



Research Article

LIFE HISTORY AND PATHOGENICITY OF RENIFORM NEMATODE ON COTTON VAR. MCU 5

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Abstract: The life cycle of reniform nematode, *Rotylenchulus reniformis* was completed in 17-23 days from egg to egg stage in cotton (MCU 5). Pre and post infection life cycle were completed in 7 days and 12 days respectively. Immature females-initiated infection by protruding the anterior portion of the body through epidermis and feed the phloem tissue of younger roots. Eggs were laid on root surface in gelatinous matrix. The maximum numbers of eggs per egg mass observed were 83-90. A pot experiment for studying the pathogenicity of *R. reniformis* showed negative correlation between inoculum level and plant growth but the nematode inoculum level and nematode reproduction showed positive correlation.

Keywords: Reniform nematode, Biology, Pathogenicity, Cotton

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Introduction

Reniform nematode is an important semi endo parasite that has a wide host range next to root knot nematode affecting economically valuable crops in sub-tropical and tropical areas. Reniform nematode, *R. reniformis* was first reported from pineapple field on the roots of cowpea in Hawaii [8]. In India, found this nematode firstly on coffee roots [10]. Cotton is a favoured host of *R. reniformis* and usually high populations of the nematode are found associated with the poor growth of plants. The nematode penetrates through the epidermis and cortex then feeds on phloem. The present investigation was undertaken to study the pre and post infection development, Histopathology and pathogenicity of nematode infection under Indian conditions considering the value of the crop and paucity of information.

Materials and Methods

The life history and pathogenicity studies were carried out in pot culture experiments under glass house conditions at Department of Nematology, Tamil Nadu Agricultural University, Coimbatore on cotton var. MCU 5.

Life history

Pre infection life cycle

The pre infection life cycle of *R. reniformis* were studied by detaching the egg mass from roots of cotton (MCU 5) maintained as pure culture in earthen pots and transferred into cavity block. Single egg was placed in a cavity slide containing a drop of distilled water, placed under compound microscope to observe the embryonic development using "Hanging drop technique". Each stage was pictured in image analyzer at hourly basis for the first day and thereafter at 6 hours interval till the eggs was hatched. Number of eggs per egg mass was counted by teasing the matrix under stereo zoom microscope.

Post embryonic life cycle

The fifteen days old cotton plants were inoculated with 100 infective stage

juveniles (J4) of *R. reniformis* in the ratio of 1:1 (male : female). The plants were gently uprooted at the intervals of 2,3,4,5,10,12,15 and 25 days of inoculation and the roots were stained using acid fuchsin lactophenol solution. The stained roots were observed under microscope for penetration and development of *R. reniformis*.

Histopathology

The cotton roots both healthy and reniform nematode infested roots were collected from potted plants and fixed in FAA followed by processing in tertiary butyl alcohol method [6]. Then infiltrated and embedded in paraffin wax. Transverse sections of roots were cut with a hand rotary microtome at 10-15µ thickness and stained with safranin aniline blue.

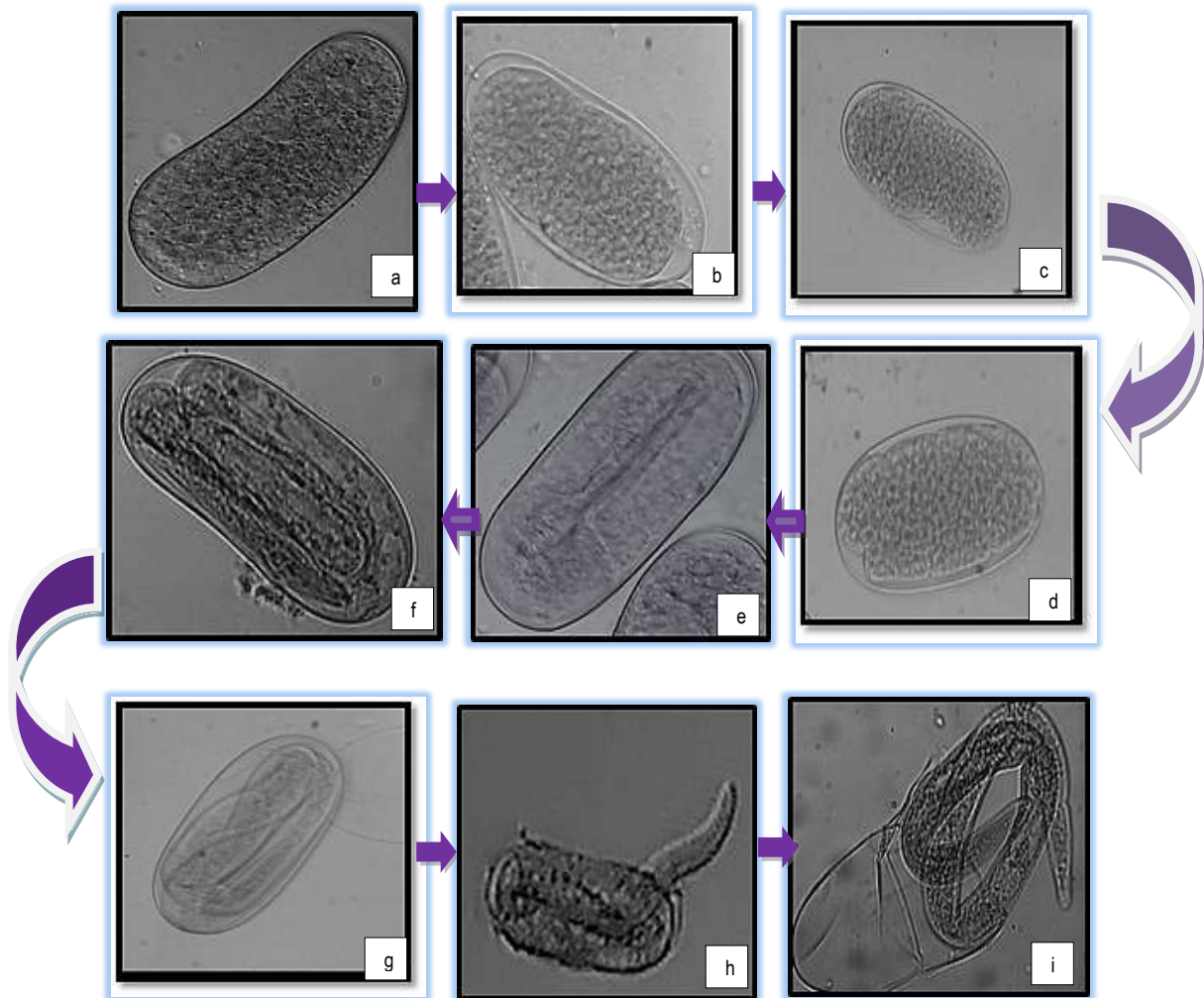
Pathogenicity

To establish pathogenicity of *R. reniformis* in cotton (MCU 5), Five kg sterile earthen pots containing 15 days old plants in a sterile pot mixture were inoculated with J4 stage of *R. reniformis* as per treatment schedule comprising 500,1000,2000,4000,8000 juveniles in 10ml suspension through 3cm deep near the rhizosphere. In addition, control plants were maintained for comparison. Pots were arranged in randomized block design with four replications. After 90 days the experiment was terminated by recording the observations on various growth characters of cotton and nematode reproduction characters.

Results

Embryonic development [Table-1]

The nematode lays single celled or rarely two celled eggs measuring 85-90µ. Single cell stage lasts for only 1-2 hours then divides transversely to form two equal blastomeres, anterior S1 and posterior P1, with a polar body. The second transverse division takes place for 3-4 hours. Anterior cell divides into two giving rises to anterior and posterior cell as B and A respectively.

Fig-1 Embryonic development stages in the eggs of *R. reniformis*

a. Undifferentiated cell b. Two cell stage c. Four cell stage d. Eight celled stage e. Blastula f. Gastrula g. J1 inside the egg h. Initiation of hatching i. Hatched of J2

The third division splits P1 into P2 and S2 transversely and embryo consists of four blastomeres in 7 hours. The anterior most cells B then undergoes a longitudinal division giving rise to fifth cell. The embryo reaches the eighth cell stage in about 24 hours.

Table-1 The embryonic developmental stages of *R. reniformis* eggs

Embryonic developmental stages	Time taken (hrs/day)
2 celled stage	2-4
4 celled stage	6-8
8 celled stage	18-20
Blastula	20-30
Gastrulation	26-36
Vermiform	36-40
J ₁ stage	42-48
Emergence of J ₂	72-96
Period	72-96 hrs

The embryo reaches "tadpole stage" in about 2 days then the elongation process starts from both anterior and posterior ends forming a vermiform body in about 4 days this forms the first stage juvenile which moves within the eggshell. The first moult takes place in about 24 hours i.e., 7 days after laying undifferentiated egg cell. Moulded cuticle can be seen through eggshell and artificially [4]. The stylet of second stage juvenile was seen after 2-3 days showing four folds inside the egg. The fully developed second stage juvenile is very active and the pressure exerted on the egg wall by vigorous movement the shell breaks freeing out the juvenile as shown in [Fig-1].

Post embryonic development

The measurements of different life stages were tabulated [Table-1]

Second stage juvenile

The second stage juvenile is vermiform distinguished by truncate head with well-developed stomato stylet (12-16µ long) with knobs oesophageal glands overlapping the intestine laterally. Tail generally tapering with round terminus. Immediately after hatching second stage juvenile observed did not show any morphological features to sex separation. The juvenile remains active for 3-4 days and then cessation of movement occurs prior to moulting. Firstly, the moulting process is indicated by dissolution of stylet followed by Oesophagus, median bulb and glands within 4-5 days after hatching juvenile reaches third stage.

Third stage juvenile

The third stage juvenile are inactive and enclosed inside the cuticle of second stage juvenile which is clearly observed in anterior region. The male and female can be easily distinguished by morphology.

Third stage female

Head region is truncate with an oral aperture pocketed in a small groove. Basal plates are represented by thickening on either side of oesophagus. Stylet is not distinct. In oesophagus, the corpus is expanded in the anterior end with a slightly swelled median bulb. Isthmus is not distinct. Glands overlap the intestine laterally. Tail is bluntly pointed. Genital primordium is broad at the centre and tapers towards both the ends. Moulting occurs in 2-3 days.

Third stage male

Except genital primordium and tail shape, all morphological features resemble the female. The genital primordium is 30-40 µ. Tail is broader holding the cloacal region.

Fourth stage juvenile

The fourth stage is also inactive and enclosed inside the moulted skin of second and third stage juvenile.

Fourth stage female

Head truncate without any sclerotization and stylet. The ovaries are branched and outstretched with vaginal canal partitioning the middle. The juvenile moults after 3-4 days to become an immature female.

Fourth stage male

The genital primordial is elongated and has series of cells. In the tail region a minute sclerotized structure is seen showing a pair of spicules supported by gubernaculum. After 3-4 days fourth stage male moults and becomes adult.

Immature female

Immature female is the infective stage and is very active. Sclerotized head with well-developed robust stylet, oesophagus well developed which ventrally overlaps the intestine. Tail tapers towards the end.

Immature male

Males are not parasitic resides in soil. At the posterior region possess a pair of spicules and a bursa. Tail tapers towards the end.



Fig-2 Post embryonic developmental - Pre-infection stages

a. Hatched juvenile J2, b. J3 stage, c. J4 stage/pre adult, d. J4 stage with retention of previous stage cuticle

Post infection development [Fig-3]

Immature females start infecting the roots of cotton with two days of inoculation. Immediately after infection the area around the vulval region starts swelling and infection occurs. The ovary starts elongating continuously forming a horse shoe shaped on alongside of vagina. The body of female enlarges and acquires a typical kidney shape in 3 days with a slender neck and starts laying eggs on 4th day. The experiment on cotton used as host, an average of 83-90 eggs were found per egg mass, egg laying was completed in about 3-4 days and completes its life cycle in 17-23 days [Table-2].

Table-2 The duration of different life stages of *R. reniformis* on cotton

SN	Stages	Duration (days)	
I	Embryonic development	3 days	
1.	Single celled egg to second stage juveniles	3 days	
II	Pre-infection development	Females	Male
1.	Second stage juveniles	2-3 days	2-3 days
2.	Third stage juveniles	3-4 days	1-2 days
3.	Fourth stage juveniles	3-4 days	2-3 days
		8-11 days	6-8 days
III	Post -infection development		
1.	Swollen female	3-4 days	
2.	Fully developed female (reniform shaped)	3-4 days	
3.	Egg laying	2-4 days	
	Period	8-12 days	

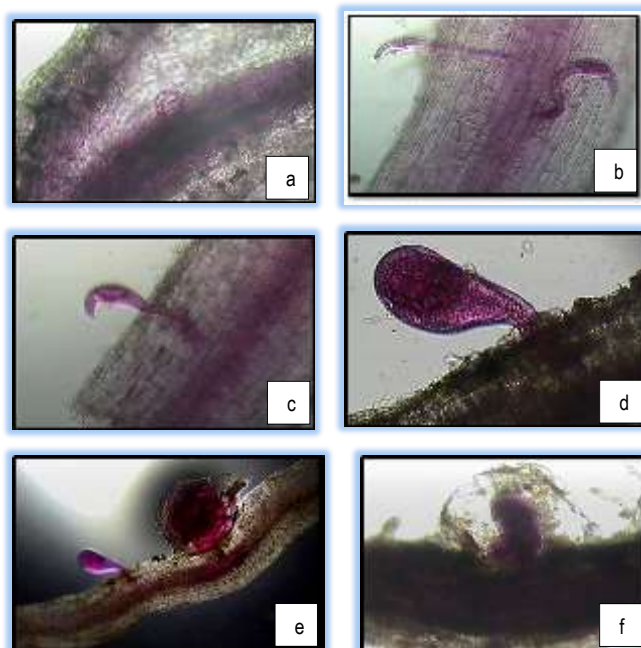


Fig-3 Post embryonic development of reniform nematode Post- infection development

a: Penetration of J4/ pre-adult into cotton roots after 48 hrs of inoculation
b: Slight swelling of juvenile in the vulval region after 3 days of inoculation (DAI)
c: Development of the adult female after 5DAI
d: Formation of reniform shape of the females after 7DAI
e: Secretion of gelatinous matrix through vulva after 10DAI
f: Egg laying into the gelatinous matrix after 12DAI

Histopathological observation

Histopathological studies show that the immature females initiate infection by protruding the anterior region of the nematode body into the root through the epidermis via the cortical parenchyma to feed the phloem tissues of young roots. The infested tissues near the head region stained darker than uninfested tissues. Xylem was seen untouched. The sequence of changes in root tissue can be clearly observed from sections of infested root. The hollow part forming a passage from epidermis to phloem shows the presence of nematode and proliferated cell close to the feeding site stained darker and showed cell wall thickenings shown in [Fig-4].

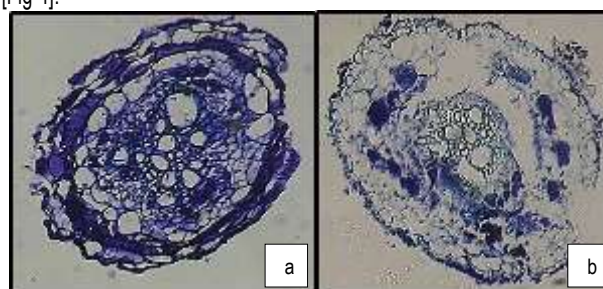


Fig-4 Cross section of cotton roots.

Transverse section of young healthy root with compact cortical cells.
Transverse section of young infested root with hollow cortical region.

Pathogenicity test

After six weeks of inoculation, effect of *R. reniformis* on plant growth were visible in infested plants inoculated with 4000 and 8000 J4. In the advanced stage of infestation, leaves started drying in the margins. The leaf tips turned brown dried and become brittle. The drying spread to entire leaf and eventually shedding of leaves. The uninoculated plants and those inoculated with 500 and 1000 (J4) nematodes produced more side shoots from the main stem in 100 days old plants. In contrast the plants inoculated with 2000 nematodes were devoid of leaves at this stage.

The flowers produced were shed and bolls produced were very smaller in size. The flowering started earlier in control than inoculated plants. The data on plant growth and inoculum density were presented [Fig-5].

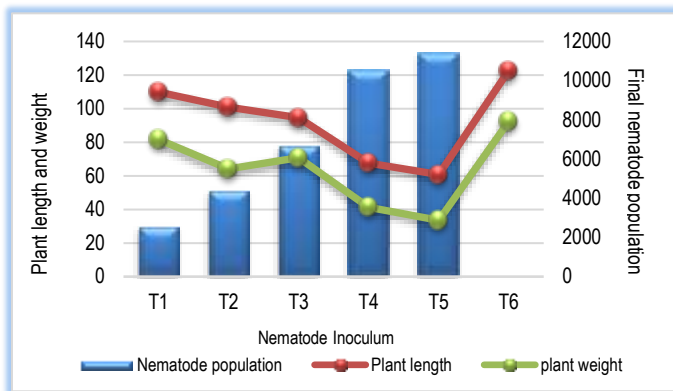


Fig-5 Pathogenicity of reniform nematode on growth parameters of cotton. The relationship between initial nematode inoculum and plant length, number of bolls, plant weight was statistically significant. The final population in treatment is given in [Fig-5] was highest in plants inoculated with 8000 (J4) nematodes and lowest in plants inoculated with 500 (J4) nematodes. A significant linear relationship was found between initial and final population.

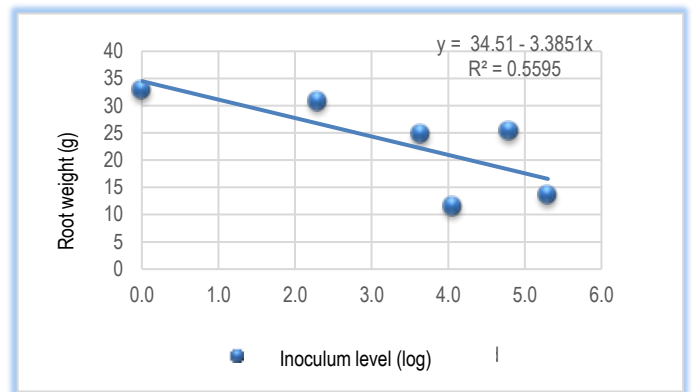
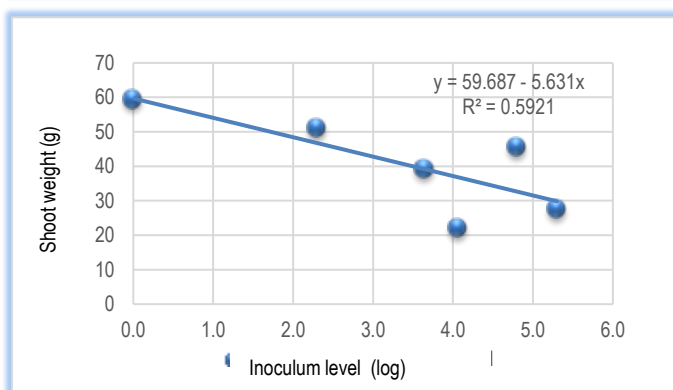
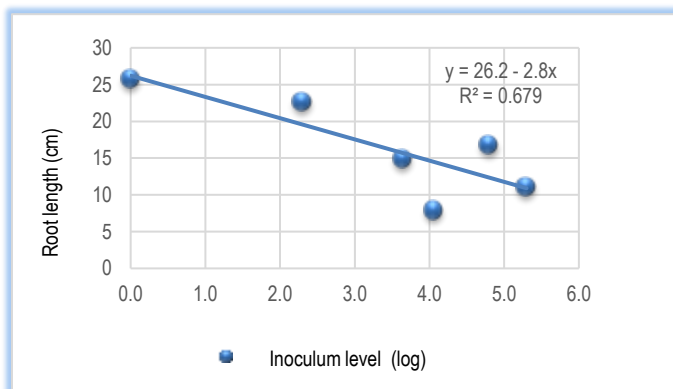
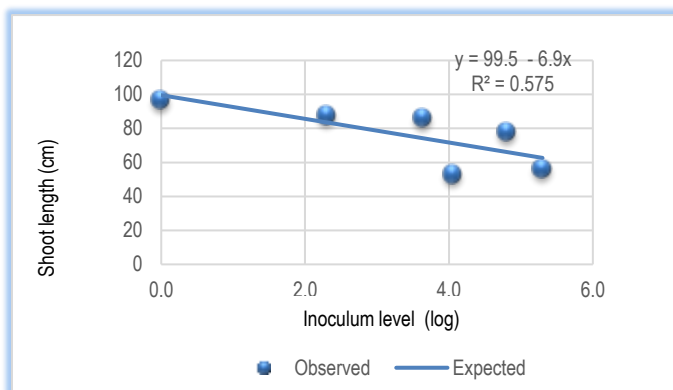


Fig-6 Effect of reniform nematode, *R. reniformis* inoculum levels on plant growth parameters of cotton variety MCU 5

Regression analysis was carried for growth parameters and the equation were as follows

Shoot length $Y = 99.5 - 6.9x$

Shoot weight $Y = 59.6 - 5.6x$

Root length $Y = 26.2 - 2.8x$

Root weight $Y = 34.5 - 3.3x$

Regression line of [Fig-6] shows that there was a negative correlation between plant growth and increasing nematode inoculum levels.

Discussion

The results on life history of *R. reniformis* in the study agree with the findings [8, 9]. Studies confirmed that this nematode could complete its post embryonic development in water and develops into infective females or adult males without feeding. Differentiation of male and female were seen in third juvenile stage and was described [8]. The enlarged tail with a minute projection is very significant in third stage males of reniform nematode and has not been reported in other nematode genera. The histopathological studies have shown that the *R. reniformis* mainly feeds on phloem tissues of young cotton seedlings. The infected tissues at infection site shows hypertrophy, hyperplasia, thickening of cell walls, enlarged nuclei and nucleoli. These changes were brought about by many plant parasites on their host [3,12]. *R. reniformis* is a cortical feeder in cowpea [8] and in contrast, feeding on phloem of cotton roots [1]. The observations on pathogenicity showed that the nematode *R. reniformis* was found highly pathogenic to cotton var. MCU 5. The results on plant growth characters of infested plants are reliable [2, 11] and studied pathogenicity of *R. reniformis* on different hosts [5]. In the present study, shedding of leaves after drying and showed delayed flowering and reduced boll size and this result agrees with the findings [7] in cotton the quality of lint and fibre gets deteriorated due to nematode infestation.

Application of research: Studies related to pathogenicity and efficacy of reniform nematode in cotton.

Research Category: Nematology

Abbreviations: J4: Infective juveniles, J2: Second stage juveniles

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Author Contributions: All authors equally contributed

Study area / Sample Collection: Department of Nematology, Tamil Nadu
Agricultural University, Coimbatore

Cultivar / Variety name: *Gossypium hirsutum* - MCU 5 (Cotton)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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