 't' Value	2.26			2.26			2.26		

Fig.8. Effect of *M. incognita* on fresh and dry shoot weight of French bean

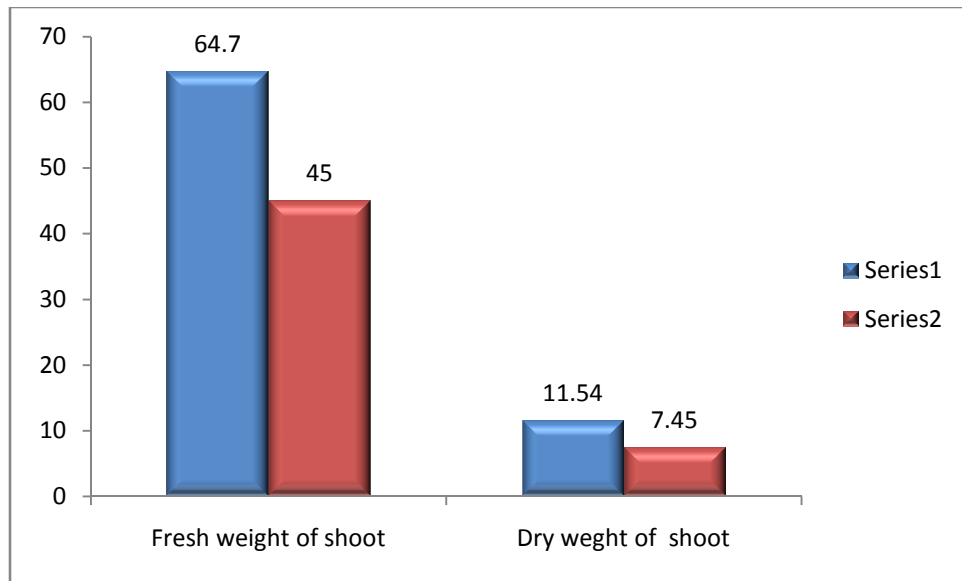


Fig.9. Effect of *M. incognita* on fresh and dry root weight of French bean

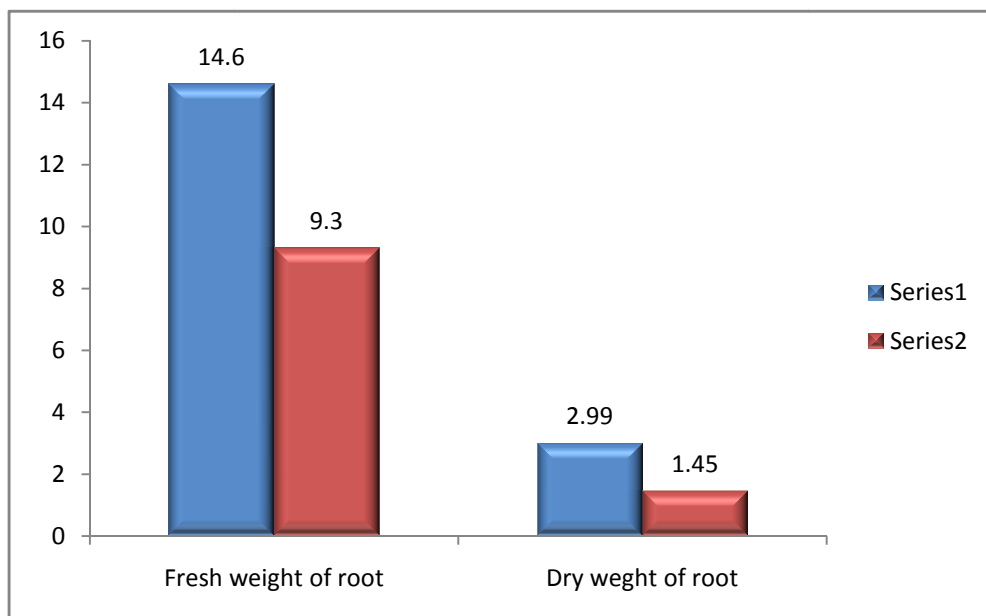
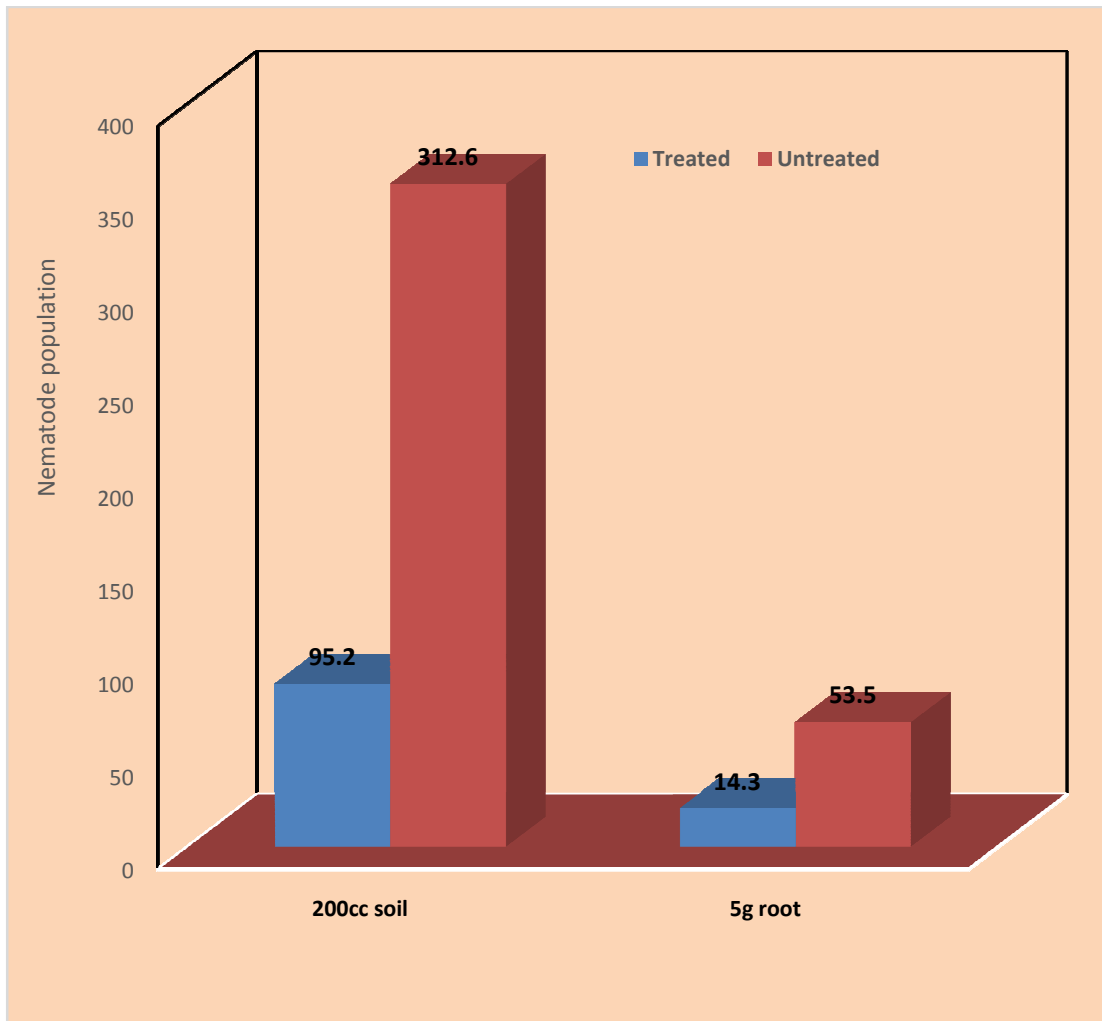


Fig. 10 Final population of *M. incognita* of French bean



CHAPTER-5

DISCUSSION

5. DISCUSSION

Root knot nematode, *Meloidogyne incognita* is a major constraint in growing the French bean crop successfully in tropical and sub tropical regions. In Odisha it is also an important commercial vegetable crop grown mainly in the districts like Kandhamal (Raikia), Koraput (Patangi, Semiliguda, Nandapur), and Cuttack (Athgarh) both during kharif and rabi season. The damage to the crop is subtle with characteristics galling on roots. The plants become chlorotic and fruit size and production is adversely affected. A loss of 19.38% to 43.48% in French bean due to root knot nematode had been earlier reported by Reddy, 2008 from different regions of the country.

It was evident from the observation that the reduction in plant growth character of French bean was directly proportional to the inoculum level of *Meloidogyne incognita*. With increasing level of the inoculums from 10 to 10000 J₂ of *Meloidogyne incognita* there was a corresponding increase in the percentage reduction at plant growth characters like length, fresh and dry weight of the shoot as well as root growth characters like length, fresh and dry weight of roots.

Significant reduction in plant growth was noticed at and above 1000 J₂/ plant. It was also observed that the reduction in all growth parameters was not significant between the inoculum levels of 1000 and 10000 J₂/plant. In these treatments the plants showed stunted growth, yellowing of leafs, shedding of basal leaves. It was also noticed that with an increase in level of inoculum from 10-1000 J₂ there was a significant progressive increase in root infection by root knot nematode as indicated by the number of galls, number of egg masses as well as the population of nematodes. Further increase to 10,000 J₂ in the inoculum resulted in more increase in root galls but not significant with 1000 level. The nematode population in soil increased with increasing inoculum levels (up to 1000 J₂/plant). The rate of nematode multiplication as indicated from reproduction factor was highest (107.4) at the minimum inoculums level (10 J₂/ plant) and lowest (2.05) at the maximum inoculums level (10000 J₂/plant). Thus the rate of nematode multiplication showed a decline trend with the increase in the initial inoculum level suggesting it to be a density depending

phenomenon. (Prasad & Chawal, 1992, Triantophyllu & Hirschmann, 1960) explained the variations in the growth rate of *M.incognita* to be caused by competition for food and overcrowding and thus the development was retarded. This might be due to the destruction of root system by the parasitism of root knot nematode which led the competition for food and nutritive among the developing nematodes and also due to inability of juveniles to find out new infection sites for subsequent generations. The high rate of multiplication at low levels of inoculum, on the other hand, could possibly be due to positive factors live abundance of food, lack of competition and the inability of host to support these levels of population (Das 2013).

Wallace (1963) and Oostenbrink (1966) were at the opinion that the increase in the nematode population and subsequent reduction in yield of any crop are directly influenced by initial density of nematodes in the soil. This view holds true with the present finding where in plant growth was proportionately affected with the increase in the initial inoculum levels of the nematode. These results are also in agreement with those reported by Dhawan and Sethi (1976) for *M.incognita* on egg plant and Vara Prasad (1977) on sugarbeet.

It can be concluded from the finding that the damaging thresh hold level of *M.incognita* on French bean was found to be as 1000 J₂/ plant. This is in agreement with Mohan and Mishra (1996) in French bean, Ahmed *et al.* (2013) and Das (2013) in chilli.

Attempts made in this investigation on estimation of avoidable yield losses in sick plots of Department of Nematology due to *M.incognita* in French bean showed significant differences in root knot nematode population both in 5g of root and 200 cc of soil and root knot indices between the untreated and treated plots. It was noted that nematode population (soil as well as root) and root knot index suppressed in plots subjected to nematicidal treatment. This was due to efficacy of carbofuran in controlling root knot nematode and also for its utility as an effective nematicide (Jain *et al.*, 1994). The nematicidal treatment reduced the population of nematodes by 69.55% in soil 73.27% in roots. The root knot index at harvest was 4.00 in untreated plots compared to 2.2 in treated plots indicating the 45.00% reduction. The increased final population of nematode because of the presence of the suitable host and

decreased population in treated plot because of efficient killing effect of the applied chemicals were reported by Bhatti and Jain (1977)

The yield of French bean was recorded to 18.77% less than the plots remained unprotected with nematicide. However in treated plots the yield increased to the 3.09 kg indicating 23.10% increase over the untreated plots.

Significant differences noticed with regard to plant growth characters. An increase of 43.27% in fresh shoot weight and 37.29% of plant height in treated plants over the untreated plot were recorded. All these results represent the outcome of an interaction of plant growth rate with nematode attack and population increase (Reddy, 1985).

The nematode root infection renders plants unfit to absorb water and nutrients and other essential elements from soil leading to poor plant growth and yield losses (Gowen et al, 2005, Hollis, 1963).The mean final population 200cc soil in untreated plots increased because of presence of suitable host and it decreased in treated plots because of efficient killing effect of the nematicide applied.

CHAPTER-6

SUMMARY & CONCLUSION

6. SUMMARY AND CONCLUSION

French bean, *Phaseolus vulgaris L.* is one of the most important leguminous vegetable grown for its tender fleshy green pods, shelled green and also for dry beans. It has been found to be infested with root knot of varying degrees due to root knot nematodes, *Meloidogyne incognia* in different French bean growing areas of Odisha. Singh & Reddy (1981) has observed 20% yield loss in this crop due to root knot nematode. Keeping in view about the association and incidence of root knot nematode in French bean the study was under taken to determine the pathogenicity potential and yield loss in French bean due to this nematode.

The investigation on pathogenicity study of *M.incognita* on French bean was carried out in pots. Seeds of French bean were sown in 15cm earthen pots containing 1.0kg of autoclaved sterilised soil. Fifteen days after germination one healthy seedling per pot was maintained and pots were inoculated with 10, 100, 1000 and 10000 freshly hatched second stage juveniles of *M.incognita*. The uninoculated plants served as control. Each treatment was replicated four times, observation on plant growth parameters and nematode multiplication were recorded at 60 days of inoculation.

Progressive reduction in plant growth parameter viz., height, fresh and dry weight of shoots and roots was noticed with the increase in the inoculum level of *M.incognita* from 10 to 10000. The highest reduction was observed in plants inoculated with 10000J₂ of *M.incognita* whereas least reduction was recorded in 10 J₂ of *M.incognita* per plant.

Shoot length, fresh and dry weight of the plants showed significant reduction at and above 1000 J₂/ plant. The reduction in these characters were 22.00, 25.40 and 29.42% at 1000 J₂/ plant and 25.14, 36.06 and 36.77% at 10000 J₂/ plant respectively. Similarly trends were also observed as regards to root length, fresh and dry weight. The reduction in root growth parameters were 33.92, 34.00 and 30.55% at 1000 J₂ / plant and 38.92, 36.00 and 40.55% at 10000 J₂ / plant. Increase in the level of nematode inoculum from 10 to 10000 J₂/ plants resulted significant increase in

number of galls and number of egg masses per plant. Maximum galls and egg masses were produced at a level of J_2 followed by 1000 J_2 per plant indicating that there were no statistical differences between these two levels of inoculum. A significant linear relationship was noticed between the initial population (P_i) and the final population (P_f) of *M.incognita*. The rate of nematode multiplication showed a declining trend with the increase in the initial inoculum level suggesting it to be a density dependent phenomenon. From this finding it is concluded that the damaging threshold levels of root knot nematode in French bean was 1000 J_2 of *M.incognita*/ plant.

The attempt was made in the study to apprise the loss of French bean yield (Green pods) due to *M.incognita* in a such plot of Department of Nematology, OUAT by growing the French bean c.v contender in paired treatment experiment with ten replication having plot size of 3.0m × 2.0 m. One plot in each pair was protected from the nematodes by applying carbofuran 3G @ 3 kg ai/ha. Observation on shoot length, root length, fresh and dry weight of shoots and roots. Root knot nematode on both in 200 cc soil and on 5g of roots, root gall index and yield per plot were recorded. The data were analysed using paired 't' test. The result clearly revised that due to application of carbofuran significant higher yield was recorded in treated plot (3.09 kg) as compared to untreated are (2.51 kg) with an increase by 23.10% over untreated control incurring 18.77% avoidable loss. The incidence of root knot nematode decreased the plant height, fresh and dry weight at shoots by 27.17, 30.45 and 35.44 %, respectively and fresh and dry root weights by 36.3 and 51.84%, respectively.

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