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Gajja Bharath Goud
Research Scholars, Department of Nematology, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Nishi Keshari
Associate Professor, Department of Nematology, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Gurram Mallikarjun
Research Scholars, Department of Nematology, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Palukuru Usha
Research Scholars, Department of Nematology, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author:
Gajja Bharath Goud
Research Scholars, Department of Nematology, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Life cycle completion of rice root knot-nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.), variety, Pusa Bhagwati under Bihar condition

Gajja Bharath Goud, Nishi Keshari, Gurram Mallikarjun and Palukuru Usha

Abstract
The Rice root-knot nematode (*Meloidogyne graminicola*) has become a major concern worldwide, attaining the status of a 'National Pest' due to its serious impact. India contributes a substantial 23% to the world's rice production, the infestation by *M. graminicola* results in a significant setback, accounting for roughly 16% of the annual global rice production of 160 million tonnes with an estimated loss value of 320 million USD annually. The current study was done to investigate the life cycle of *M. graminicola* on paddy cv. Pusa Bhagwati. The results revealed that within 24 hours of inoculation, infective second-stage juveniles entered rice roots, initiated feeding, and became sessile as the body size increased. The duration of the second (J2), advanced juveniles (J3/J4), preadult female, and adult female stage lasted for 1-7, 8-19, 20-22, and 23-30 days, respectively, at a temperature of 25 ± 2°C. Females laid about 250-300 eggs in an egg sac inside the root tissues. The total duration of life cycle including the preparasitic stage, was 28-30 days.

Keywords: *Meloidogyne graminicola*, rice, life cycle, *Oryza sativa*, Pusa Bhagwati

Introduction
Rice is the third most important cereal crop globally, right after wheat and maize. Its significance is evident in addressing challenges related to food security. In agriculture, worldwide, plant parasitic nematodes cause a major loss of more than $US 80 billion (Nicol *et al*., 2011) [10]. Rice, grown in different environmental condition, is attacked by different nematode species like *Ditylenchus angustus*, *Meloidogyne* spp., *Hirschmanniella* spp., *Aphelenchoides besseyi* and *Pratylenchus* spp.. Endoparasitic root-knot nematodes (*Meloidogyne* spp.) are foremost economically damaging genus of plant parasitic nematodes in rice. Among them, rice root-knot nematode has gained attention during the recent times because of its damage potential to rice particularly under water deficient conditions (Dutta *et al*., 2012) [3]. It is a pest of Global importance (Mac Gowan and Langdon, 1989) [7] and reported to cause 17-30% yield loss in upland rice (Prasad *et al*. 1986; Rao *et al*.1977) [11, 12]. The yield loss of rice root nematode is very severe upto 20% to 90% (Jain *et al*., 2012) [6].
The rice root knot nematode, *M. graminicola* is an important pathogen, impacting rice and wheat and possibly vegetable production in South-East Asia (Pokharel *et al*., 2010) [16]. Among the plant-parasitic nematodes (PPN) belonging to the poaceae crops globally, rice root-knot nematode (*M. graminicola*) is common among them. The main host races of *M. graminicola* in the family of poaceae includes rice, wheat, barley, oat and sorghum (Vaish and Pandey, 2012) [14]. There are several reports indicating variability in the host range among different populations of *M. graminicola* (Yik and Birchfield, 1979) [15]. Rice Root knot nematode infections often appear as patch patterns in a field, with the infected plants showing symptoms such as growth reduction, less vigor, yellowing of leaves and severe yield loss (Mantelin *et al*., 2017) [8]. A peculiar symptom of rice root knot nematode is the galls look like hook at the root tip of young roots. Hence, studying the life cycle of the rice root-knot nematode is essential for developing strategies to protect crops, manage pests, minimize economic losses, and promote sustainable agricultural practices.

Materials and Methods
The current study was carried in the Department of Plant Pathology &Nematology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar.

Nematode culture

The *M. graminicola* population used in this study was isolated from infected rice roots collected from various villages during field survey. Plants were uprooted, and egg masses were either carefully detached or dissected from the galled roots. Egg masses hatched and approximately 1000 J2s were used to infect Paddy (PB1121) roots in pots. After 30 days of infection, nematode females and egg masses were extracted (Hussey & Barker, 1973) [5]. Using perineal patterns (Eisenback *et al*., 1980) [4], *M. graminicola* identity was confirmed. Egg masses were collected (Hussey & Barker, 1973) [5] from the infected roots at 30 days post-inoculation (dpi), surface sterilized with 0.1% HgCl2 for 1 min, followed by rinsing in sterile water. Egg masses were kept for hatching in double-layered tissue paper placed on a wire mesh in a Petri plate containing sterile distilled water. The inoculum used for this was freshly hatched second stage juveniles.

Life cycle study of *M. graminicola*

The study was carried using rice (Pusa Bhagwati). The seed was surface sterilized as mentioned above. Young seedlings of 3-5 days were transplanted into 6-inch earthen pots containing 1 kg sterilized soil. Each seedling was inoculated with 1000 J_2, 15 DAT. Plants were watered to field capacity level. The seedlings were uprooted every 24 hours and brought to the laboratory for observation. The developments in the roots stained with 0.1% acid fuchsin lactophenol were observed under binocular microscope for every 24 hours. The observations like, preparasitic second stage (J_2), third stage (J_3) and fourth stage juvenile (J_4) and adult females of *M. graminicola*, were recorded. The whole experiment was replicated three times.

Results and Discussions

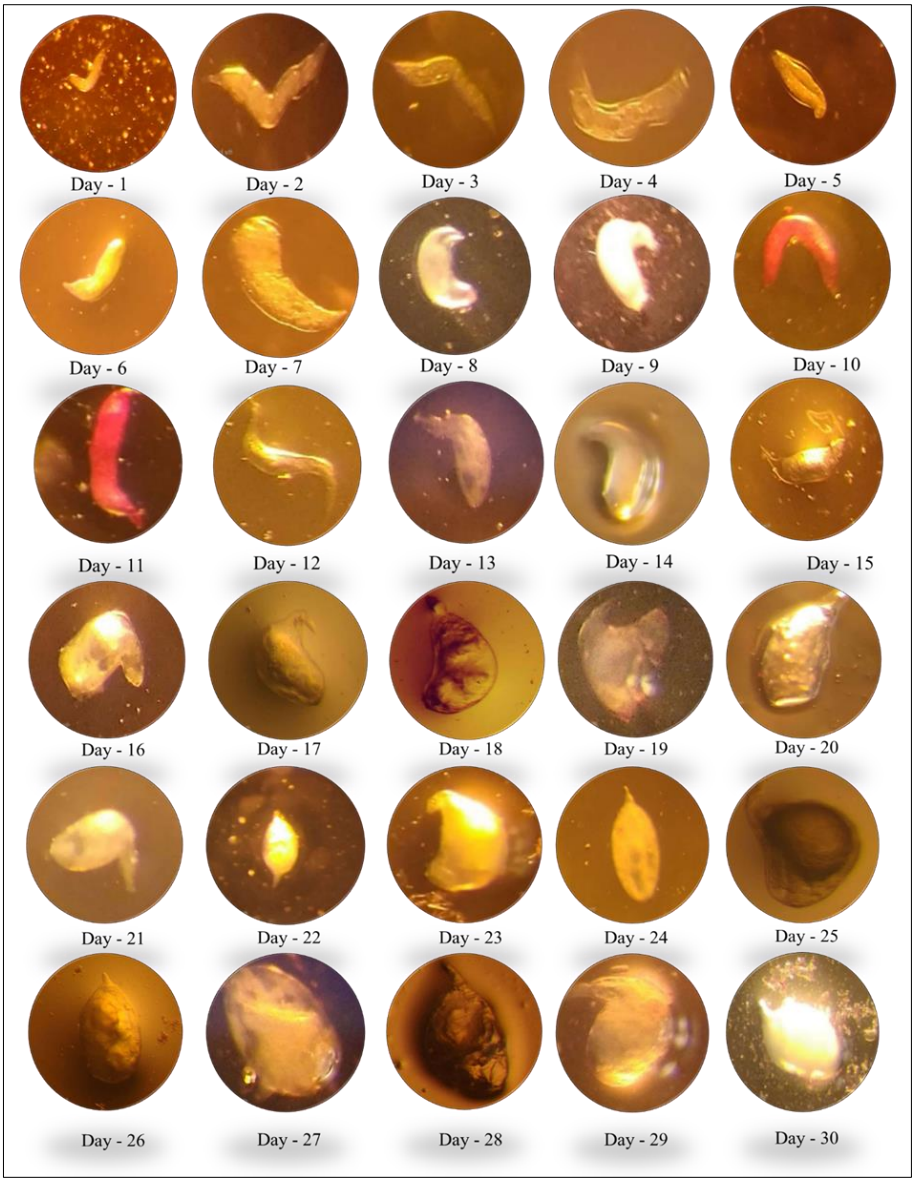
Life cycle of *M. graminicola* on rice (Pusa Bhagwati)

Life cycle of rice root-knot nematode, *M. graminicola* in rice, variety - "Pusa Bhagwati", was studied. All stages of the nematodes were recorded during the study(Fig. 2). Second stage juveniles (J_2) were found to penetrate the root surface within 24 hours of inoculation. Gall formation was initiated after 7-9 days. Size of the J_2 increased for every 24 hours and moulting of the J_2 occurred on the 7th day and third stage juvenile (J_3) appeared on the 8th day. J_3/J_4 are found on the 14th day, revealed clear appearance of spike tail. J_3 and J_4 stages have no functional stylet, hence they do not feed and are enclosed in a cuticular sheath from the preceding J_2 stages. Preadult females are formed on the 20th day, flask shaped, posterior region of the body gradually increased in width. Formation of adult female was observed on the 23rd day. Adult female increased in size with spiked appearance on 25th day and eggs laid in the gelatinous matrix in the cortex very close to the epidermal surface. On 29th day, root tissues became enlarged enough to contain the egg mass around the nematode. Each egg mass had about 250-300 eggs embedded inside the root tissues. On 30th day, adult female with egg mass, was seen. The life cycle of *M. graminicola* on *Oryza sativa* (Pusa Bhagwati) was completed in 30 days at 25± 2 °C. These results are in line with the findings of Narasimhamurthy *et al.* in 2018 [9] where they reported duration of second stage juvenile as 1-7 days and duration of advanced stage juveniles as 8-19 days. Emergence of adult female and adult male was on 23rd and 26th day respectively. The total life of *M. graminicola* was finally found to be 30 days. *M. graminicola* can reproduce through amphimixis as well as meiotic parthenogenesis completes its life cycle in 26-51 days in different periods of the year. In Bangladesh, its life cycle was completed in 19 days at 22-29 ºC (Bridge and Page, 1982) [1]. It was reported that *M. graminicola* can complete its life cycle in 15-19 days (Dabur *et al.*, 2004) [2]. The life cycle of *M. graminicola* takes 24 days for its completion, according to Singh *et al*. (2006) [12]. Hence, this indicates that to decide the length of the life cycle it depends upon the temperature.

Table 1: Duration of different stages in the life cycle of rice-root knot nematode *Meloidogyne graminicola* (at ambient temperature 25 ± 2 °C)

Developmental stage	Day	Remarks
Second Stage Juvenile (J_2)	1	Second stage infective juveniles (J_2) penetrated the roots behind root tip
	3	J_2 feeding the roots and gradually increase in size
	7	Moulting of the second stage juvenile started
Advanced Juvenile stages (J_3/J_4)	8	Appearance of third stage juvenile (J_2) with continuous feeding
	10	Body size increased and spike tail stage begins to appear
	13	Size of the advanced stage juvenile increased
	14	Spiked stage was prominent
	15	Size of the young females increased
	16	Young females with spiked appearance
	17	Body size increased, young female with spiked appearance
	18	Young female with clear spiked appearance
	19	Moulting of the advanced stage juvenile
Preadult Females	20	Developing females became typical flask shaped
	21	the posterior region of the body increased in width
	22	Body size of the female increased in width
Females	23	Formation of adult female
	24	Adult female with spiked appearance
	25	The posterior end of the females embedded in the cortex and eggs are laid very close to the epidermis in gelatinous matrix in the cortex
	26	Adult Males along with females in the galls
	30	Adult female with egg masses, each egg mass with 250-300 eggs

Fig 1: *M. graminicola* infected rice roots showing terminal hook shaped galls used for inoculum.



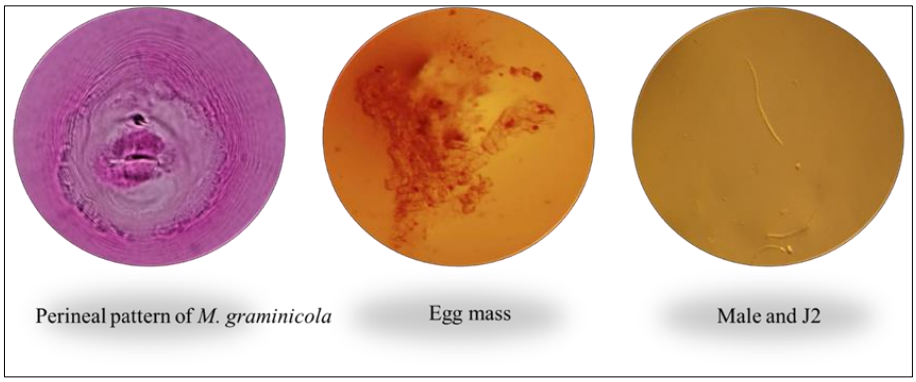


Fig 2: Different life cycle stages of *Meloidogyne graminicola* in rice

Conclusion

The life cycle of *Meloidogyne graminicola* in rice is completed in 30 days at temperature 25 ± 2 °C. The duration of second, advanced juveniles (J_3/J_4), preadult female and adult female stages lasted for 1-7, 8-19, 20-22 and 23-30 days respectively.

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