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Study on infection and development of Root-Knot Nematode, *Meloidogyne javanica* on Mungbean

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Abstract
Mungbean root-knot nematode (*Meloidogyne javanica*) has emerged as a major threat throughout the world and it has occupied a place of 'National Pest' owing to its severity. This is a major constraint in successful mungbean cultivation leading to the significant loss to the pulse grower. The present study was carried to aware of the biology and life cycle of *Meloidogyne javanica* under glasshouse condition at Department of Plant Pathology, G. B. Pant University of Agriculture & Technology, Pantnagar by using cultivar "Pant mung-8". The results revealed that in life cycle of *Meloidogyne javanica* one generation of nematode could be completed in 30-34 days. In sown plants, second stage juveniles (J2) readily penetrated roots, developed to adult females in 20 days and started laying eggs in 24 days. The details of life cycle are as follows: penetration (24 hours), gall formation (48 hours), J2 moulting (9th day), J3 stage (10th or 11th day), J3 moulting (15th day), J4 stage (17th day), J4 moulting (18-24th day), adult male (19th day), adult female (20th day), egg laying (24th day), gelatinous matrix (27th day) and J2 juveniles (30th day) were released.

Keywords: *Meloidogyne javanica,* root-knot nematode, life cycle, mungbean, *Vigna radiata*

Introduction
Root-knot nematodes (*Meloidogyne* spp.) are believed to be damaging approximately 50% of the global food grain production and thus, have been regarded as one of the most destructive plant pathogens (Sasser and Freckman, 1987) [1]. These soil borne pathogens cause the formation of root galls, resulting in the reductions of growth by troubling the nutrient and water uptake, increased wilting, mineral deficiency and poor yield of diseased plant (Abad *et al*., 2003) [2]. *Meloidogyne javanica* is one of the important pests among *Meloidogyne* genus attacking a wide host range of leguminous crops and cultivars (Bernard *et al.,* 1990 [3]; Saka, 1990 [4]; Devi and Kumari, 2014 [5]) and significantly reduce the yield of leguminous crops grown in infested soils. The pathogenic effect of root-knot nematodes on growth parameters, yield and nutrient uptake of leguminous crops have been reported by several workers and it is documented as a potential threat to various leguminous plants (Gupta *et al.,* 1986 [6]; Bhagawati and Phukan, 1991 [7]; Kalita and Phukan, 1993 [8]). Yield losses in different pulse crops caused by root-knot nematodes may range from, 13-16 per cent in mung bean (Khan *et al.,* 2002 [9]). The mung bean (mung or green gram or golden gram) is the seed of *Vigna radiata* (L.) R. Wilczek. Mungbean an important pulse crop, is used as vegetable, fodder and grains. It is severely affected by root-knot nematode, *Meloidogyne* spp. (Hussaini and Seshadri, 1975 [10]). Being an important pulse crop attacked by nematode it is important to study the life cycle of the pathogen and interaction with host and environmental conditions. Therefore an experiment was conducted to know the biology and life cycle of mungbean root-knot nematode (*Meloidogyne javanica*) infesting mungbean in glasshouse condition.

Materials and Methods
Identification of *Meloidogyne javanica*
Roots of mung bean infected with root knot nematode were recovered from field which were washed and stained with acid fuchsin lactophenol solution. After cooling upto normal temperature,the rootswere washed with runningwater; theserootswerekept in plain lactophenol solution overnight fordestaining. Rootswere dissectedunder stereomicroscope and adult female nematodes were takenoutand placed in lactophenol. The perinealsectionswere examined under the microscope, 15 perineal patterns were examined and compared with description given by Seinhorst (1966) [11] and Eisenback (1985) [12]. All specimen were found to be *M. javanica.*

Study of biology and life cycle of *Meloidogyne javanica*

The development and life cycle of *M. javanica* was studied in earthen pots in glasshouse of the Department of Plant Pathology, College of Agriculture, G.B.P.U.A.T, Pantnagar. A population of *Meloidogyne javanica,* isolated from mung bean was propagated on tomato, *Lycopersicon esculentum* (L.) Mill. Earthen pots of 15 cm diameter were filled with previously sterilized soil, five surface sterilized mung bean seeds of Pant mung-8 were sown in each pot. After 10 days, the seedlings were thinned to one per pot.

Roots with egg masses were removed from the soil two months after infestation of the soil with this nematode and then the eggs were extracted by sodium hypochlorite method as described by Hussy and Barker (1973) [13]. The newly emerged J2 were collected 24 hours later and concentrated in aliquots of 100 J2 in 5 ml aqueous suspensions. Each aliquot was poured around the root zone of 10 days old seedlings and watered, as and when required at saturation level. The pots were then kept for 1 day for penetration and then the plants were carefully removed from the pots, roots were thoroughly washed with tap water to free of soil and remove nematodes that have not penetrated.

The seedlings were transplanted in 15 cm pots containing sterilized sand and placed in the greenhouse (15-30 °C). Two plants were daily extracted and stained in acid fuchsin. Nematodes and root penetrated by nematode larvae were examined by dissecting them from the plant tissue and mounting them in glycerine on glass slides and observing under stereomicroscope. The occurrence of each moult was used to delineate the time periods for each development stage (Eisenback, 1985) [12]. This procedure was continued until the life cycle was completed, i.e., until release of second stage juveniles from newly laid eggs by the females. Twenty five nematodes at each stage were measured and a mean value was considered in table.

Results

Isolation and Identification of the root- knot nematode (*M. javanica*)

Affected plants of mung bean with root knot nematode were collected from field and brought to laboratory for isolation and identification of nematode. The nematode were isolated as per the technique described in materials and methods, and nematode were morphologically characterized for identification. The morphological characters observed are as follows-

Female: *M. javanica* female body is nearly spherical in adult with projecting neck tapering to head, posterior end rounded or with slightly protruding perineal region. Head with one annule behind head cap, slightly wider than first body annule. Stylet is slender, dorsally curved and knobs are rounded. Posterior cuticular pattern (perineal pattern) round or oval to pear-shaped, dorsal arch varying from rounded, to moderate height, sometimes dorsally flattened. Striae smooth to wavy, tail tip is often marked by an irregular whorl. Typically lateral fields are well defined by a double incisures bordering a definite break in the striae and clearly dividing pattern into dorsal and ventral sectors. Lateral fields may be visible for some distance from tail, but do not extend into the neck region or may be poorly marked. Phasmids are usually distinct (Fig.1 B, C).

Male: *M. javanica* male has vermiform body, Head region is not offset from body. Outline in lateral view is characteristic with first annule projecting antero-laterally, that is well separated by constriction from a wider basal annule which may be subdivided into two annules either completely or partially giving the appearance of one annule on one side of the body and two on the other (Fig.1 A). In dorsal or ventral view head outline rounded, amphidial apertures are distinct. Stylet knobs are rounded, not prominent. Excretory pore distinct, duct visible for some way along body (Fig.1 A). One testis or two, Intersexes common, displaying female characters to varying degrees ranging from a slight swelling anterior to cloaca to possession of a well-developed vulva. Tail shape is somewhat variable, digitate in lateral view, bluntly rounded in ventral view. Terminus is not clearly striated. Phasmids located in lateral field at cloacal level or slightly anterior. Spicules are slightly curved, with ventral flange distally and tips pointed.

Juvenile(J2): Second stage juveniles of *M. javanica* head is not offset, lateral view truncate cone shape with 3 annules behind head cap and more rounded in dorsal and ventral view with one annule behind the head cap. Stylet knobs are rounded, not prominent. Rectum inflated; tail tapering to sub-acute or finely rounded terminus and annules coarsening posteriorly (Fig.1 D).

Study of life cycle of root knot nematode (*M. javanica*) in mung bean

Microscopic observation of stained roots of mung bean revealed the gross morphology of infected root tissues and different developmental stages of *M. javanica* (Figure 2 and Figure 3). One day after inoculation, infective second stage juveniles were found penetrating the root at or near the root tip (Fig.3-A), Larvae passes through the root tissues intercellular and intracellularly. Direct juvenile penetration in the epidermis and cortex region make it distorted and killed nearby cells. After 2 days, second stage juveniles were observed feeding on vesicular region and cortical tissues and many juveniles were observed feeding on a single root tip (Fig.3 A-C). Juveniles were oriented in various directions, but more were arranged parallel to the longitudinal axis of the root with their posterior ends extended towards the root tip. However, the visible swelling on the infected root was observed on second day (48 hours) after inoculation (Fig.3-E). The mean length and width of the second stage juveniles which were used in inoculation of mung bean plant roots were 387 µm and 17 µm respectively. The length width measurement of J2 one day after inoculation which were penetrated, oriented in vesicular region and caused visible swelling on the roots of mung bean were 464 µm and 23 µm respectively (Table 1). From third day after inoculation, the juveniles had started to increase in size and this was very noticeable on the eighth day. The developing juvenile were now fusiform (swollen) in shape. The increase in size continued on 9th day, some of the juveniles were seen in second moulting stage (Fig.2-D). However, the larvae length were decreased and width were increased. On 10th/11th day after inoculation J2 stage of developing juvenile turned into J3 stage (spike-tail stage) after second moulting (Fig.2-E) which can be seen inside root (Fig.3-F). The measurement of the developing juveniles and third stage on 10th and 11th day after the inoculation are given in Table 1.

On 12th day after inoculation the sex differentiation start in juveniles, 7 juveniles were seen in third stage, 14 were in the

process of moulting and 4 remained in the early stage of 2nd stage. The earliest moulting of third stage was observed on 15th day, which indicated that this stage was completed in four days. Two types of developing juveniles were seen at this stage, one type with rounded posterior region which would eventually become females (Fig.2-F) and, other fusiform in appearance with spiked tail which would develop into adult male (Fig.3-G). On 16th/17th day after inoculation, J3 stage of developing juvenile turned into J4 stage after third moulting and the stylet were reformed and large median bulb can be seen in J4 and the length and width were also increased. The measurement of developing J4 on 16th day given in Table 1.

The fourth moult occurred on 18th day and was continued up to 24th day, the young male and female were formed (Fig.2 L, H). The development of the male could not be monitored easily because only few adult male were observed throughout the duration of the experiment apart from the male fourth stage juvenile was observed on 18th day after inoculation. The male nematodes were seen around mung bean roots 33 day after inoculation. It had well developed stylet with knobs and spicules were posteriorly located. It measured 1160 µm in length and 37 µm in width (Fig.2 L and Table 1).

On 20th day after inoculation, the J4 stage of developing juvenile turned into adult female, after fourth moulting. In the J4 stage, the progression from juvenile to globose adult females (Fig.2-H) becomes clearly visible and grow rapidly between 24-30 days of inoculation (Fig.3 G-H). The stylet of adult female was well developed indicating the parasitic nature of the nematode. During this period the width of adult female increased further and there was quick expansion of the female body (Table 1). The head of young female nematode was observed to be attached to the stele of the root and other part of the body were observed in the cortex.

The females started egg laying on 24th day and continue egg laying for several days. A single female nematode can produce 100-200 eggs embedded in gelatinous matrix (Fig.3 I-J). The gelatinous matrix formation starts from 27 days and continues up to 30 days while egg deposition continues up to 30-40 days of inoculation. The second stage juvenile was seen on 30th day after inoculation, which indicated the completion of life cycle of nematode.

Table 1: Development of life cycle of *Meloidogyne javanica.*

Days after inoculation	Stages	Measurement (µm) n = 25						Ratio Length: Width
		Length			Width			
		Min.	Max.	Avg.	Min.	Max.	Avg.	
0	II Stage juveniles just after hatching	348	393	387	14	19	17	22.67
2	II Stage juveniles	351	401	386	21	25	23	16.78
10	III Stage juveniles	438	484	464	61	79	68	6.82
11	III Stage juveniles	453	499	470	78	99	90.39	5.22
16	IV Stage juveniles	472	530	497	90	114	104	4.78
18	Male	1042	1352	1160	35	38	37	31.35
20	Female	722	923	829	482	573	543	1.53

Discussion

The morphological characters observed in our study of female, male and second stage larvae are very similar to *M. javanica*, characterized by (Orton, 1972) [14]. During present investigation the temperature at glasshouse was ranging between 15.1-31.89 ºC. Observation on life cycle of *M. javanica* revealed that the root knot nematode could complete its life cycle i.e. from juvenile stage (J2) to second generation juvenile stage (J2) within 30-34 days, in which temperature plays an important role in completing the life cycle. Any increase or decrease in range of optimum temperature (25-30 ºC) can increase or decrease the duration of life cycle.

The present findings are in agreement with Bird (1959) [15] who observed that growth of *M. javanica* larvae in tomatoes was slow at first, but after about 14 days (after second moulting), juveniles get thickened and possess the spiked tail characteristic of *Meloidogyne* Spp. The third and fourth moults rapidly followed within the moulted cuticle of the second-stage juvenile. Under optimum conditions, by the 19th day all juveniles were in the final moult or were adults. Egg deposition began between the 20th and 27th days, followed 2 days later by egg laying. Egg deposition increased rapidly from the 30th to 40th day, the egg sac becomes usually bigger than the female which produced it. However, Khan and Ashraf (2006) [16] reported that *M. javanica* required 29 days to complete its life cycle on lettuce at a temperature ranging between 18-25°C. The *M. javanica* required 27 days to complete the life cycle on broccoli at a temperature range of 28–35°C (Khan *et al.,* 2010) [17].

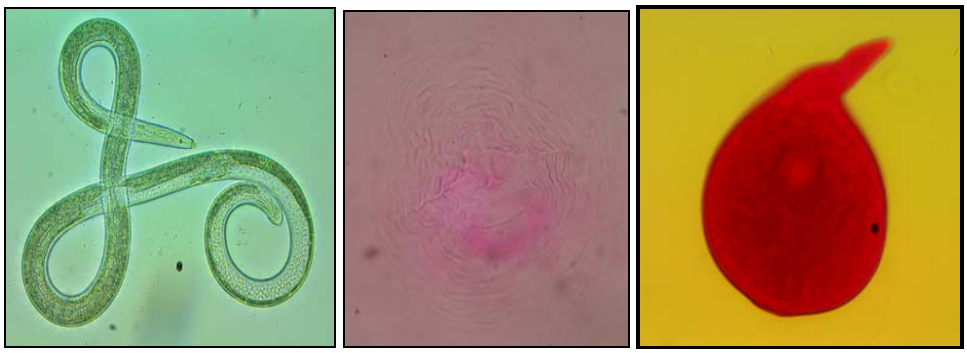

Fig 1: (A) Adult Male, (B) Perineal Pattern of female, (C) Female, (D) Second Stage Juvenile

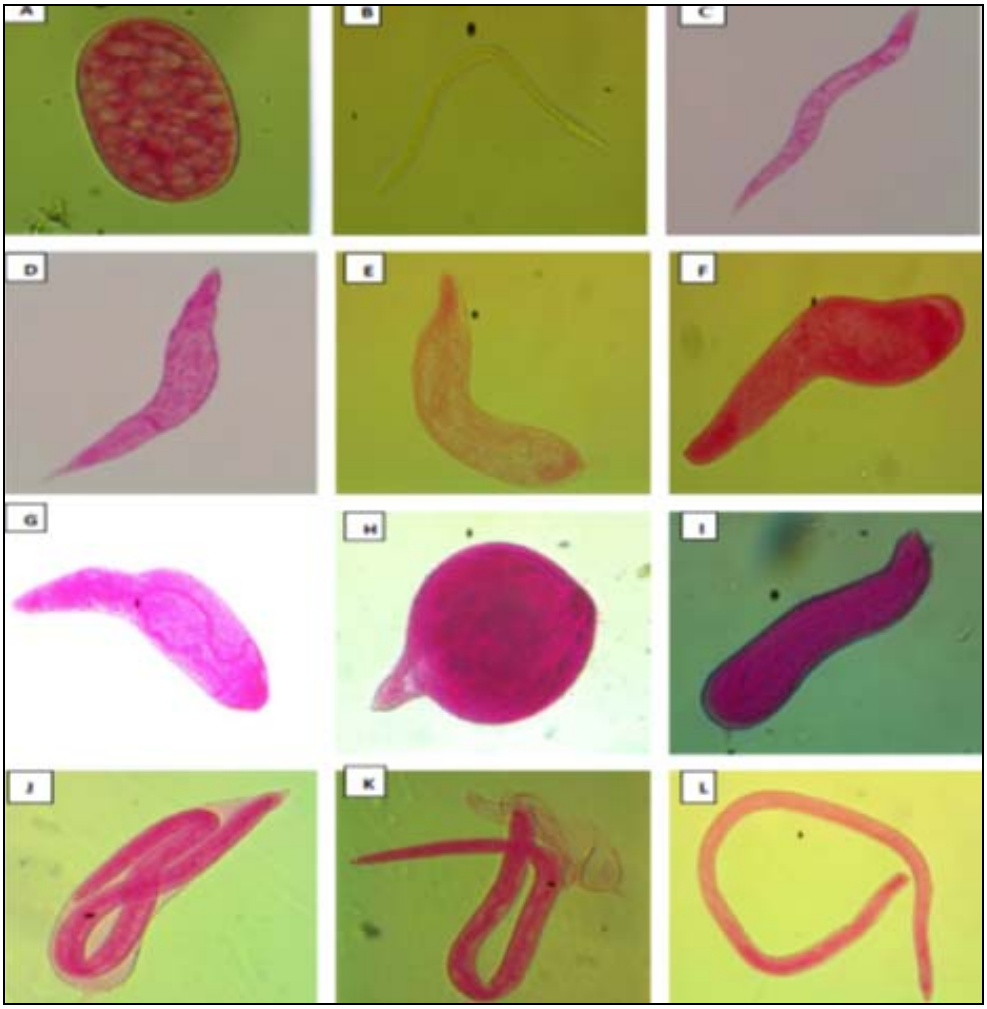

Fig 2: Developmental stages of *M. javanica* in mung bean seedlings (A) Egg; (B) J2 before inoculation; (C) Developing J2 after 2 days of inoculation; (D) J2 moulting after 7 days; (E) J3 developing spike tail stage; (F) Developing J4; (G) Development of sex; (H) Mature female; (I-J) Developing Male; (L) Mature male.

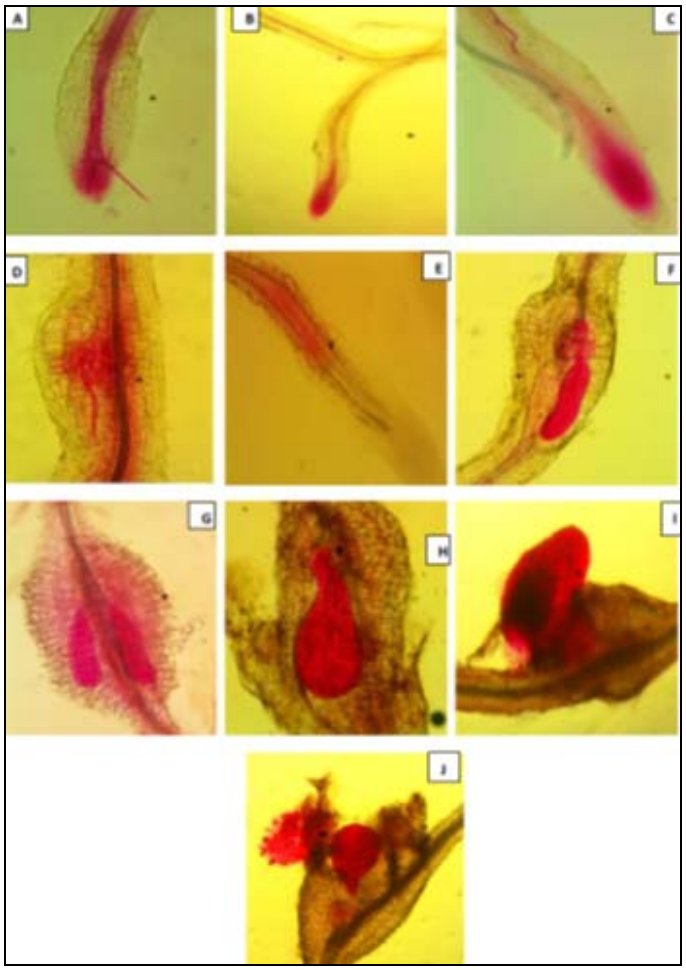

Fig 3: Developmental stages of *M. javanica* in mung bean seedlings. (A) Penetration of J2 in the root near root tip; (B-C) Migration of J2 through cortex ; (D) Sedentary stage J2; (E) Slight swelling of the root; (F) Spike tail stage of larvae; (G) Adult female and male; (H) Fully grown male within root; (I) Gelatinous matrix with eggs and female; (J) Egg laying female with egg mass.

Fig 4: Infected Root of Mungbean

Conclusion

It can be concluded from the study that temperature can be very helpful in managing the root-knot nematode without much use of agrochemicals. Optimum temperature for growth favours rate of multiplication of nematodes, whereas unfavourable temperature slow down or inhibit rate of multiplication. Hence, following the principle of avoidance the crop can escape from high nematode infestation and multiplication resulting in yield loss.

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