Life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield) on rice

By

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CERTIFICATE – I

This is to certify that this thesis entitled, **"Life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield) on rice"**, submitted for the degree of **Master of Science**, in the subject of **Nematology** to the CCS Haryana Agricultural University, is a bonafide research work carried out by **Vinod Kumar,** Admission No. **2013A98M(R)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that this thesis entitled, **"Life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield) on rice",** submitted by **Vinod Kumar,** Admission No. **2013A98M(R)** to the CCS Haryana Agricultural University in partial fulfillment of the requirement for the degree of **Master of Science,** in the subject of **Nematology** has been approved by the Student's Advisory Committee after an oral examination on the same.

MAJOR ADVISOR **EXTERNAL EXAMINER**

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PLACE: Hisar **Vinod Kumar**

Dated:

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	percent
/	:	per
@	:	at the rate
^{0}C	:	degree Celsius
C. D.	:	critical difference
cc	:	cubic centimeter
CCS HAU	:	Chaudhary Charan Singh Haryana Agricultural University
cf	:	cited from
cm	:	centimeter
CRD	:	completely randomized design
DAI	:	days after inoculation
dia.	:	diameter
DPI	:	days post-inoculation
et al.	:	*et allii* (and others)
Fig.	:	figure
g	:	gram
h	:	hours
i.e.	:	*id est* (that is)
kg	:	kilo gram
ml	:	milliliter
mm	:	millimeter
N.S.	:	non-significant
No.	:	number
viz.	:	*videlicet* (namely)

CHAPTER- I

INTRODUCTION

Rice is the most important cereal crop worldwide since it provides food security for more than half of the world's human population. It is the world's most important staple food and is cultivated in around 162 million ha annually with an annual global production of 464 million metric tonns (FAO, 2013). It is the most important cereal crop in Asia where more than 90 per cent of the world's rice is grown and consumed. India is the largest producer and consumer of rice in the world. The rice-wheat cropping system (RWCS) is the backbone of India's food security. This system covers about 33 per cent of the total rice area, 42 per cent of the total wheat area in four countries (India, Pakistan, Bangladesh and Nepal), and accounts for one quarter to one third of the total rice and wheat production.

In India, rice is grown in almost all the states and occupies first position among the cereals in respect of both area and production. West Bengal is the highest rice producing state while Tamil Nadu has first place in productivity. The states of Punjab and Haryana are the major contributor in the national food basket. It is the second most cultivated cereal after wheat.

The cultivation of rice is affected by several biotic and abiotic stresses. A number of ecto and endo-parasites of root, stem and foliar parts e.g., white tip nematodes, *Aphelenchoides besseyi*, rice stem nematode, *Ditylenchus angustus* and rice root nematode, *Hirshmanniella spp.* are already reported as major pests of rice from India (Prasad *et al.* 1987). *Meloidogyne graminicola* was first described in 1965 from grasses and oats in Louisiana (Golden and Birchfield, 1965). *M. graminicola* has a wide host range with rice being a major economically important host. The root-knot nematode is making its importance felt in almost all the rice growing areas. Among them, the rice root-knot nematode (*M. graminicola*) is considered as the major problem in rainfed, upland and lowland rice fields.

A number of nematodes namely, *A. besseyi*, *D. angustus*, *M. graminicola* and *Hirschmanniella* spp. cause damage to the tune of 10.54 per cent in rice alone (Jain *et al*., 2007). *M. graminicola* was reported to cause upto 21 per cent yield loss in rainfed and well drained soils throughout the country (Prasad *et al*., 1987). In upland rice, this nematode was reported to cause 16-32 per cent loss in grain yield due to incomplete filling of kernels (Biswas & Rao, 1971; Rao & Biswas, 1973). Rice root-knot nematode, *M. graminicola,* is a pest of international importance and it is reported to cause 17-30 per cent yield loss due to poorly filled kernels (MacGowan & Langdon, 1989). It is a serious problem in the nurseries and upland rice but has been found to be widespread in the deep water and irrigated rice also in many states of India (Prasad *et al*., 1985; Bridge *et al*., 1990; Jairajpuri & Baqri, 1991).

This nematode was reported from north Indian plains which has been a traditional wheat growing areas of the region as this nematode has also been found damaging the wheat crop (Kaur, 2005). It was first reported from Haryana in 1993 (Gaur *et al*., 1993). But later on, its occurrence has been noticed from Kaithal, Karnal, Kurukshetra, Sonepat, Jhajjar, Rewari, Bhiwani, Sirsa and Hisar districts. *M. graminicola* has now become a major constraint in rice production due to rice cropping intensification and increased scarcity of water (Prasad & Somasekhar, 2009, Somasekhar & Prasad, 2009) throughout the country, including Haryana.

M. graminicola is a meiotic parthenogen, with a haploid chromosome number of 18. The species was identified as *M. graminicola* according to the perineal pattern and other morphological characters (Mulk, 1976; Eisenback, 1985). This nematode feeds on the vascular tissues and interferes with water and nutrient uptake and also translocation. The eggs are laid within the cortex of the root, unlike other *Meloidogyne* spp., which allows the juveniles to remain in the maternal gall and re-infect the same root (Bridge & Page, 1982). Root-knot nematode affected plants show depletion in vigour, stunted growth, chlorotic and curled leaves in nurseries and main field. The nematode infection is characterized by the formation of small galls (hook shaped) near the tip of the roots. Excessive branching of affected roots occurs.

Monocropping of rice or rotation with crops susceptible to these nematodes resulted in high build up of nematode population in soil causing considerable yield losses to both rice and rotation crop in rice based agriculture. The major cause for such high incidence of nematode infection is attributed to the presence of light textured soil, the non-availability of ample water and transplantation of infected seedlings.

Quite a good information is available on rice root-knot nematode infecting paddy in traditional rice growing areas of eastern, north eastern and southern India (Rao *et al*.,1986), however not much work has been done on population of *M. graminicola* prevalent in northern India. The basic aspects of pathogenicity and biology of this pest has not been dealt with particularly under Haryana conditions expect few aspects of life cycle development by Dabur *et al*., (2004) and that too on scented rice. No such work has been done on any non-scented rice variety for comparison. As rice is being grown in various soil types prevalent in different agro-climatic zones of Haryana, so such studies have to be carried out in different textured soils as the relationship between content of soil and nematode is linear (Rao & Israel, 1972). Therefore, systematic work on life cycle development and pathogenicity on scented (basmati) and non-scented (non-basmati) was necessitated keeping in view the importance of this

nematode. Thus, the present studies were undertaken to ascertain pathogenic level of *M. graminicola* and also its life cycle studies on both types of rice with the following objectives:

Objectives

1. To study life cycle and development of root-knot nematodes, *Meloidogyne graminicola* on scented and non-scented rice
2. To study pathogenicity of *Meloidogyne graminicola* in different soil types on scented and non-scented rice

CHAPTER- II

REVIEW OF LITERATURE

In this chapter, relevant literature on "life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* on rice" has been reviewed.

2.1 Life cycle of *M. graminicola* on different types of rice and other crops

Life cycle of *M. graminicola* is very short hence; they complete a number of generations in crop season leading to a very high population. The life cycle duration also demonstrates the reproductive and damaging potential of this nematode species. Although life cycle duration is dependent on temperature and other environmental factors.

Rao and Israel (1973) revealed that larvae of *M. graminicola* penetrated into roots of rice in 5 hours and continued to invade roots of rice till the 12^{th} day following inoculation. The second stage larva developed and moulted in 5 days, third larval stage appeared in 3 days, the fourth stage on the 7^{th} day. Males were first observed on the 8^{th} day and females with ovisacs on the 11^{th} day. The life cycle was completed in 26-34 days during early summer months while this period was extended by 5 days in June, by 12 days in October to December and by 16 days in July to September. Bridge and Page (1982) revealed that larvae of *M. graminicola* invaded roots and completed its life cycle within 19 days at an ambient temperature of 22-29 ^{0}C; third and fourth-stage juveniles were present in roots after eight days, young females after twelve days, mature females with eggs after fifteenth day, and mature females with embryonated eggs and second stage juveniles by nineteen days.

Dabur *et al.,* (2004) revealed that larvae of *M. graminicola* started entry on 5^{th} day of sowing in roots of scented rice variety and their number increased with the advancement of time, on 8^{th} day of sowing, j_3 and j_4 stages were seen in the roots. All the larval stages as well as adult males and females were recorded on 10^{th} day of sowing while j_2 of second generation were recorded on 24^{th} day.

As far as their penetration in wheat is concerned, Kaur (2005) found that under pot condition, j_2 of *M. graminicola* penetrated the roots of wheat variety PBW 343 after 7 days of sowing near the root tips. Gall formation took place after 15 days of sowing. After 25 days of sowing, 4 females along with j_2 and j_3 were observed. Eggmass was also recorded though it was small. After 45 day of sowing, new gall formation took place on fresh roots. Kanwar *et al.,* (2008) also studied the life cycle of *M. graminicola* on wheat at two different sowing times under greenhouse conditions. They observed that j_2 penetrated roots easily and developed to adult females in 24 days and started laying eggs in 38 days after inoculation in October sown plants, however, fewer j_2 penetrated and developed slowly to adulthood in 110 days in November sown plants.

Senthilkumar *et al.,* (2007) found that j_2 of *M. graminicola* penetrated rice roots after 24 h, the third stage (j_3) developed on the 6th day, fourth stage (j_4) on 9th day and 7-9 days into pre-adults. Males and females were observed on 23rd and 28th day. The life cycle was completed in 25-30 days in rice at 30±2 ^{0}C at greenhouse conditions. Similar pattern in the life cycle of *M. graminicola* was observed by Rao *et al.*, (1984) who reported that the duration of development of j_2, j_3 and j_4 were 5, 3 and 7 days respectively. Males developed in about 3 days and egg masses took about 22 days

Jaiswal and Singh (2010) reported that life cycle of *M. graminicola* was completed in 15 days in rice. The second moult of the feeding (j_2) began on 4th day and third-stage juveniles (j_3) were first seen on 5th DAI. The j_3 moulted on 6th day and fourth-stage juveniles (j_4) were noted on 7th day. Males were observed on day 9 and young females were seen on day 10. Egg-laying was first observed on day 11 and j_2 were detected on 15 DAI.

Cannayane and Anita (2011) studied the life cycle of *M. graminicola* on rice under field conditions. The second stage (j_2) developed into third-stage juveniles (j_3) in 1-6 days, the fourth stage (j_4) in the 7-11 days, fourth stage (j_4) to adult on 12-18 days and egg mass production on 19-22 days while Dutta *et al.,* (2011) studied invasion, development and reproduction of *M. graminicola* in tomato and rice and reported that invasion increased in tomato from 1-3 days post-inoculation (DPI), however, significantly more j_3 were found in rice at 4 DPI. At 6, 10 and 15 DPI, significantly more j_4 were found in rice than tomato. Females were found in significantly greater numbers in rice than tomato at 20, 25 and 30 DPI, and at 30 DPI, no females were observed in tomato. Eggs of *M. graminicola* were found in significantly greater number on rice than on tomato at 20 and 25 DPI. Greater number of eggs was observed in rice at 30 DPI and no eggs were detected in tomato at this time point.

Singh *et al.,* (2011) observed that one generation of *M. graminicola* was completed in 23 days on wheat during February-March. In October sown plants, j_2 penetrated roots, which developed into females in 15 days and started laying eggs in 16 days. The second stage (j_2) penetrated roots after 24 h, gall formation (48 h), second stage juveniles (2nd day), j_2 moulting (7th day), j_3 stage (8th and 9th day), j_3 moulting (11th day), j_4 stage (12-13th day), j_4 moulting (15th day), adult male (14th day), adult female (15th day), egg laying (16th day) and j_2 juveniles (23rd days) were released. Cabasan *et al*., 2012 studied migration, penetration, development and reproduction of *M. graminicola* on rice and reported that the penetration of j_2 was higher at 3-7 days after inoculation.

In the year 2014, Fernandez *et al.,* observed that life cycle of *M. graminicola,* was studied in an indoor growth chamber on rice cultivar (UPLRi-5). At 29/26 °C under simulated non-flooded conditions, infective juveniles (j_2) had penetrated the rice roots close to the tip at 3 DAI. The j_2 that had penetrated into the roots developed into third-stage juveniles (j_3) at 4 DAI and into fourth-stage juveniles (j_4) at 9 DAI. Adult females were observed at 13 DAI.

Egg deposition started at 14 DAI. Second-generation j_2 were observed at 20 DAI. At 36/32 °C under simulated non-flooded conditions, the j_2 that had penetrated into the roots also developed into j_3 and j_4 at 4 and 9 DAI, respectively. Adult females were observed at 11 DAI. Second-generation j_2 were observed at 19 DAI. At 29/26 °C under non-flooded and flooded conditions, the life cycle was completed in 20 and 19 days, respectively and at 36/32 °C, under non-flooded conditions also in 19 days.

2.2 Pathogenicity of *Meloidogyne graminicola* in different soil types on rice.

2.2.1 Pathogenicity of *M. graminicola* on rice and other crops

Rao and Biswas (1973) studied the effect of varying degree of incidence of root-knot nematode on the rice under inoculated conditions. For every unit increase of 1000 larvae in inoculum, there was an estimated reduction of 2.6 per cent in grain yield when plants were inoculated at 12 days age, 30 per cent in plants inoculated at 32 days and 42 per cent in plants inoculated at 64 days age. Rao and Israel (1972) studied the influence of initial inoculum of *M. graminicola* on the final nematode population in rice. When 12 days-old plants were inoculated with 1-4 egg masses/seedling, the rate of increase, in the final population at the end of 30 days, was much higher as compared to that obtained with an initial inoculum level of 8-32 egg masses per seedling.

Roy (1976) predicted the effect of different levels of inoculum (4000, 8000 and 16000 j_2/pot) of *M. graminicola* on rice plants (cv. IR 8) and found that height, weight of shoot and roots were progressively decreased with the increased inoculum levels. However, there was no significant difference between treatments with 0-4000 j_2/pot. Height of the plants inoculated with 8000 and 16000 larvae were significantly less than control, and all the treatments were at par. However, there was not much difference in the percentage of galls/ plant and their sizes between the different treatments of inoculum.

Prasad *et al.,* (1990) studied the effect of *M. graminicola* infection under simulated rainfed lowland on different rice varieties *viz*. CN 492, CR 1018, CR 1030, FR 13A and Jaladhi-1 at different inoculum levels of 0, 1 and 2 nematodes/g soil. Seedling mortality was significantly higher at 2 nematodes/g of soil in all the varieties. Plant height was reduced by 5.1 to 19 per cent and 4.2 to 55.4 per cent in the nematode infected plants at 5 and 15 days after submergence respectively. Jaladhi-1 and CR 1018 recorded highest number of egg masses and adults.

Plowright and Bridge (1990) carried out experiment on the effect of *M. graminicola* on rice seedling (var. IR36) in pot conditions. A high initial population density (Pi) of 80 second stage juveniles (j_2)/ml of soil caused seedling death at 10 days after sowing; 80 per cent of seedlings had died after 32 days. The initial high population density caused wilting of seedlings along with severe reduction in growth parameter while low population caused only reduction in growth parameters. The fecundity of females was inversely related to Pi. Soomro

and Hague (1992a) conducted a greenhouse experiment to study the effect of *M. graminicola* on root growth of wheat and sorghum. Growth of both the plants was effectively reduced by nematode invasion but sorghum suffered more as compared to wheat, total root length of sorghum was reduced by 67 per cent as compared to 54 per cent reduction in wheat after inoculation. It was evident that *M. graminicola* can slow the production and elongation of roots thereby reducing total root length of plants. The highest number of galls and nematodes occurred on sorghum roots. Under glass house conditions, Soomro and Hague (1992b) studied the root morphology and growth of rice and *Echinochloa colonum*. Invasion by nematode juveniles reduced total root length, length of axes and laterals in both host, while the number of axes in the infested rice roots increased due to nematode invasion. Growth of root was checked and secondary laterals grew on the galls by the nematode invasion, up to 11.6 per cent in rice and 27.3 of total laterals on *Echinochloa colonum* were growing on the actual galls formed due to invasion by nematodes.

Soomro and Hague (1993) in a pot experiment found that at different inoculum levels of (0, 125, 500, 1000, 2000, 4000 and 8000 j_2/pot), plant growth parameters were adversely affected. Maximum reduction in growth parameters were observed at 8000 j_2/pot and the seedlings did not tolerate even the lowest inoculums of 125 j_2/pot. Clear differences among growth parameters were recorded at 30 days after inoculation when shoot and root weights and the number of tillers were significantly smaller. Reproduction rate *i.e*. final population (Pf)/initial population (Pi) decreased with an increased inoculum levels. Mian and Khan (1995) conducted an experiment using a series of inoculum levels (0, 10, 100, 500, 1000, 5000 and 10000 j_2/pot) on post-penetration development of *Meloidogyne graminicola* in rice root. Significantly higher numbers of galls, developing larvae and mature females with eggs/g root were recorded at the highest level of inoculum as compared to all other levels. Prot and Matias (1995) conducted a greenhouse experiment to study the pathogenicity of *M. graminicola* on rice (var. UPL15) on different soil types (clay and sandy loam). Rice seedlings were inoculated with (0, 10, 100, 1000 and 10000 j_2/kg of soil) after 3 days of transplanting the seedlings and observed that shoot, leaves, dry weight and flag leaf area were more in clay soil than sandy loam. They also observed that dry shoot weight and dry root weight decreased with increased Pi (< 0.01) and there was significant interaction between water regime and Pi on the number of panicles ($P < 0.01$) and the grain yield ($P < 0.05$). Soil types and Pi did not affect the percentage of unfilled spikelets.

Tarafder and Mian (2001) recorded 500 j_2/3 kg of *M. graminicola* to be pathogenic on onion. They further observed that number of galls, population of nematodes and all developmental stages increased with an increased level of inoculation in the range of 100-10000/3 kg of soil. The effects of different pre-plant population levels (Pi) of *M. graminicola* on onion (var. Yellow Granex 49) in pots and of different percentage of galled roots in a

naturally infested field were evaluated. Leaf weight and root length of onion seedlings decreased with increased Pi, while low Pi mildly stimulated plant height at the vegetative growth stage. Age of transplant and Pi influenced growth and yield of onion at maturity. Onion yield and root and leaf weights decreased as the age of the transplants increased. Growth and yield decreased with increased Pi. Bulb weight was reduced by 7 to 82 per cent and diameter by 10 to 62 per cent when plants were inoculated with 50 to 10,000 second-stage juveniles. Onion bulbs from the field were reduced by 16, 32, and 35 per cent in weight and by 6, 17, and 18 per cent in diameter when the percentage of roots galled was 10, 50, and 100 per cent, respectively (Gergon *et al.,* 2002).

Poudyal *et al.,* (2005) found that there was 97 per cent reduction in the yield of rice cv. Musali. Rice seedlings were inoculated at 0, 0.1, 0.2, 0.4, 1, 2, 5 and 10 j_2/g of soil in pot. Plant height and tiller were unaffected, but root length, panicle number, total panicle length grain, number and weight declined significantly with increased nematode density. However, reduction of 31 per cent occurred at Pi=0.1 j_2/g soil. The j_2 population in the soil as well as in root and root gall index did not differ significantly with inoculum levels and Pi of 5 j_2/g soil had the highest j_2 and egg density. The pathogenic effect of *M. graminicola* in rice seedlings showed that the first visible symptom in the form of swelling of roots occurred 40 h after inoculation. Initiation of yellowing symptom at different levels of inoculum of *M. graminicola* in rice seedlings began 5, 7, 9 and 15 days after inoculation at Pi 9000, 6000, 3000 and 1000 j_2/pot. Reduction in fresh weight of shoot and root were observed 8 days after inoculation, yellowing and stunting were first noted on days 9 and 10, respectively (Singh *et al*., 2006).

Jaiswal *et al.,* (2011b) conducted an experiment to test the pathogenic effect of *M. graminicola* on growth of rice seedlings. Maximum reduction in all the plant growth parameters was recorded at 5000 j_2/ kg of soil. Significant reduction in shoot length, root length, fresh shoot and root weight was observed at 1000 j_2/kg of soil and considered as damaging threshold level of *M. graminicola.* Shoot and root length was decreased by 63.5 and 43.4 per cent at 5000 j_2/kg of soil, respectively. Root galls also increased significantly with the increased levels of nematode inocula with maximum at 2 j_2/g being at 2000 j_2/kg of soil. Wilting of rice seedling was also observed at 5000 j_2/kg of soil.

2.2.2 Effect of soil texture on root-knot nematode, *M. graminicola* on rice and some other crops

Rao and Israel (1972) studied the movement of infective j_2 of *M. graminicola* and revealed that sandy soil provided free movement to nematode with an increase in sand content. Clay loam soil was found less suited as compared to sandy soil. With an increase in sand content of soil, there was an increase in root growth, root-knot development and egg mass production by the nematode, the relationship between the sand content and the activity

of the nematode was found being linear. *M. graminicola* significantly reduced ($P < 0.01$) growth of deep water rice before flooding and to a greater extent after flooding ($P < 0.001$).

Soriano *et al.,* (2000) studied the effect of different water regimes and two soil types on the pathogenicity of *M. graminicola* under greenhouse conditions on six rice cultivars, *viz.* IR72 and IR74 were more tolerant than IR20 and IR29 under intermittent flooding. In clay loam soil, shoot and leaf dry weight as well as grain weight were reduced by nematode under all water regimes except continuous flooding. The investigation showed that rice tolerance cultivars of *M. graminicola* vary with water regime. Continuous flooding until the later stage of rice growth appears to help defer increased in the nematode population.

Pokharel, (2009) studied the effect of different fields with different soil types in rice. The significantly larger nematode population densities observed in the fields with light soil as compared to heavy soil. Similarly, significantly larger root-knot nematode populations were observed in both roots and soils in the fields with light soils as compared to heavy soils. However, significantly larger soil and root population densities of the nematode were observed in light-textured soils as compared to heavy textured soils.

Jaiswal *et al* (2011a) carried out a greenhouse experiment on the effect of rice (cv. MUT 7029) for determining the infestation level *M. graminicola.* Dilution of infested soil with sand in 1:1 ratio resulted heavy infestation of *M. graminicola* and recorded maximum numbers of root galls while minimum root galls were achieved at using of 1:63 ratio. The number of root galls decreased with increased soil or atmosphere temperature. Maximum galls were recorded during the month of March followed by April and June while minimum roots galls was recorded during the month of May.

Vaish and Pandey (2012) carried out investigation on the effect of *M. graminicola* on barley and found that growth parameters and grain yield was reduced. The disease was more severe in sandy loam as compared to loam and clay soil. Yield loss was much higher in patches (36 - 73.4 per cent) in respect of soil type.

CHAPTER-III

MATERIALS AND METHODS

Present investigations were carried out on life cycle and pathogenicity of *Meloidogyne graminicola* on rice. The above study was carried out in screen house of the Department of Nematology, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The procedure adopted and materials used in achieving the objectives of the study are given in detail in this chapter.

3.1 Experimental site and season

Studies on the life cycle and pathogenicity of *M. graminicola* on rice were conducted in the screen house of Department of Nematology, CCS HAU, Hisar during kharif, 2014.

3.2 Propagation of pure culture of *M. graminicola* eggs and j_2

The inoculums of *M. graminicola* used for various experiments were propagated from the culture of *M. graminicola* maintained in screen house of the Department of Nematology, CCS HAU, Hisar on rice plants. Pure cultures were raised in screen house in earthen pots filled with steam-sterilized sandy loam soil. Healthy seedlings of rice (var. Pusa 1121) were transplanted in the pots. Second-stage juveniles (j_2) of *M. graminicola* were obtained from eggs from the pure culture maintained in the department, the seedlings of rice in pots were inoculated with these j_2.The cultures were allowed to multiply for 2-3 generations and were further sub-cultured periodically. For the conformation of purity, 5-10 matured females were extracted from these roots by teasing them under a stereoscopic binocular microscope with the help of needle and forceps for preparation of perineal pattern for identification and confirmation of species of *M. graminicola* (Mulk, 1976; Eisenback, 1985).

3.3 Collection of j_2 for inoculation

The galled portions of the rice roots taken from culture pots were washed gently in water to remove the adhering soil. The galled roots were chopped into small pieces and incubated in a mist chamber. The contents of 400 and 500 mesh sieves were collected in the beaker and examined under stereoscopic microscope for the presence and density of eggs. The egg suspension was poured over 2-ply facial tissue paper by Modified Baermann Funnel technique to obtain j_2 which were collected daily for further use. Sufficient amount of water was added to keep the eggs just submerged. The water from the Petri plates was collected daily and replaced with fresh water. The daily catch of j_2 was checked for the presence and density of j_2. The number of juveniles was counted per ml solution with three replications. The assembly was maintained till hatching ceased.

3.4 Collection of soils

Different types of soils such as sandy loam and loamy sand were collected from HAU farm and Clay loam soil from a nearby village near Hansi (Hisar).

3.5 Characteristics of soils used for different experimentation

The physico-chemical properties and texture of various types of soils were got analysed by Department of Soil Science, CCS HAU, Hisar which are as follow:

Table 1. Texture, composition and other characteristics of different soils

Soil type	Percentage of			pH	E.C. ($dSm^{-1)}$	Organic carbon (%)	Availability (kg/ha)	
	Sand	Silt	Clay				Phosphorus	Potash
Clay loam	36.0	25.0	39.0	8.3	0.61	0.49	9	270
Loamy sand	82.1	9.6	8.3	7.9	0.32	0.39	12	300
Sandy loam	74.0	15.0	11.0	7.9	0.29	0.75	22	342

3.6 Soil sterilization

Different types of soils collected viz., sandy loam, loamy sand and clay loam from different sites was brought to Nematology laboratory and sterilized in autoclave at 15 lbs pressure with 121±1^{O}C for one hour. Each soil type was then allowed to dry for one day and then filled in 15 cm diameter earthen pots (one kg capacity).

3.7 Host and its varieties

a) Scented rice (var. Pusa 1121)

b) Non-scented (var. PR 114). The seeds of these two varieties were procured from Regional Rice Research Station, Kaul, CCS HAU and was used in all the experiments.

3.8 Experimental details:

3.8.1 Life cycle and development experiments

For non-scented rice (var. PR 114)

Date of sowing: 12 July 2014

Date of inoculation: 17 July 2014

For scented rice (var. Pusa 1121)

Date of sowing: 20 July 2014

Date of inoculation: 25 July 2014

3.8.2 Pathogenicity experiments

For non-scented rice

Date of sowing: 29 July 2014

Date of inoculation: 4 Aug 2014

Date of termination: 15 Sep 201

For scented rice Pusa 1121

Date of sowing: 15 Aug 2014

Date of inoculation: 20 Aug 2014

Date of termination: 1 Oct 2014

3.9 Inoculation of second stage juveniles of root-knot nematode, *M. graminicola*

Nematode inoculation was done by carefully removing the soil around the roots of plants in each pot to ensure direct and easy approach of juveniles to root system. The larval (j_2) suspension was bubbled continuously for 10-15 seconds for uniform quantity and poured on exposed root system with pipette according to the desired population, required for the both experiment. For life cycle experiment, $500j_2$/kg and for pathogenicity experiment, different inoculum levels *viz.,* 0, 10, 100, 1000 and 10000 j_2/kg soil for each soil types and both the variety of rice (var. Pusa 1121and var. PR 114). The roots were immediately covered with soil and light watering was done in the pots.

3.10 Application of fertilizers

The recommended doses of fertilizers, i.e., nitrogen (N), phosphorus (P), and zinc (Zn) @ 150, 60 and 25 kg in the form of urea, single super phosphate and zinc sulphate, respectively were incorporated in each pot at the time of sowing. The amounts of fertilizers to be applied were calculated on the basis of weight of soil/pot. Nitrogen was applied in two split doses, i.e.*;* half of dose of nitrogen and full dose of phosphorus and zinc were applied at the time of sowing. The other half of nitrogen dose was applied one month after sowing.

3.11 General care of plants

The plants were observed and watered daily depending upon the temperature and rainfall. Hand hoeing with a khurpa was done at desired intervals for removing weeds etc. To check the incidence of insect pests and diseases, chemical spray was done as and when required.

3.12 Studies on life cycle and development of *M. gramincola* on scented rice (var. Pusa 1121).

The experiment was conducted in screen house conditions to study the life cycle of rice root-knot nematode*, M. graminicola*. The experiment was conducted using the variety namely scented rice (var. Pusa 1121). Seeds of the above variety were soaked in tap water for 24 hours and the sprouted seeds were sown in sterilized sandy loam soil. Four days after sowing, the seedlings were inoculated with freshly hatched *M. graminicola* @ $500j_2$/kg soil. Each treatment was replicated three times with completely randomized design. Regular watering was done according to the crop requirement and from 24 hours onwards after inoculation, started taking the observations.

Observations to be recorded:

The plants were uprooted according to the treatment schedule:

A. Time taken for penetration: Observations were recorded daily upto 3 days and subsequently on alternate day's upto 15 days.

B. Time taken to complete different developmental stage of the nematode, i.e., j_2-j_3, j_3-j_4 and j_4-adults.

C. Time taken to complete the life cycle (j_2- j_2).

At each interval, three plants were uprooted without any damage to roots system and brought to the laboratory. These roots were washed gently to remove adhering, soil particles. The roots were stained in 0.1% acid fuchsin lectophenol. After that, roots were again washed under running water followed by placing the roots in plain lectophenol for destaining for 24 hours. Roots were observed under stereoscopic microscope for teasing out the different developmental stage. Further, these stages were mounted on glass slides in plain lectophenol. Matured females were mounted in glycerine jelly.

3.13 Studies on life cycle and development of *M. gramincola* on non-scented rice (var. PR 114)

The experiment was conducted in screen house conditions to study the life cycle of rice root-knot nematode, *M. graminicola*. The experiment was conducted using the non-scented rice (var. PR 114). Seeds of the above variety were soaked in tap water for 24 hours and the sprouted seeds were sown in sterilized sandy loam soil. Four days after sowing, the seedlings were inoculated with freshly hatched *M. graminicola* @ 500j_2/kg soil. Each treatment was replicated three times with completely randomized design. Regular watering was done according to the crop requirement and from 24 hours onwards after inoculation, started taking the observations.

Observations to be recorded: The same observations as in experiment No. 3.12 were recorded for this experiment also

3.14 Studies on Pathogenicity of *M. graminicola* in different soil types on scented rice (var. Pusa 1121)

This experiment was conducted in the screen house of the Department of Nematology, CCS HAU, Hisar to assess the effect of different inoculum levels (mentioned below) of *M. graminicola* on the growth parameters of rice plants and to determine the pathogenic level of the nematode on rice in three soil types. Steam sterilized soils of different types such as sandy loam, loamy sand and clay loam were filled in 15 cm dia. earthen pots (one kg capacity). Three seeds of rice were sown per pot and after germination, one plant was retained.

Levels of nematode inoculation:

1. 0 (Non-inoculated check)
2. 10 j_2/kg soil
3. 100 j_2/kg soil
4. 1000 j_2/kg soil
5. 10000 j_2/kg soil

After four days of sowing, freshly hatched j_2 of *M. graminicola* were inoculated in seedlings grown under three different types of soil in a series mentioned as above. The pots without nematodes were treated as non-inoculated check. The juveniles were inoculated as per treatment schedule. Each treatment was replicated three times and the statistical design was Factorial CRD.

Observations recorded:

Forty days after inoculation, each plant was uprooted carefully from soil. The roots were retrieved carefully and kept in a basin of water to clear it from adhering soil particles and recorded the following observations: plant growth characteristics (shoot length, fresh shoot weight (wt), dry shoot (wt), fresh and dry root weight (wt), and also on nematode multiplication such as number of galls, number of eggs/plant and number of j_2/kg of soil. Roots were spread in the big sized Petri plate which contained water and recorded the observations on number of galls with the help of the hand lens.

Observations were recorded on the following parameters:

3.14.1 Shoot length:

The shoot length of rice plants was measured from surface of soil to the base of growing bud with the help of a measuring scale in cm.

3.14.2 Fresh shoot weight:

Fresh shoot weight was taken on electrical balance after removing the plants from pots and was taken in g.

3.14.3 Fresh root weight:

Fresh root weight was taken on an electrical balance after removing the excess water by pressing the roots between blotting papers, in g.

3.14.4 Root length:

Root length of rice plants measured from end point to stem to root tip with the help of a measuring scale in cm.

3.14.5 Dry shoot and root weight:

Dry weight was recorded on top pan electric balance after drying the samples in an oven at 60 ^{o} C for 3 days, in g.

3.14.6 Number of galls:

Each visible gall/knot formed by the root–knot nematodes was counted on the whole root system with the help of a hand lens.

3.14.7 Number of eggs/plant

The number of eggs/plant was counted after teasing the roots in water.

3.14.8 Final soil population:

For recording final soil population, each pot soil after depotting was analysed by Cobb's Sieving and Decanting method and nematodes extracted by Modified Baermann's

Funnel technique (Christie and Perry, 1951). The extracted nematodes per ml nematode suspension were counted under stereoscopic binocular microscope with the help of counting dish and finally the soil population/kg soil was calculated.

3.15 Studies on Pathogenicity of *Meloidogyne graminicola* in different soil types on non-scented rice (PR 114)

This experiment was also conducted in the screen house of the Department of Nematology, CCS HAU, Hisar to assess the effect of different inoculum levels (mentioned below) of *M. graminicola* on the growth parameters of rice plants and to determine the pathogenic level of the nematode on rice in three soil types. Steam sterilized soils of different types such as sandy loam, loamy sand and clay loam were filled in 15 cm earthen pots (one kg capacity). Three seeds of rice were sown per pot and after germination, one plant was retained.

Levels of nematode inoculation:

1. 0 (Non-inoculated check)
2. 10 j_2/kg soil
3. 100 j_2/kg soil
4. 1000 j_2/kg soil
5. 10000 j_2/kg soil

After four days of sowing, freshly hatched j_2 of *M. graminicola* were inoculated in roots of plants grown three different types of soil in a series mentioned as above. The pots without nematodes were treated as non-inoculated check. The juveniles were inoculated as per treatment schedule. Each treatment was replicated three times and the statistical design was Factorial CRD.

Observations recorded:

Forty days after inoculation, each plant was uprooted carefully from soil. The roots were retrieved carefully and kept in a basin of water to clear it from adhering soil particles and record the following observations: plant growth characteristics (shoot length, fresh shoot weight (wt), dry shoot wt, fresh root wt, dry root wt), and also on nematode multiplication such as number of galls and number of j_2 /kg of soil. Roots were spread in the big sized Petri plate which contained water and recorded the observations on number of galls with the help of the hand lens.

Observations were recorded on the same parameters and in the same manner as given in previous experiment No. 3.14

3.16 Statistical Analysis

The statistical analysis of data obtained in experiments 3.13 and 3.14 was done using OPSTAT software available online at CCS HAU website (www.hau.ernet.in).

CHAPTER- IV

RESULTS

The present investigations were carried out on "Life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield) on rice". The experiment was conducted under screen house conditions as per the materials and methods explained in chapter 3 and the data, thus, obtained were analysed and the results are presented in this chapter.

4.1. Life cycle and development of root-knot nematode, *Meloidogyne graminicola* on both types, i.e., scented and non-scented rice

Experiments were conducted to study the life cycle and development of root-knot nematode, *M. graminicola* on scented and non-scented rice. The plants were uprooted according to the treatment schedule and observations were recorded daily up to 3 days and subsequently on alternate days up to 15 days of penetration. Time taken to complete different development stages of the nematodes, i.e., j_2-j_3, j_3-j_4 and j_4-adults and time taken to complete the life cycle was recorded. Ambient maximum and minimum temperature and relative humidity was recorded and monitored regularly.

4.1.1. Life cycle and development of root-knot nematode, *M. graminicola* on scented rice (var. Pusa 1121)

The life cycle of root-knot nematode, *M. graminicola* in rice var. Pusa 1121 was studied under screen house conditions. The second stage active juveniles of *M. graminicola* penetrated into young roots of rice seedlings near the root tip within 24 hours (h). However, the visible swelling on the infected root was observed on second day (48 h) after inoculation (DAI).

On the 3rd day after inoculation, second stage juveniles (j_2) slightly enlarged in size (Table 2). Some of the j_2 were seen in second moulting stage. Several j_2 invaded the same root inducing 1-10 galls per plant. The j_2 that had penetrated into the roots developed into third-stage juveniles (j_3) on 7 DAI which was developed into fourth-stage juveniles (j_4) at 9 DAI. Young females were observed on 11th day and males on 13 DAI inside the root. Maximum number of adults per root system (27 females and 5 males) was recorded on 15 DAI inside the root. Males in the soil were observed on 17 DAI. Egg deposition started at 19 DAI inside the root. The eggs were deposited singly inside the roots. On 21 DAI, six j_2 of the second generation were recorded inside the root. Thus, life cycle of the root-knot nematode, *M. graminicola* from j_2-j_2 stage was completed in 21 days under screen house conditions at a temperature ranging between a maximum average of 35.0 ^{0}C and a minimum average of 25.8 ^{0}C. Some of the second generation larvae (j_2) developed further inside the old galls while others invaded new roots and induced new galls at root tips.

Table 2. Time taken for life cycle and development of root-knot nematode, *M. graminicola* on scented rice (var. Pusa 1121).

Date	Observations (Days after inoculation)	Developmental stage of nematode	Remarks	Temperature (^{0}C)		Relative Humidity (%)	
				Max.	Min.	Max.	Min.
26/07/2014	1	j_2	Penetration started	34.4	28.0	81	48
27/07/2014	2	j_2	Swelling of roots started	37.4	28.7	85	61
28/07/2014	3	j_2	-do-	36.4	26.0	100	92
30/07/2014	5	j_2	-do-	28.4	25.4	94	68
01/08/2014	7	j_3	-do-	34.6	28.0	91	59
03/08/2014	9	j_4	-do-	36.4	26.0	94	67
05/08/2014	11	Female	-do-	35.9	27.0	86	74
07/08/2014	13	Male	Hook shaped galls	35.9	28.3	79	56
13/08/2014	19	Eggs deposition	-do-	38.0	27.3	75	45
15/07/2014	21	j_2	-do-	35.4	27.5	74	50

Date of sowing: 20/07/2014; Date of inoculation: 25/07/2014

4.1.2. Life cycle and development of root-knot nematode, *M. graminicola* on non-scented rice (var. PR 114)

The life cycle of root-knot nematode, *M. graminicola* in rice var. PR 114 was also studied under screen house conditions. The second stage active juveniles of *M. graminicola* penetrated into young roots of rice seedlings near the root tip within 72 h. However, the visible swelling on the infected root was observed on 5th day after inoculation (DAI).

On the 5th day after inoculation, second stage juveniles (j_2) slightly enlarged in size (Table 3). Some of the j_2 were seen in second moulting stage. Several j_2 invaded the same root inducing 1-6 galls per plant. The j_2 that had penetrated into the roots developed into third-stage juveniles (j_3) on 9 DAI which was developed into fourth-stage juveniles (j_4) at 11 DAI. Young females were first observed on 15th day and males on 17 DAI inside the root. Maximum number of adults per root system (19 females and 3 males) was recorded on 19 DAI inside the root. Males in the soil were observed on 21 DAI. Egg deposition started at 23 DAI inside the root. The eggs were deposited singly inside the roots. On 25 DAI, five j_2 of the second generation were recorded. Thus, life cycle of the root-knot nematode, *M. graminicola* from j_2-j_2 stage was completed in 25 days under screen house conditions at a temperature ranging between a maximum average of 35.6 ^{0}C and a minimum average of 27.2 ^{0}C. Some of the second generation juveniles (j_2) developed further inside the old galls while others invaded new roots and induced new galls at root tips.

Table 3. Time taken for life cycle and development of root-knot nematode, *M. graminicola* on non-scented rice (var. PR 114)

Date	Observations (Days after inoculation)	Developmental stage of nematode	Remarks	Temperature (^{0}C)		Relative Humidity (%)	
				Max.	Min.	Max.	Min.
18/07/2014	1	j_2	No penetration	37.0	25.5	89	69
19/07/2014	2	j_2	-do-	33.4	25.5	89	59
20/07/2014	3	j_2	Penetration started	35.0	25.4	89	54
22/07/2014	5	j_2	Swelling of roots started	38.0	28.5	85	49
24/07/2014	7	j_2	-do-	37.0	27.9	85	54
26/07/2014	9	j_3	-do-	34.4	28.0	81	48
28/07/2014	11	j_4	-do-	37.4	28.7	100	92
01/08/2014	15	Female	Hook shaped galls	28.4	25.4	91	59
03/08/2014	17	Male	-do-	34.6	28.0	94	67
07/08/2014	21	Eggs deposition	-do-	35.9	27.0	79	56
11/08/2014	25	j_2	-do-	34.9	26.5	80	54

Date of sowing: 12/07/2014; Date of inoculation: 17/07/2014

4.2 Pathogenicity of *M. graminicola* in different soil types on scented and non-scented rice varieties

Pathogenicity of *M. graminicola* on rice was studied under screen house conditions using a series of initial inoculum densities of 0 (non-inoculated check), 10, 100, 1000 and 10000 j_2/kg soil. Observations on the impact of nematodes on plant growth parameters and also on nematode reproduction and multiplication were recorded forty days after inoculation and data are presented in this chapter.

4.2.1 Effect of different inoculum levels on plant growth parameters of rice (var. Pusa 1121)

4.2.1.1 Shoot length

Data in Table 4 clearly indicated that shoot length was significantly different in each soil types. Maximum and significantly highest shoot length was observed in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. Treatments of inoculum levels of 10-10000 j_2/kg soil were significantly different from each other but shoot length at 10 j_2 was statistically at par with non-inoculated check. Maximum shoot length was observed at inoculum levels of non-inoculated check. The significant reduction in shoot length was observed at 100 j_2 onwards.

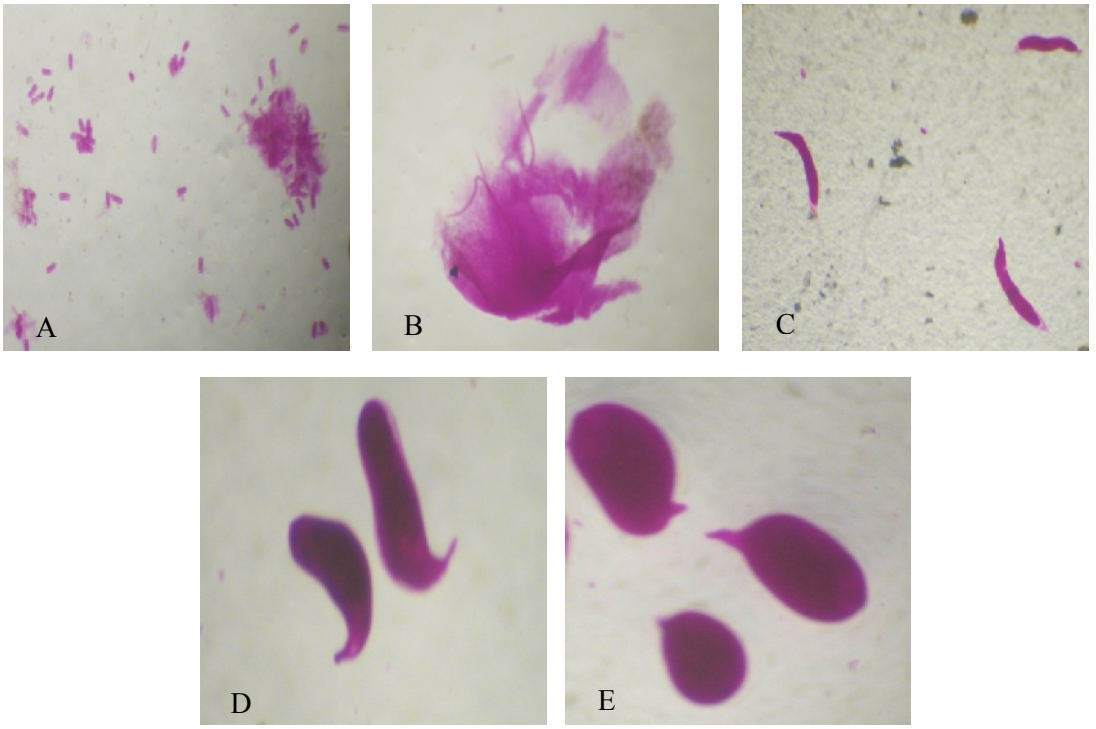

Plate 1. Developmental stages of root-knot nematode, *Meloidogyne graminicola* in root of scented rice (var. Pusa 1121).

A= Eggs 19 DAI: B= j_2 developing 24 h DAI: C=j_2 moulting 3 DAI; D=adult female after 11 DAI; E=adult female at eggs laying stage 19 DAI

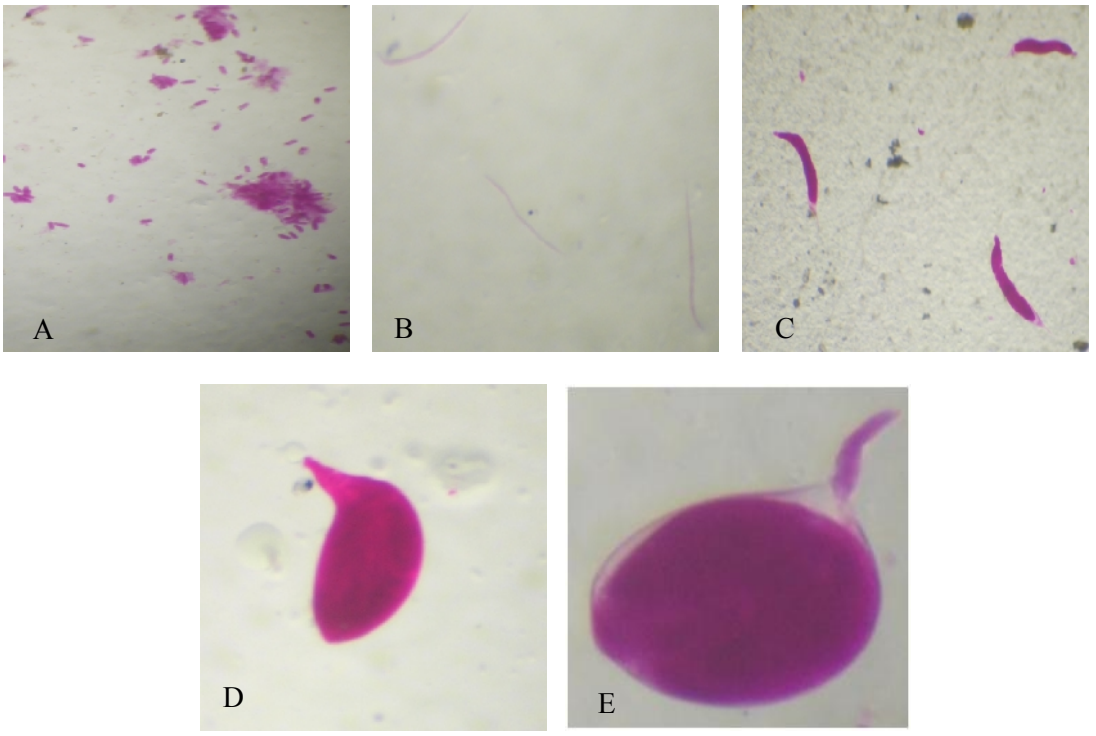

Plate 2. Developmental stages of root-knot nematode, *Meloidogyne graminicola* in root of non-scented rice (var. PR 114).

A= Eggs 21 DAI: B= j_2 developing 72 h DAI: C=j_2 moulting 5 DAI; D=adult female after 15 DAI; E=adult female at eggs laying stage 21 DAI

Table 4. Effect of different inoculum levels of *M. graminicola* on plant growth parameters of scented rice (var. Pusa 1121) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Shoot length (cm) Soil types			Mean	Fresh shoot weight (g) Soil types			Mean	Dry shoot weight (g) Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
0	53.83	46.50	41.27	47.20	7.90	5.83	5.03	6.26	2.87	2.33	2.03	2.41
10	52.47	45.37	40.20	46.01	7.50	5.60	4.88	5.99	2.57	2.27	1.80	2.21
100	41.33	34.60	31.23	35.72	4.40	3.43	2.80	3.54	1.57	1.37	1.07	1.33
1000	34.70	30.47	26.57	30.58	3.23	2.57	1.87	2.56	1.27	0.93	0.70	0.97
10000	26.73	24.77	21.70	24.40	2.30	1.87	1.03	1.73	0.83	0.70	0.40	0.64
Mean	41.83	36.34	32.19		5.07	3.86	3.12		1.82	1.52	1.20	
C.D. at 5 per cent												
Soil types		1.28				0.27				0.16		
Inoculum levels		1.65				0.34				0.21		
Interaction (Soil types v/s inoculum levels)		2.85				0.60				N.S.		

As nematode number increased from 10-10000 j_2, shoot length also decreased accordingly, irrespective of soil types. In case of interaction between soil types and inoculum levels, maximum shoot length was observed in clay loam followed by sandy loam at non-inoculated check, which was statistically at par with 10 j_2 and significantly different from all other treatments of interactions.

4.2.1.2 Fresh shoot weight

Perusal of data in Table 4 indicated that fresh shoot weight was significantly different in each soil type but maximum and significantly highest fresh shoot weight was obtained in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. Fresh shoot weight decreased as inoculum levels increased from 10-10000 j_2/kg soil. Maximum and significantly highest fresh shoot weight was obtained in non-inoculated check which was statistically at par with 10 j_2 and significantly different from all other inoculum levels.The significant reduction in shoot length was observed at 100 j_2. In case of interaction between soil types and inoculum levels, maximum fresh shoot weight was observed in clay loam soil at non-inoculated check, which was statistically at par with 10 j_2 and significantly different from all other interactions.

4.2.1.3 Dry shoot weight

It was inferred from data in Table 4 that dry shoot weight was significantly different in each soil types. Minimum and significantly lowest dry shoot weight was observed in loamy sand followed by sandy loam and clay loam soil irrespective of inoculum levels. Dry shoot weight was statistically different from each other from inoculum levels of 10-10000 j_2/kg soil but dry shoot weight at 10 j_2 was statistically at par with non-inoculated check. Maximum and significantly highest dry shoot weight was obtained at non-inoculated check. As inoculum levels increased from 10-10000 j_2, dry shoot weight decreased accordingly, irrespective of soil types. The interaction between soil types and inoculum levels was however non-significant.

4.2.1.4 Root length

The data in Table 5 depicted that root length was significantly different in each soil type. Maximum and significantly highest root length was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Treatments of inoculum levels of 10-10000 j_2/kg soil were significantly different from each other. Maximum and significantly highest root length was observed in non-inoculated check followed by 10, 100, 1000 and 10000 j_2/kg of soil. As nematode number increased from 10-10000 j_2, root length also decreased accordingly, irrespective of soil types. The significant reduction in shoot length was observed at 100 j_2 and onwards. Maximum reduction in root length was observed at 10000 j_2.The interaction between soil types and inoculum levels was however non-significant.

Table 5. Effect of different inoculum levels of *M. graminicola* on plant growth parameters of scented rice (var. Pusa 1121) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Root length (cm) Soil types			Mean	Fresh root weight (g) Soil types			Mean	Dry root weight (g) Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
0	26.53	23.63	22.00	24.06	5.87	4.27	3.47	4.53	1.30	1.00	0.93	1.08
10	23.57	20.30	19.57	21.14	5.67	3.97	3.27	4.30	1.20	0.93	0.83	0.99
100	17.80	15.70	13.23	15.58	3.53	2.50	2.03	2.69	0.80	0.57	0.50	0.62
1000	14.07	12.70	9.17	11.98	2.73	1.87	1.50	2.03	0.63	0.40	0.37	0.47
10000	9.23	7.70	6.20	7.71	1.93	1.37	0.97	1.42	0.43	0.33	0.23	0.33
Mean	18.24	16.01	14.03		3.95	2.79	2.25		0.87	0.65	0.57	
C.D. at 5 per cent												
Soil types		1.23				0.22				0.08		
Inoculum levels		1.59				0.28				0.10		
Interaction (Soil types v/s inoculum levels)		N.S.				0.49				N.S.		

4.2.1.5 Fresh root weight

Perusal of data in Table 5 indicated that fresh root weight in all three types of soils such as clay loam, sandy loam and loamy sand was significantly different from each other but maximum and significantly highest fresh root weight was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Fresh root weight was significantly different from each other from inoculum levels of 10-10000j_2/kg soil but fresh root weight at 10 j_2 was statistically at par with non-inoculated check. Maximum fresh root weight was observed at non-inoculated check followed by 10, 100, 1000 and 10000 j_2/kg of soil. Maximum reduction in fresh root weight was observed at 10000 j_2. In case of interaction between soil types and inoculum levels, maximum fresh root weight was observed in clay loam soil at non-inoculated check, which was statistically at par with 10 j_2 and significantly different from all other treatment of interactions.

4.2.1.6 Dry root weight

Data in Table 5 clearly indicated that dry root weight was significantly different from each other in different soil types such as clay loam, sandy loam and loamy sand but maximum and significantly highest dry root weight was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Dry root weight decreased as inoculum levels increased from 10-10000 j_2/kg soil. Significantly lowest and minimum dry root weights were however, observed in 10000 j_2 level of *M. graminicola*. Maximum dry root weight was observed at non-inoculated check which was statistically at par with 10 j_2 but significantly different from all other of nematode inoculation used in the experimentation. The interaction between soil types and inoculum levels was however non-significant.

4.2.1.7 Number of galls per plant

Data in Table 6 indicated that number of galls was significantly different from each other in clay loam and sandy loam but statistically at par with sandy loam and loamy sand. Minimum number of galls was observed in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. As inoculum level increased from 10-1000 j_2, numbers of galls increased. Number of galls decreased at inoculum levels 10000 j_2/kg soil. Number of galls was significantly different from each other at different inoculum levels but minimum number of galls was observed at 10 j_2. In case of interaction between soil types and inoculum levels, minimum number of galls was observed in clay loam soil at 10 j_2 which was significantly different from all other treatments of interactions. Maximum number of galls was observed at loamy sand at 1000 j_2 which were significantly different from other treatment of interactions.

4.2.1.8 Number of eggs per plant

Perusal of data in Table 6 indicated that numbers of eggs per plant were significantly different from each other in different soil types but minimum numbers of eggs per plant were recorded in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels.

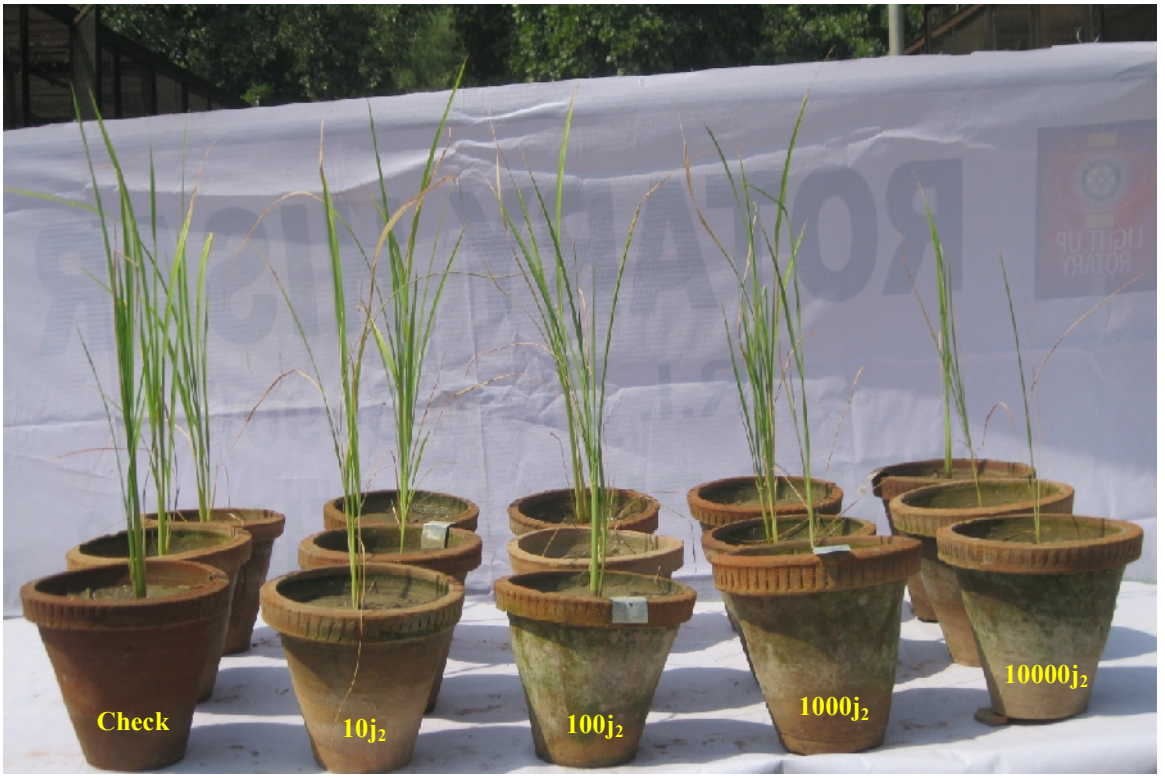

Plate 3. Effect of different inoculum levels of *M. graminicola* on growth of scented rice (var. Pusa 1121) in clay loam soil

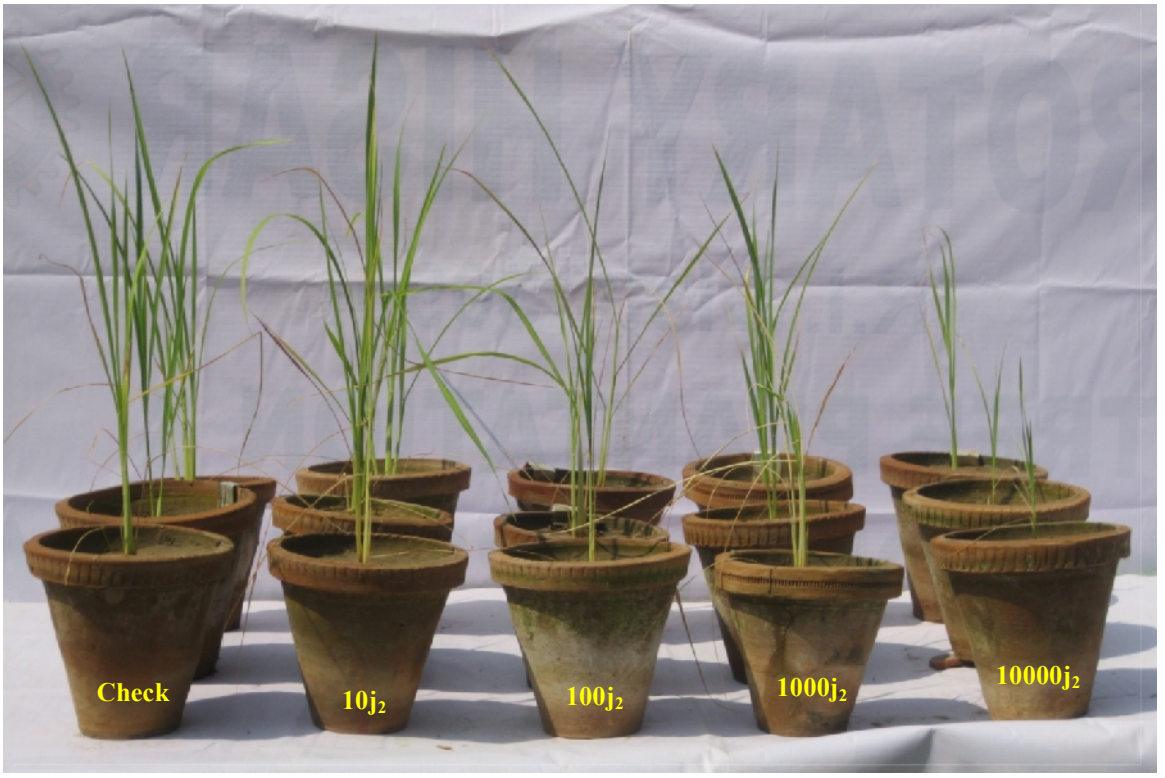

Plate 4. Effect of different inoculum levels of *M. graminicola* on growth of scented rice (var. Pusa 1121) in sandy loam soil

Table 6. Effect of inoculum levels on reproduction and multiplication of *M. graminicola* in scented rice (var. Pusa 1121) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Number of galls/plant Soil types			Mean	Number of eggs/plant Soil types			Mean	Final soil population (j_2)/kg soil Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
10	15.00 (3.99)	22.00 (4.79)	25.00 (5.09)	20.67 (4.62)	455.00 (21.31)	655.00 (25.50)	705.00 (26.42)	605.00 (24.41)	45.00 (6.74)	60.00 (7.77)	78.00 (8.88)	61.00 (7.80)
100	79.00 (8.91)	122.00 (11.08)	145.00 (12.05)	115.33 (10.68)	2070.00 (45.40)	3280.00 (57.08)	4050.00 (63.34)	3133.33 (55.27)	245.00 (15.67)	292.00 (17.10)	350.00 (18.65)	295.67 (17.14)
1000	117.00 (10.84)	168.00 (12.99)	192.00 (13.89)	159.00 (12.57)	3825.00 (61.78)	5290.00 (72.73)	6000.00 (77.46)	5038.00 (70.66)	690.00 (26.28)	970.00 (31.14)	1150.00 (33.85)	932.67 (30.43)
10000	63.00 (7.98)	70.00 (8.38)	71.00 (8.47)	68.00 (8.28)	1585.00 (39.76)	1740.00 (41.54)	2010.00 (44.75)	1778.33 (42.02)	117.00 (10.82)	154.00 (12.44)	175.00 (13.85)	148.67 (12.17)
Mean	68.50 (7.93)	95.50 (9.31)	108.25 (9.87)		1983.00 (42.06)	2741.25 (49.22)	3191.00 (52.99)		274.25 (14.88)	369.00 (17.11)	438.00 (18.66)	
C.D. at 5 per cent												
Soil types		(0.61)				(3.39)				(1.10)		
Inoculum levels		(0.71)				(3.91)				(1.27)		
Interaction (Soil types v/s inoculum levels)		(1.22)				N.S.				(2.19)		

Since the observations recorded were nil in non-inoculated (0 j_2) check, so this treatment is not depicted in the Table

Figures in parentheses are √ n transformed value

In case of inoculum levels minimum and significantly lowest number of eggs per plant was observed in 10 j_2 which was significantly different from all other inoculum levels but number of eggs decreased at inoculum level of 10000 j_2/kg soil. As inoculum levels increased, number of eggs per plant also increased but in case of 10000 j_2/kg soil, number of eggs per plant decreased. There was non-significant interaction between soil types and inoculum levels.

4.2.1.9 Final nematodes population (j_2/kg soil)

It is clear from data in Table 6 that final nematode population (j_2/kg soil) was significantly different from each other in different soil types. Maximum and significantly highest nematode population was observed in loamy sand followed by sandy loam and clay loam irrespective of inoculum levels. As inoculum levels increased from 10-1000 j_2, final nematode population increased significantly but decreased at inoculum level of 10000 j_2/kg soil. There were significant differences in nematode population at each inoculum levels. The minimum and significantly lowest number of nematodes was observed at 10 j_2/kg soil. In terms of interaction between soil types and inoculum levels, minimum and significantly lowest nematode population was observed in clay loam soil at 10 j_2, which was significantly different from other treatments of interaction. Maximum and significantly highest nematode population was observed in loamy sand at 1000 j_2, which was also significantly different from all other treatments.

4.2.1.10 Reproduction factor (RF):

Figure 1 clearly indicated that reproduction factor of *M. graminicola* was maximum in loamy sand soil followed by sandy loam and clay loam irrespective of inoculum levels. Reproduction factor decreased as inoculum levels increased from 10-10000 j_2/kg soil. Maximum reproduction factor was found in loamy sand at 10 j_2 and minimum in clay loam at 10000 j_2.

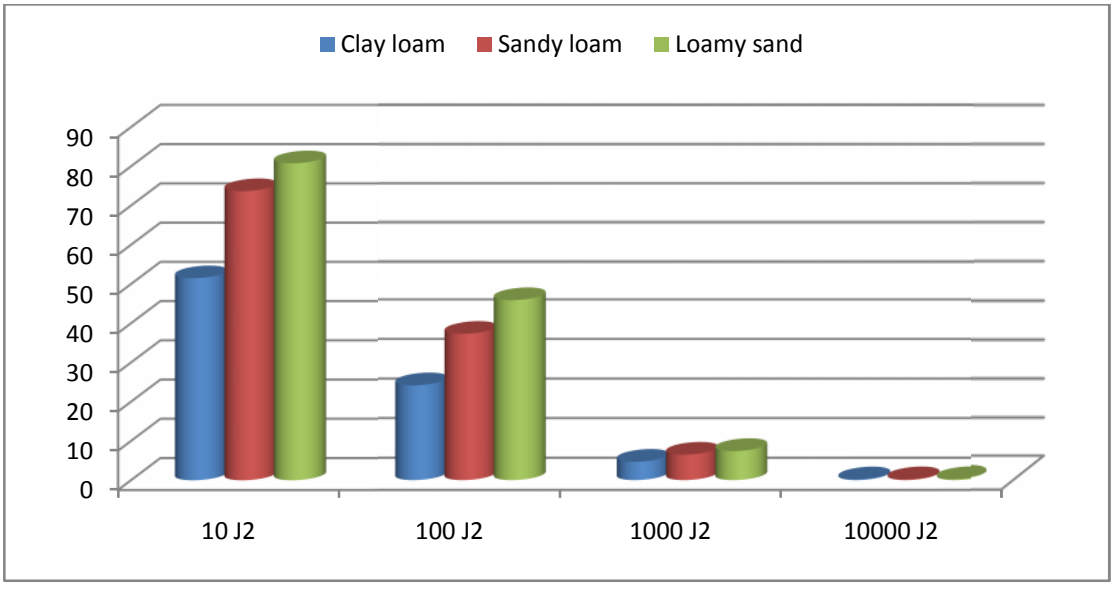

Fig. 1. Reproduction factor of *M. graminicola* at various inoculum levels in different soil types on scented rice (var. Pusa 1121)

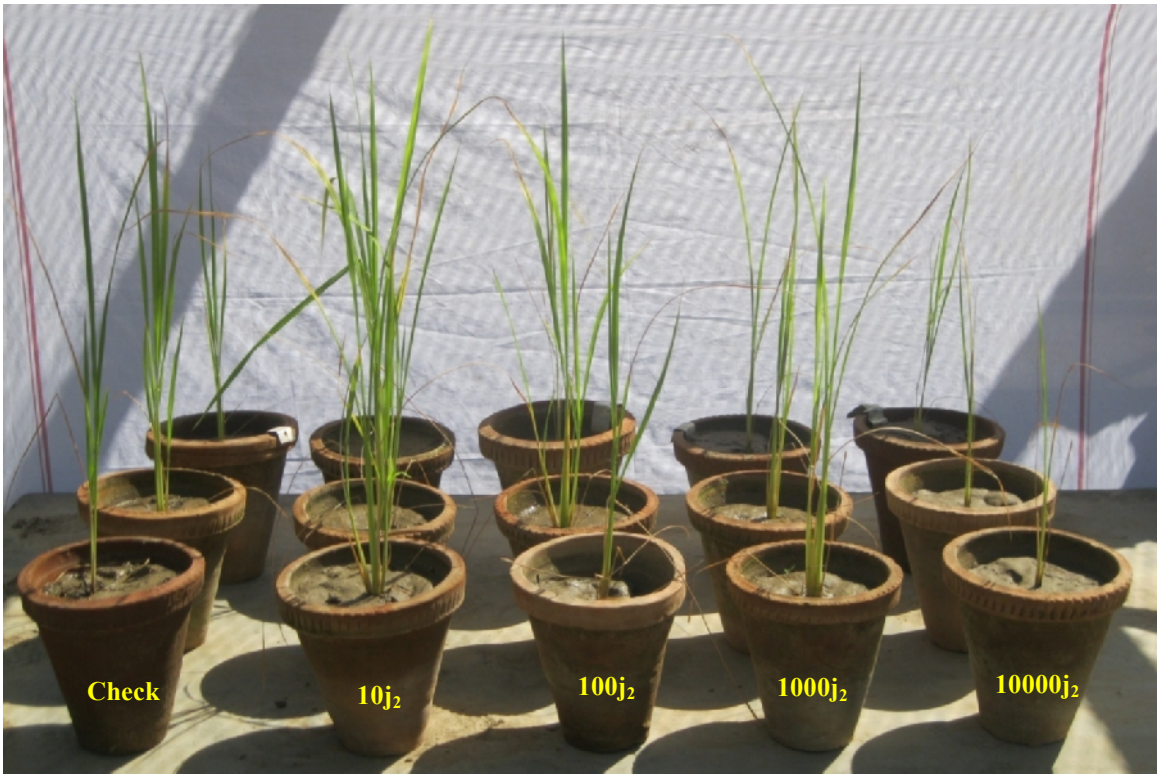

Plate 5. Effect of different inoculum levels of *M. graminicola* on growth of scented rice (var. Pusa 1121) in loamy sand soil

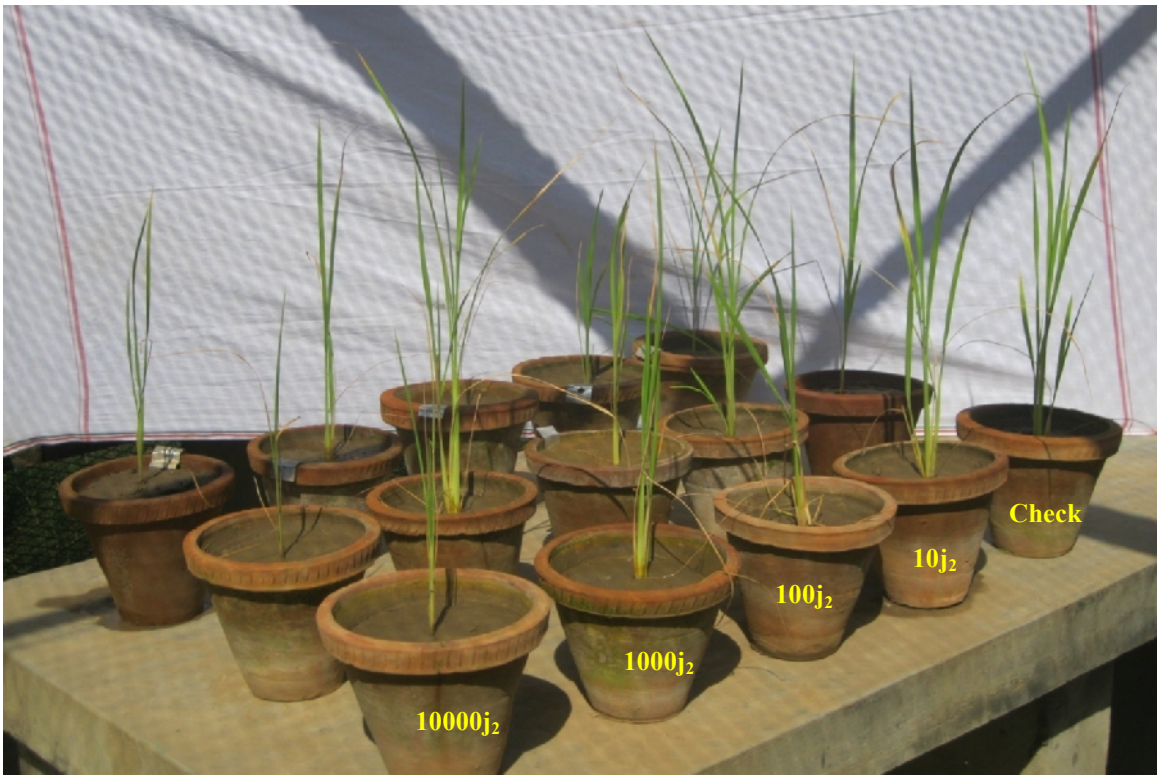

Plate 6. Effect of different inoculum levels of *M. graminicola* on growth of non-scented rice (var. PR 1121) in clay loam soil

4.2.2 Effect of different inoculum levels on plant growth parameters in rice (var. PR 114)

4.2.2.1 Shoot length

Data in Table 7 clearly depicted that the first factor (soil types) as well as second factor, i.e., inoculum levels, showed statistically significant results while interaction between these two factors i.e. soil types and inoculum levels proved non-significant. Highest and significantly maximum shoot length was observed in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. However, each soil types i.e. clay loam, sandy loam and loamy sand were significantly different from each other. The maximum and significantly highest shoot length was found in non-inoculated check (0 nematode) which was statistically at par with inoculum level of 10 j_2 /kg soil. The significant reduction in shoot length was observed at 100 j_2 which was statistically different from 0, 10, 1000 and 10000 j_2. The minimum and significantly lowest shoot length was observed in 10000 j_2 followed by 1000 and 100 j_2 which were significantly different from each other.

4.2.2.2 Fresh shoot weight

The data in Table 7 indicated that fresh shoot weight was significantly different in each soil type but maximum and significantly highest fresh shoot weight was obtained in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Fresh shoot weight decreased as inoculum levels increased from 10-10000 j_2/kg soil. Fresh shoot weight was significantly different from each other from inoculum levels of 10-10000j_2/kg soil but fresh root weight at 10 j_2 was statistically at par with non-inoculated check. Maximum reduction in fresh shoot weight was observed at 10000 followed by 1000 and 100 j_2. Maximum and significantly highest fresh shoot weight was obtained at non-inoculated check. The interaction between soil types and inoculum levels was however non-significant.

4.2.2.3 Dry shoot weight

It was inferred from data in Table 7 that dry shoot weight was significantly different in each soil type. Maximum and significantly highest dry shoot weight was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. The weight was maximum and significantly highest in non-inoculated check and 10 j_2 which were statistically at par with each other but different from other inoculum levels. This weight decreased as inoculum levels increased from 10-10000 j_2/kg soil. In case of interaction between soil types and inoculum levels, maximum and significantly highest dry shoot weight was observed in clay loam followed by sandy loam at non-inoculated check which were statistically at par with each other and significantly different from all other treatment of interactions.

Table 7. Effect of different inoculum levels of *M. graminicola* on plant growth parameters of non-scented rice (var. PR 114) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Shoot length (cm) Soil types			Mean	Fresh shoot weight (g) Soil types			Mean	Dry shoot weight (g) Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
0	54.70	48.83	42.50	48.68	8.50	7.23	6.13	7.29	4.13	2.70	2.23	3.02
10	52.50	46.43	40.60	46.51	8.33	6.97	5.90	7.07	3.80	2.43	2.03	2.76
100	42.27	35.57	31.90	36.58	6.60	4.20	3.70	4.83	2.40	1.53	1.33	1.76
1000	35.00	28.27	26.33	29.87	4.30	2.83	2.23	3.12	1.57	0.93	0.83	1.11
10000	26.40	23.67	21.80	23.96	3.03	1.83	1.13	2.00	1.13	0.70	0.43	0.76
Mean	42.17	36.55	32.63		6.15	4.61	3.82		2.61	1.66	1.37	
C.D. at 5 per cent												
Soil types		1.83				0.43				0.23		
Inoculum levels		2.37				0.55				0.29		
Interaction (Soil types v/s inoculum levels)		N.S.				N.S.				0.51		

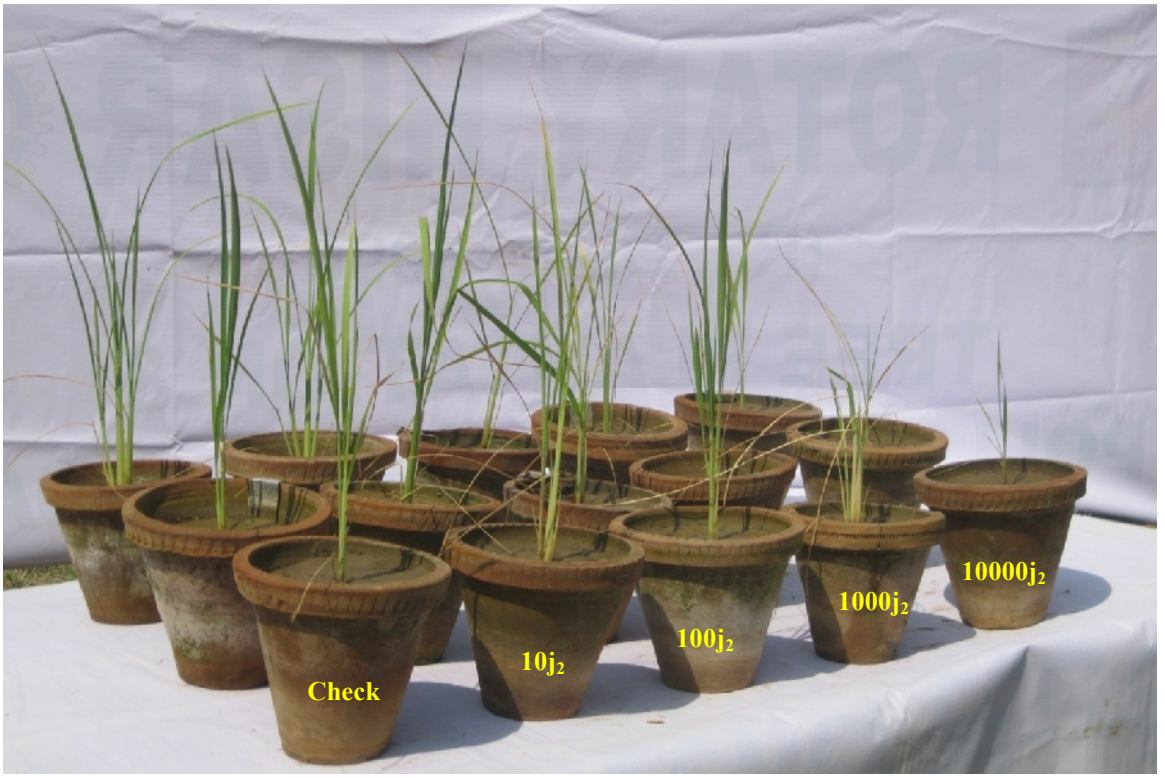

Plate 7. Effect of different inoculum levels of *M. graminicola* on growth of non-scented rice (var. PR 114) in sandy loam soil

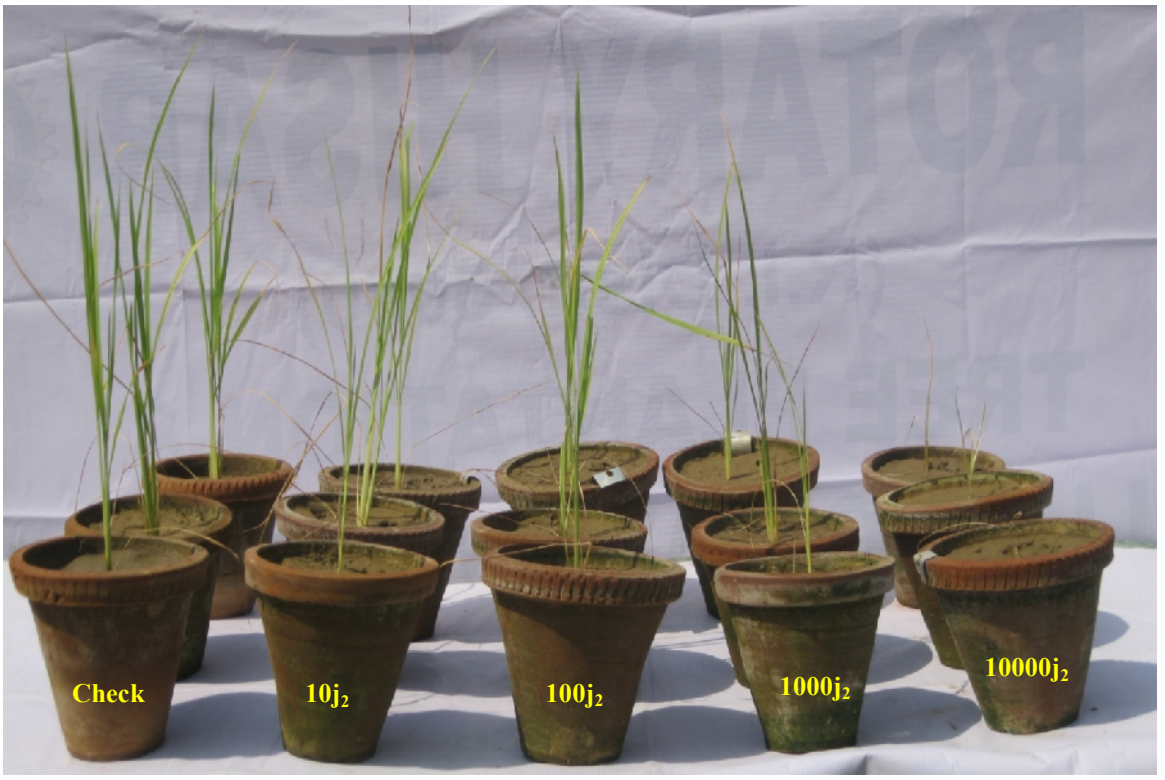

Plate 8. Effect of different inoculum levels of *M. graminicola* on growth of non-scented rice (var. PR 114) in loamy sand soil

4.2.2.4 Root length

The perusal of data in Table 8 indicated that root length was significantly different in each soil type. Maximum and significantly highest root length was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Treatments of inoculum levels of 10-10000 j_2/ kg soil were significantly different from each other. Maximum root length was observed in non-inoculated check which was statistically at par with 10 j_2. As nematode number increased from 10-10000 j_2, root length also decreased accordingly. The interaction between soil types and inoculum levels was however non-significant. Significant reduction in root length was started at the lowest inoculums, i.e., 10 j_2 which lasted upto 10000 j_2 with significant difference.

4.2.2.5 Fresh root weight

The data in Table 8 clearly indicated that fresh root weight was significantly different among various treatments of soil types. Maximum and significantly highest dry root weight was observed in clay loam soil followed by sandy loam and loamy sand which significantly different from each other irrespective of inoculum levels. The weight was significantly highest in non-inoculated check. Fresh root weight at 10 j_2 was statistically at par with non-inoculated check. Treatments of inoculum levels of 10-10000 j_2/kg soil were significantly different from each other. This weight decreased as inoculum levels increased from 10-10000 j_2/kg of soil. As far as interaction between soil types and inoculum levels is concerned, maximum fresh root was observed in clay loam followed by sandy loam and loamy sand at non-inoculated check, which were significantly different from all other treatment of interactions but in case of sandy loam and loamy sand at 10 j_2, these were statistically at par with non-inoculated check.

4.2.2.6 Dry root weight

It was inferred from data in Table 8 that dry root weight in all three types of soils such as clay loam, sandy loam and loamy sand was significantly different from each other, but maximum and significantly highest dry root weight was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. Dry root weight decreased as inoculum levels increased from 10-10000 j_2 /kg soil. The maximum and significantly highest dry root weight was found in non-inoculated check which was statistically at par with inoculum level of 10 j_2 /kg soil. Maximum reduction in dry root weight was observed at 10000 j_2. In case of interaction between soil types and inoculum levels, maximum dry root weight was observed in clay loam followed by sandy loam and loamy sand soil at non-inoculated check followed by 10 j_2.

4.2.2.7 Number of galls per plant

Perusal of data in Table 9 indicated that significantly lowest and minimum number of galls was observed in clay loam followed by sandy loam and loamy sand.

Table 8. Effect of different inoculum levels of *M. graminicola* on plant growth parameters of non-scented rice (var. PR 114) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Root length (cm) Soil types			Mean	Fresh root weight (g) Soil types			Mean	Dry root weight (g) Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
0	31.47	28.03	24.17	27.89	9.07	6.13	5.10	6.77	1.97	1.40	1.10	1.49
10	30.50	27.63	23.83	27.32	8.07	5.77	4.83	6.22	1.83	1.23	1.07	1.38
100	21.60	18.27	16.50	18.79	5.40	3.83	2.73	3.99	1.10	0.80	0.60	0.83
1000	15.53	14.13	12.57	14.08	3.53	2.83	1.67	2.79	0.77	0.60	0.40	0.59
10000	10.77	9.67	7.67	9.37	2.03	1.70	1.03	1.59	0.47	0.40	0.23	0.37
Mean	21.97	19.55	16.95		5.62	4.05	3.07		1.23	0.89	0.68	
C.D. at 5 per cent												
Soil types		1.15				0.44				0.12		
Inoculum levels		1.48				0.57				0.15		
Interaction (Soil types v/s inoculum levels)		N.S.				0.99				0.26		

Table 9. Effect of inoculum levels on reproduction and multiplication of *M. graminicola* in non-scented rice (var. PR 114) in different soil types

(Average of three replications)

Inoculum levels (j_2/kg soil)	Number of galls/plant Soil types			Mean	Number of eggs/plant Soil types			Mean	Final soil population (j_2)/kg soil Soil types			Mean
	Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand		Clay loam	Sandy loam	Loamy sand	
10	10.00 (3.30)	17.00 (4.23)	21.00 (4.67)	16.00 (4.07)	315.00 (17.66)	490.00 (21.93)	620.00 (24.86)	475.00 (21.48)	38.00 (6.17)	50.00 (7.07)	85.00 (9.26)	57.67 (7.50)
100	55.00 (7.62)	80.00 (8.98)	103.00 (10.18)	79.33 (8.93)	1555.00 (39.26)	2145.00 (46.10)	2800.00 (52.75)	2166.67 (46.04)	205.00 (14.32)	280.00 (16.74)	310.00 (17.61)	265.00 (16.23)
1000	90.00 (9.62)	124.00 (11.17)	145.00 (12.07)	119.67 (10.95)	2700.00 (51.86)	3850.00 (61.98)	4320.00 (65.48)	3623.33 (59.78)	625.00 (25.02)	810.00 (28.41)	995.00 (31.50)	810.00 (28.31)
10000	40.00 (6.37)	48.00 (6.98)	61.00 (7.85)	49.67 (7.07)	1185.00 (34.34)	1335.00 (36.45)	1555.00 (39.26)	1358.33 (36.68)	110.00 (10.53)	160.00 (12.67)	190.00 (13.79)	155.33 (12.33)
Mean	48.75 (6.73)	67.25 (7.84)	82.50 (8.69)		1438.7 (35.78)	1995.00 (41.61)	2323.00 (45.59)		244.50 (14.01)	325.00 (16.22)	395.00 (18.04)	
C.D. at 5 per cent												
Soil types		(0.54)				(3.72)				(1.11)		
Inoculum levels		(0.62)				(4.30)				(1.29)		
Interaction (Soil types v/s inoculum levels)		N.S.				N.S.				N.S.		

Since the observations recorded were nil in non-inoculated (0 j_2) check, so this treatment is not depicted in the Table

Figures in parentheses are √ n transformed values

Each soil types were significantly different from each other. As inoculum levels increased from 10-1000 j_2/kg soil, number of galls was increased correspondingly but number of galls decreased significantly at highest inoculum levels of 10000 j_2/kg soil. Significantly highest and maximum numbers of galls were however observed in 1000 j_2 level of *M. graminicola* which was significantly different from all other levels of nematode inoculation used in the experimentation. Each inoculum levels were significantly different from one other. In terms of number of galls the interaction between inoculum levels and soil types was, statistically found to be non-significant.

4.2.2.8 Number of eggs per plant

The data in Table 9 clearly indicated that number of eggs per plant was significantly different from each other but minimum and significantly lowest numbers of eggs were recorded in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. In case of inoculum levels, significantly highest and maximum number of eggs was observed in 1000 j_2, which was significantly different from all other levels. Number of eggs at 10, 100 and 1000 j_2 was also significantly different from each other. As inoculum levels increased, number of eggs per plant also increased but number of eggs decreased at inoculum level of 10000 j_2. There was non-significant interaction between soil types and inoculum levels. Minimum and significantly lowest eggs were recorded in 10 j_2 levels which increased abruptly at other levels up to 1000 j_2.

4.2.2.9 Final nematodes population (j_2 per kg soil)

Data in Table 9 clearly indicated that final nematode population (j_2/kg soil) was significantly different from each other in different soil types. Minimum and significantly lowest nematode population was observed in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. As inoculum levels increased from 10-1000 j_2, final nematode population increased significantly but decreased at inoculum level of 10000 j_2/kg soil. There were significant differences in nematode population at each inoculums level. The minimum and significantly lowest number of nematodes was observed at 10 j_2/kg soil. Significantly maximum number of nematodes was found in 1000 j_2 which was significantly different from 100 and 10000 in terms of interaction. The interaction between inoculum levels and soil types was statistically found to be non-significant.

4.2.2.10 Reproduction factor (RF):

Figure 2 clearly depicted that reproduction factor of *M. graminicola* was minimum in clay loam followed by sandy loam and loamy sand soil irrespective of inoculum levels. Reproduction factor decreased as inoculum levels increased from 10-10000 j_2/kg soil. Minimum reproduction factors was obtained in clay loam at 10000 j_2 and maximum in loamy sand at 10 j_2.

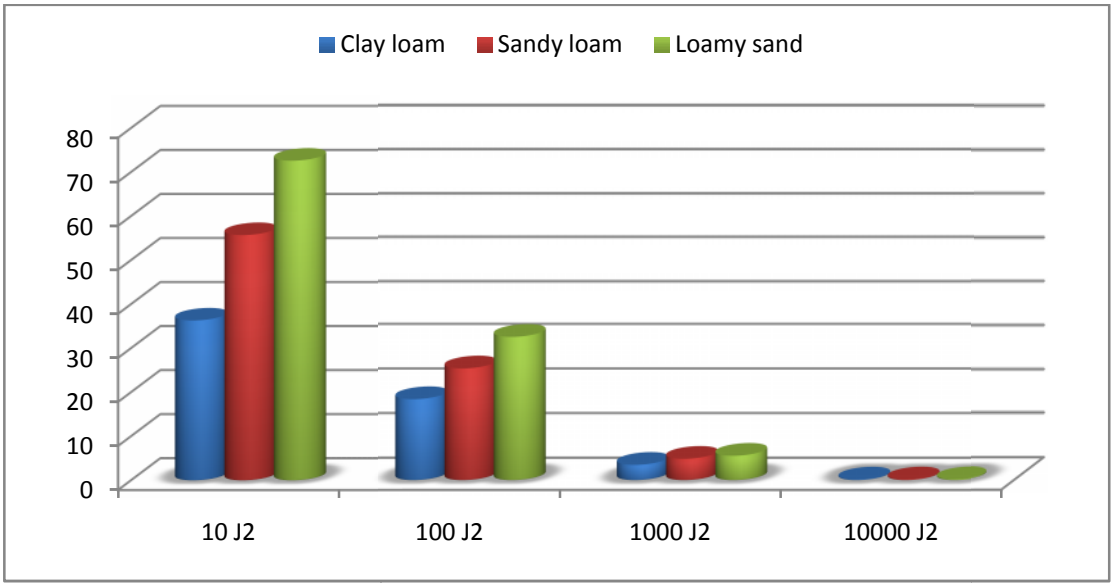

Fig. 2. Reproduction factor of *M. graminicola* at various inoculum levels in different soil types on non-scented rice (var. PR 114)

CHAPTER-V

DISCUSSION

Rice is the most important cereal crop worldwide since it provides food security for more than half of the world's human population. In India, it has become an economic pest of rice especially in light texture soils and irrigated upland conditions where it cause enormous damage in nurseries and main fields.

Quit a good information is available on rice root-knot nematode infecting paddy in traditional rice growing areas. However, not much work has been done on population of *M. graminicola* prevalent in northern India. The basic aspect of pathogenicity and biology of this pest has not been dealt with in detail particularly under Haryana conditions except few aspects of life cycle development on scented rice. No such work has been done on any non-scented rice variety for comparison. Therefore, systematic work on life cycle development and pathogenicity was studied and results so obtained are discussed in this chapter.

5.1 Life cycle and development of root-knot nematode, *Meloidogyne graminicola* on scented and non-scented varieties of rice

Life cycle of root -knot nematode, *M. graminicola* was studied in scented rice (var. Pusa 1121) and non-scented rice (var. PR 114) under screen house conditions. The second stage juveniles (j_2) of *M. graminicola* penetrated young roots of rice seedling near the root tip within 24 hours in scented rice (var. Pusa 1121) while in non-scented rice (var. PR 114), time taken for penetration was prolonged to 72 hours. Second stage juveniles (j_2) slightly enlarged in size on 3 DAI in scented rice as compared to 5 DAI on non-scented rice. After penetration, the j_2 developed into third stage juvenile (j_3) on 7 DAI, in var. Pusa 1121 whereas this period was 9 DAI in var. PR 114. Third stage juveniles further developed into fourth stage juvenile (j_4) in 9 DAI in scented rice while in non-scented rice, on 11 DAI. Young females were observed on 11 DAI and males on 13 DAI in var. Pusa 1121 as compared to 15 and 17 DAI in variety PR 114. Males in the soil were observed on 17 DAI in scented rice while in non-scented rice, 21 DAI. Egg deposition inside the roots was started at 19 DAI in scented rice while in non-scented rice, the period was 23 DAI. Thus, the life cycle of *M. graminicola* from j_2-j_2 was completed in 21 days at average maximum temperature of 35.0^{o}C and average minimum temperature of 25.8 ^{o}C in scented rice as compared to 25 days at 35.6 ^{o}C and 27.2 ^{o}C in non-scented rice under screen house conditions.

The results are in confirmity with the results of Bridge and Page, (1982) who observed the completion of life cycle of *M. graminicola* to be 19 days at an ambient temperature of 22-29 ^{o}C. Similarly, Senthilkumar *et al*., 2007 also reported the completion of life cycle in 25-30 days at 30 ^{o}C but in the present experimentation, the highest average temperature was 35.0 ^{o}C for

scented, 35.6 ^{o}C for non-scented rice. As regards the penetration of j_2 and its further development from j_2-j_3 and j_3-j_4, our observations are in conformity with those of Rao *et al*., 1984 and Cannayane and Anita, 2011. For the completion of life cycle, a difference of time period of 4 days in scented and non-scented rice could be attributed to differential rate of nematode development as the temperature was more or less same for experimentation of both types of rice. However, our results showed that temperature did not substantially decrease or increase the rate of development of *M. graminicola* on both the rice varieties. These results of the present study are in full conformity with those of Fernandez *et al*, 2014.

The egg deposition was observed on 19 and 21 DAI in scented and non-scented rice respectively in the present studies. These observations are in conformity with those of Dutta *et al.,* 2011 who reported this time to be 20 days. As this nematode is a pest of rice-wheat cropping system, therefore, this nematode affected wheat crop also. Period to complete one generation of this nematode on rice in present study was 21-25 days on both types of rice varieties. Similar results were reported by Singh *et al.*, 2011 though on wheat crop during the month of Feb-March. Contrary to it, Kanwar *et al.*, 2008 reported this period to be 75-90 DAI during Jan-Feb indicating thereby role of temperature prevailing at this time in wheat crop.

5.2 Pathogenicity of *M. graminicola* in different soil type on rice

Pathogenicity of root-knot nematode, *M. graminicola* was studied under screen house conditions in different soil types such as clay loam, sandy loam and loamy sand by using a series of different inoculum levels such as 0 (non-inoculated check), 10, 100, 1000 and 10000 j_2/kg soil on scented rice (var. Pusa 1121) and non-scented rice (var. PR 114).

In scented rice, the data revealed that maximum and significantly highest plant growth was observed in clay loam soil as compared to sandy loam and loamy sand irrespective of inoculum levels. As far as effect of different inoculum levels is concerned, the significant reduction in shoot and root parameters started at 100 j_2/kg soil while the plant growth at 10 j_2 was statistically at par with non-inoculated check (0 j_2). Maximum plant growth was observed at non-inoculated check followed by 10 j_2, plant growth parameters were decreased as inoculum levels increased from 10-10000 j_2. The interaction between soil type and inoculum levels was significant in shoot length, fresh shoot weight and fresh root weight. Plant growth parameters were maximum and significantly higher in interaction of clay loam soil at non-inoculated check, which was statistically at par with 10 j_2. Nematode reproduction and multiplication was more in loamy sand as compared to sandy loam and clay loam. As inoculum levels increased from 10-1000 j_2/kg soil, nematode reproduction and multiplication was increased correspondingly but reproduction and multiplication decreased significantly at highest inoculum levels of 10000 j_2/kg soil. In case of interaction between soil types and inoculum levels, number of galls/plant and final nematode population in soil was significant while number of eggs/plant was non-significant. Minimum number of galls/plant

and final nematode population in soil was observed in clay loam soil at 10 j_2 which was significantly different from all other treatments of interactions. Significantly maximum reproduction and multiplication was observed at 1000 j_2 in loamy sand followed by sandy loam and clay loam. The reproduction factor of *M. graminicola* was maximum in loamy sand at 10 j_2 and minimum in clay loam at 10000 j_2.

In non-scented rice, the data revealed that minimum and significantly lowest plant growth was observed in loamy sand soil as compared to sandy loam and clay loam irrespective of inoculum levels. Significant reduction in growth parameters was found at 100 j_2/kg soil, while the plant growth at 10 j_2 was statistically at par with non-inoculated check. Minimum plant growth was observed at 10000 followed by 1000 and 100 j_2. Plant growth parameters were decreased as inoculum levels increased from 10-10000 j_2. The interaction between soil type and inoculum levels was significant in dry shoot weight, fresh root weight and dry root. Plant growth parameters were maximum and significantly higher in interaction of clay loam soil at non- inoculated check, which was statistically at par with 10 j_2. Nematode reproduction and multiplication was more in loamy sand as compared to sandy loam and clay loam. As inoculum levels increased from 10-1000 j_2/kg soil, nematode reproduction and multiplication was increased correspondingly but reproduction and multiplication decreased significantly at highest inoculum levels of 10000 j_2/kg soil. The interaction between soil types and inoculum levels was however non-significant. Significantly minimum reproduction and multiplication was obtained in clay loam and maximum in loamy sand at 10 j_2. The reproduction factor of *M. graminicola* was minimum in clay loam at 10000 j_2 and maximum in loamy sand at 10 j_2.

The highest growth of rice plants was observed in clay loam followed by sandy loam and loamy sand in both types of rice i.e. scented (var. Pusa 1121) and non-scented (var. PR 114) irrespective of the inoculum levels indicating the preference of rice to grow well in clay loam because of high clay content of 39.0 per cent in clay loam as compared to 11.0 per cent in sandy loam and 8.3 per cent in loamy sand (as per soil analysis). These results are in conformity with those of Prot and Matias, 1995 who also observed higher growth of rice plants in clay loam soil. The amount of sand present in all types of soil is also contributing for the lower growth of plants. Loamy sand had 82.1 per cent sand which was least favoured by rice to grow well followed by sandy loam and clay loam which had sand content of 74.0 and 36.0 per cent, respectively. So this difference in the growth of plants may be attributed to difference in soil texture, water holding capacity and nutrient availability of their soil. In its contrast, the multiplication and reproduction of *M. graminicola* was significantly highest in loamy sand followed by sandy loam and least in clay loam. This reverse trend of lowest plant growth in loamy sand but highest multiplication and reproduction of nematode speaks well of the amount of sand content which was highest in loamy sand followed by sandy loam and

clay loam. Higher amount of sand content is favourable for this nematode in particular and other plant parasitic nematodes in general. As it is well established fact that coarse textured soils having high sand content had more pore space (because of the increase in the diameter of soil particles) for the movement and developments of the nematode. These results are in conformity with those of Rao and Israel, 1972 who observed that coarse and medium soils with particles size above 0.053 mm in diameter and sandy soils allowed free movement of infective larvae and invasion into roots of the rice plant. In present study, clay loam soil was least favoured by this nematode for its multiplication and reproduction. The same trend was observed by Rao and Israel, 1972, Prot and Matias, 1995 and Pokharel, 2009. Rao and Israel, 1972 correlated the nematode development with sand content of soil. They further observed that in fine soils, migration of larvae was 13.5 per cent whereas in coarse textured soils, the migration of larvae was 76.1 per cent while in medium soil, larval migration was 47.0 per cent.

With the increase in sand content of the soil, there was an increase in number of galls, number of eggs per plant and final soil population of the nematode, showing thereby the relationship between the sand content and high activity of the nematode. The difference in the growth of scented and non-scented rice varieties grow differently in same soil types may be due to the difference of growth pattern of both type of rice. The growth of non-scented rice (var. PR 114) was slightly more than that of scented rice (var. Pusa 1121) as it clear from the data of plant growth parameter of non-inoculated treatments. The multiplication and reproduction of this nematode was more in scented rice as compared to non-scented rice which may be attributed due to the reaction of the variety towards *M. graminicola*. The biochemistry and physiology of variety Pusa 1121 may be more favourable to the nematode as compared to variety PR 114, which is less susceptible though the nematode multiplied well in both the varieties.

The role of the abiotic factors particularly temperature prevailing at the time of infestation in also be considered as factor for difference in the varietal response. Rice is particularly sensitive to temperature and even various cultivars within rice differ in their root production and extension responses to temperature which in turn will affect their response to nematode attack, irrespective of the soil types. Plant growth parameters having 10 j_2/kg soil had statistically at par growth with non-inoculated check (no nematode inoculation), significant reduction in growth parameters was observed from inoculum level of 100 j_2/kg soil and onwards. It can be inferred that inoculum level of 100 j_2/kg soil proved to be pathogenic due to reduced growth of rice plants at this level. This low inoculum level of 100 j_2/kg soil speaks of nematode to be very severe and pathogenic on both type of rice as in other species of root-knot nematode, 1000 j_2/kg soil is considered to be threshold level. The short life cycle duration, high reproduction potential, many generations in a single crop season, are the factors which are considered to be important for the severity of this nematode even at low inoculum

level. Poudyal *et al*, 2005 also concluded that the response of plant to nematode invasion will depend on the status of the host and the nematode population. Nematode effects on plant growth and yield are generally proportional to the numbers of infective nematode per unit of soil at planting. There is a population density below which no loss in plant growth and yield occurs. These results are in conformity with findings of Prasad *et al*, 1990, Soomro and Hague, 1993 and Poudyal *et al*, 2005 who observed 200 and 125 j_2/kg soil to be pathogenic level. Jaiswal *et al.*, 2011 also observed reduction in plant growth with the increase in the initial inoculum levels from 50-5000 j_2/kg soil, but observed 1000 j_2 as damaging level of *M. graminicola* on rice. At the same time, Mian and Khan, 1995 observed 5000 and 10000 larvae/plant to be necessary to have higher number of galls and eggs irrespective of soil types. There was significant increase in multiplication with corresponding increase in nematode inoculum levels starting from 10-1000 j_2/kg soil in both type of rice but the results were more pronounced in var. Pusa 1121 showing it to be more susceptible as compared to var. PR 114. The highest multiplication was observed at 1000 j_2/kg soil which reduced drastically at 10000 j_2 levels in both types of rice. The probable reason for this reversal was the high density of the nematode in a limited space in soil. Occurring of these conditions might have competition for space, nutrition and other requirement of the nematodes as it is clear from debilitation of roots having 10000 j_2 in the form of lowest root growth. Due to sharp decline in the growth parameters of the roots at 10000 j_2 level, there might be mortality of nematodes due to overcrowding. Rao and Israel, 1972 also observed the high rate of reproduction of *M. graminicola* in rice at low levels of inocula, could possibly be due to the positive factors like abundance of food, lack of competition, ability of the host to support these levels of population. While the reduction in nematode developmental parameters at high levels of inocula is considered to be due to the negative density factor like crowding of endoparasites in the roots.

CHAPTER-VI

SUMMARY AND CONCLUSION

Investigation were carried out to study life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* on both types of rice i.e. scented and non-scented. These experiments were conducted in screen house of department of Nematology, CCS HAU, Hisar.

In life cycle experiment, life cycle of *M. graminicola* was studied on var. Pusa 1121 and var. PR 114. The j_2 of *M. graminicola* penetrated rice roots after 24 h in scented rice while in non-scented rice 72 h. The third stage (j_3) appeared in the 7 DAI in scented rice as compared to non-scented rice in 9 DAI. Young females were first noticed on 11 DAI and 15 DAI for scented and non-scented rice, respectively. Further egg deposition started as 19 DAI and 23 DAI inside the root in scented and non-scented rice, respectively. Nematode completed its life cycle in 21 days in scented rice and 25 days in non-scented rice.

The experiment on pathogenicity of *M. graminicola* was carried out in three different types of soil (clay loam, sandy loam and loamy sand) by using different inoculum levels such as 0 (non-inoculated check), 10, 100, 1000 and 10000 j_2/kg soil. The results revealed that growth of both varities rice (scented and non-scented) was significantly improved in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. In case of inoculum levels, maximum plant growth was observed as non-inoculated check which was statistically at par with 10 j_2. Plant growth parameters were decreased significantly as inoculum levels increased from 10-10000 j_2 irrespective of soil types. Significant reduction in growth parameters was observed from inoculum level of 100 j_2/kg soil onwards.

The interaction between soil type and inoculum levels was significant in all plant growth parameters except dry shoot weight, root length and dry root weight for scented rice and shoot length, fresh shoot weight and fresh root weight for non-scented rice. Observations on nematode reproduction and multiplication revealed that maximum and significant reduction in number of galls, number of eggs per plant and final nematode population was observed in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. Nematode multiplication and reproduction was increased as inoculum level increased from 10-1000 j_2 but decreased abruptly at 10000 j_2. Maximum and significantly highest multiplication and reproduction was observed in 1000 j_2 of *M. graminicola*. Nematode growth parameters at 100 j_2 were significantly different from non-inoculated check and from other inoculum levels which can be termed as pathogenic level of *M. graminicola* on rice.

On the basis of these findings, following conclusion was drawn:

- The second stage of *M. graminicola* penetrated the rice seedlings near the root tips within 24 h in scented rice (var. Pusa 1121) while in non-scented rice (PR 114) in 72 h.

- Formation of young females first observed on 11 DAI (scented rice) as compared to non-scented rice in 15 DAI.
- The life cycle of *M. graminicola* from j_2 -j_2 stage completed in 21 days in scented rice while in 25 days in non-scented rice.
- Growth parameters of rice plants were maximum and significantly highest in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels in both types of rice.
- Significantly reduction in growth parameters was started from inoculum level of 100 j_2/ kg soil irrespective of soil types but plant growth decreased by increasing the inoculum levels from 10-10000 j_2 in both rice varieties.
- Nematode multiplication and reproduction was significantly highest in loamy sand followed by sandy loam and clay loam irrespective of rice varieties.
- Nematode multiplication and reproduction decreased significantly at highest inoculum levels of 10000 j_2/kg soil.

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ABSTRACT

Title of Thesis	:	**Life cycle and pathogenicity of root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield) on rice**
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Admission Number	:	2013A98M(R)
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Total Number of Pages in Thesis	:	38 + iv
Number of Words in Abstract	:	363

Key words: Development, life cycle, *Meloidogyne graminicola*, pathogenicity root-knot nematode and soil type

Investigations were carried out on life cycle and pathogenicity of *Meloidogyne graminicola* on scented and non-scented rice varieties under screen house conditions. In life cycle and development experiment, life cycle of *M. graminicola* was studied on var. Pusa 1121 and. PR 114. The j_2 of *M. graminicola* penetrated into rice roots after 24 h in scented rice while in non-scented rice in 72 h. The third stage (j_3) appeared on 7 DAI in scented rice as compared to non-scented rice in 9 DAI. Young females were first noticed on 11 DAI and 15 DAI for scented and non-scented rice, respectively. Further, egg deposition started as 19 DAI and 23 DAI inside the roots in scented and non-scented rice, respectively. Nematode completed its life cycle in 21 days in scented rice and 25 days in non-scented rice. The experiment on pathogenicity of *M. graminicola* was carried out in three different types of soil (clay loam, sandy loam and loamy sand) by using different inoculum levels such as 0 (non-inoculated check), 10, 100, 1000 and 10000 j_2/kg soil. The results revealed that growth of both varieties of rice (scented and non-scented) was significantly improved in clay loam soil followed by sandy loam and loamy sand irrespective of inoculum levels. In case of inoculum levels, maximum plant growth was observed in non-inoculated check which was statistically at par with 10 j_2. Plant growth parameters were decreased significantly as inoculum levels increased from 10-10000 j_2 irrespective of soil types. Significant reduction in growth parameters was observed from inoculum level of 100 j_2/kg soil onwards. Observations on nematode reproduction and multiplication revealed that maximum and significant reduction in number of galls, number of eggs per plant and final nematode population was observed in clay loam followed by sandy loam and loamy sand irrespective of inoculum levels. Nematode multiplication and reproduction was increased as inoculum levels increased from 10-1000 j_2 but decreased abruptly at 10000 j_2. Maximum and significantly highest multiplication and reproduction was observed in 1000 j_2 of *M. graminicola*. Nematode growth parameters at 100 j_2 were significantly different from non-inoculated check and other inoculum levels which can be termed as pathogenic level of *M. graminicola* on rice.

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