Plant parasitic nematodes associated with *Bt* cotton

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The present study on the plant nematodes associated with *Bt* cotton crop growth in northern Karnataka, India covered several aspects like a nematode random survey in *Bt* cotton-growing areas of the state, nematode community analysis, pathogenicity and biology of a dominant nematode and reaction of *Bt* cotton cultivars and hybrids to that nematode. A random survey undertaken in the *Bt* cotton-growing districts of northern Karnataka for the associated nematodes in cotton rhizosphere soil and root samples showed the presence of plant pathogenic nematodes, mainly reniform nematode (*Rotylenchulus reniformis*), lesion nematode (*Pratylenchus* sp.) and some dorylaimid pathogens. Other plant pathogenic species were present in less numbers. Community analysis showed that these nematodes were important in the cotton-growing areas surveyed. Pathogenic nature of reniform nematode on a *Bt* cotton cultivar was demonstrated in greenhouse studies. The nematode required 27–29 days for the completion of one generation under Dharwad conditions. Many inter- and intraspecific *hirsutum Bt*-hybrids were not found susceptible to the reniform nematode infection, but MRC-7918 and Tulsi-117 were found to be moderately susceptible.

Keywords: *Bt* cotton, nematodes, pathogenicity, resistance, *Rotylenchulus reniformis*.

INDIA has the largest area under cotton cultivation in the world, where more than 80% of cotton grown is *Bt*-cotton. Presently, Karnataka occupies about 5% acreage of *Bt*-cotton in our country. Nematodes in cotton have always been of concern to nematologists worldwide because of their potential damage inflicted upon the crop. As such, the annual cotton yield loss due to damage by plant parasitic nematodes on a worldwide basis is estimated[1] to be 10.7%. The earliest records of plant pathogenic nematodes on cotton in India are by Luthra and Vasudeva[2] in 1939 followed by Thirumalachar[3], who reported the occurrence of root knot nematode, *Meloidogyne* spp. and reniform nematode, *Rotylenchulus reniformis* on cotton. Rashmi and Lingaraju[4] brought out the role played by the latter in non-*Bt* cotton. As such, India loses cotton yield worth Rs 234.5 million every year[5]. Hitherto, no work has been carried out in India concerning plant nematodes associated with *Bt* cotton and their effect on plant/crop performance barring a study[6] on the effect of *Bt*-cotton genotypes on the life cycle of reniform nematode. There is a need to look into the role played by plant parasitic nematodes affecting *Bt* cotton in India.

A nematode random survey of the major *Bt* cotton-growing areas of North Karnataka (places/locations surveyed are presented in Table 1) was carried out by collecting soil and root samples for different plant parasitic nematodes associated with the crop. Samples were collected from rhizospheres of cotton crop at the flowering stage (110–120 days). Composite soil samples were collected with the help of a scoop from a depth of 15–20 cm from the soil surface. Each sample consisted of 10–15 soil cores. Root samples were also collected along with the soil. Samples were either analysed on the day of collection or after storing them for a couple of days under refrigerated conditions. For analysis, 200 cm^3 of soil was taken from each of the composite soil samples. Roots and root bits collected from the plants were observed for the presence of galls and lesions.

For the estimation of nematode population in the soil and community analysis, Cobb's sieving and decanting technique was followed: the soil (200 cm^3) was taken in a container and mixed thoroughly with water. Hard particles and stones, if any, were removed by stirring the suspension, and the soil was then passed through a set of sieves of 250, 45 and 37 μm pore size. The sievates were collected on a tissue paper spread over a wire gauze, which was then placed in a petri dish containing enough water. This assembly was kept still for three days; care was taken to prevent drying of the tissue paper. The nematode suspension collected in the petri dish was examined by means of a research stereo binocular microscope. Different plant parasitic nematodes present in the suspension were identified to the genus level. Their numbers present in the suspension were determined by taking the average number of nematodes present in five different 1 ml aliquots of nematode suspension. Ecological indices of different nematode species in the samples were calculated using the formulae of Norton[7]:

$$\text{Absolute frequency} = \frac{\left(\begin{array}{c}\text{Number of samples}\\ \text{containing a species}\end{array}\right)}{\left(\begin{array}{c}\text{Number of samples}\\ \text{collected}\end{array}\right)} \times 100.$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\left(\begin{array}{c}\text{Sum of frequencies}\\ \text{of all species}\end{array}\right)} \times 100.$$

$$\text{Relative density} = \frac{\left(\begin{array}{c}\text{Number of individuals of a}\\ \text{species in a sample}\end{array}\right)}{\left(\begin{array}{c}\text{Total of all individuals}\\ \text{in a sample}\end{array}\right)} \times 100.$$

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Table 1. List of the locations surveyed

Location	Variety	Stage of the crop	Previous crop
Belgaum district			
Angadi	Niraj *Bt*	Boll formation	Cotton
Budarkatti	Kanaka *Bt*	Boll formation	Cotton
Dushnur	Bunny *Bt*	Flowering	Cotton
Nayanagar	Bunny *Bt*	Boll formation	Cotton
Samphur	Brahma *Bt*	Flowering	Maize
Sutalgatti	Double *Bt*	Flowering	Sunflower
Dharwad district			
Main Agricultural Research Station, Dharwad	Mallika *Bt*	Square formation	Maize
Agricultural Research Station, Hebballi	Bunny *Bt*	Flowering	Cotton
Hangarki	Mallika *Bt*	Square formation	Green gram
Garag	Mallika *Bt*	Square formation	Peas
Jeergewad	Kavery Jackpot *Bt*	Square formation	Cotton
Shirkol	Bunny *Bt*	Flowering	Cotton
Tadakod	Bunny *Bt*	Flowering	Green gram
Gadag district			
Govnal	Kanaka *Bt*	Boll formation	Maize
Doddur	Kanaka *Bt*	Flowering	Cotton
Kotaba	Mallika *Bt*	Flowering	Cotton
Nelluri	Bunny *Bt*	Flowering	Maize
Haveri district			
Devgiri	Brahma *Bt*	Flowering	Soyabean
Hosaritti	Brahma *Bt*	Flowering	Sunflower
Kakoal	Tulsi-4	Flowering	Sunflower
Shiggoan	Mahyco	Boll formation	Cotton
Yalavigi	Bunny *Bt*	Flowering	Cotton

$$\text{Absolute density} = \frac{\left(\begin{array}{c}\text{Number of individuals}\\ \text{of a species in a sample}\end{array}\right)}{\left(\begin{array}{c}\text{Volume or mass}\\ \text{units of the sample}\end{array}\right)} \times 100.$$

$$\text{Prominence value} = \frac{\left(\begin{array}{c}\text{Absolute density} \times\\ \sqrt{\text{absolute frequency}}\end{array}\right)}{100}.$$

For pathogenicity studies, a pot culture experiment was conducted at the greenhouse of the Department of Plant Pathology, College of Agriculture, Dharwad to evaluate the effect of different levels of inoculum of the reniform nematode on the growth parameters of cotton plants and to determine the pathogenic level of the nematode. The experiment was conducted using *Bt* cotton plants of the cultivar MRC-7918, which, according to an earlier observation supported a large population of the reniform nematode. The inoculum was obtained from the reniform nematode culture maintained on castor plants in the greenhouse of the department. The seeds of the said cultivar were sown in pots (45 cm diameter) filled with sterilized soil and sand mixture in 2 : 1 proportion. Upon germination, the seedlings were thinned to one per pot. Seven days after the date of sowing or at two-leaf stage, suspensions containing the desirable number of infective juveniles of reniform nematode were inoculated onto the exposed roots of the seedlings and later covered with sterilized soil. The number of juveniles present in 1 ml of the suspension was determined by taking an average number of juveniles present in five different 1 ml aliquots of the suspension. Uniform quantity of this suspension containing the desired number of juveniles was used for inoculation. The inoculum levels maintained for the pathogenicity test were: A check; associated check (suspension without juveniles), 10, 50, 100 and 1,000 infective juveniles per treatment. Four replications were maintained for each treatment. Pots were placed on the greenhouse benches and suitably randomized according to completely randomized design (CRD). Just before the completion of the experiment, plant height, number of squares and bolls for all the treatments were recorded. Sixty days after the imposition of the treatments, plants were carefully depotted by inverting the pots into a container filled with water. The root system was gently retrieved after loosening the soil. Observations were recorded in respect of shoot and root fresh and dry weights, number of females or egg sacs of the reniform nematodes per gram of the root system and number of juveniles per 200 cm^3 of soil collected from the cotton root zone.

Table 2. Prevailing *Bt* cultivars and their reaction to reniform nematode

Cultivar and its *Bt* gene	Cotton type	Females or eggmasses/ plant (root system)	Disease index or grade
BG-I (*Cry 1Ac*)			
MRC-6918	*Gossypium hirsutum* × *G. barbadense*	2	2
RCH-2	*G. hirsutum* × *G. hirsutum*	3.5	2
RCH-708	*G. hirsutum* × *G. barbadense*	3.5	2
Chiranjeevi	*G. hirsutum* × *G. hirsutum*	0.5	1
Miracle	*G. hirsutum* × *G. hirsutum*	6	2
Tulsi-4	*G. hirsutum* × *G. hirsutum*	0.5	1
Tulsi-9	*G. hirsutum* × *G. hirsutum*	2.5	2
Tulsi-117	*G. hirsutum* × *G. hirsutum*	6	2
Encounter	*G. hirsutum* × *G. hirsutum*	8	2
NCS-207	*G. hirsutum* × *G. hirsutum*	8	2
Bunny	*G. hirsutum* × *G. hirsutum*	6.5	2
BG-II (*Cry 1Ac* + *Cry 2Ab*)			
MRC-7918	*G. hirsutum* × *G. barbadense*	12.5	3
RCH-2	*G. hirsutum* × *G. hirsutum*	0.5	1
Kashinath	*G. hirsutum* × *G. hirsutum*	3.5	2
Chiranjeeevi	*G. hirsutum* × *G. hirsutum*	8	2
Miracle	*G. hirsutum* × *G. hirsutum*	1	2
Tulsi-4	*G. hirsutum* × *G. hirsutum*	4	2
Tulsi-9	*G. hirsutum* × *G. hirsutum*	1.5	2
Tulsi-117	*G. hirsutum* × *G. hirsutum*	10	3
Encounter	*G. hirsutum* × *G. hirsutum*	4	2
NCS-954 (Kanaka)	*G. hirsutum* × *G. hirsutum*	3	2
Bunny	*G. hirsutum* × *G. hirsutum*	4	2
Brahma	*G. hirsutum* × *G. hirsutum*	5	2
MRC-7201	*G. hirsutum* × *G. hirsutum*	9	2
MRC-7351	*G. hirsutum* × *G. hirsutum*	1.5	2
CPD-8-1 (Sharada)	*G. hirsutum* × *G. hirsutum*	24	4
	SEm ±	1.541	
	CD @ 5%	4.47	

Biology tests were conducted to determine the number of days taken by the reniform nematode to complete its life cycle and to record its developmental stages on *Bt* cotton under Dharwad conditions. Seeds of the cultivar MRC-7918 were sown in 27 styrofoam cups filled with sterilized soil and sand mixture in 1 : 1 proportion. After germination, one seedling was maintained per cup. Seedlings were inoculated with suspension containing infective juveniles of the reniform nematode as follows: Depressions were made in the soil near the base of the seedlings and uniform quantity of the suspension containing 100 infective juveniles was inoculated into these depressions and later covered with soil. The soil was later moistened lightly after inoculation. Observations were recorded by randomly picking three styrofoam cups (which constituted three replications) containing cotton plants which were uprooted by gently inverting the cups and tapping them on their back. Roots were retained by cutting away the stem portion and placed in a basin of water to clear it from adhering soil particles. These were later stained by dipping them in hot, boiling acid fuchsin stain of 0.05–1.0% for 2 min (ref. 8). The solution was allowed to cool; roots were later drained off the stain and placed in petri plates containing glycerol. These destained roots were later observed to record the number of developed juveniles as well as the developmental changes of the nematodes.

Pot studies were conducted in the glasshouse to test the reaction of 25 different *Bt* cultivars of *Bt* cotton to the reniform nematode. Inter- and intra-specific cultivars belonging to *Gossypium hirsutum* and *G. barbadense* were used to study their susceptibility or resistance reaction to the reniform nematode. These genotypes had either *Cry 1Ac* or a combination of *Cry 1Ac* and *Cry 2Ab* genes meant for resistance against the bollworm complex. Two seeds of each cultivar were sown in 10 cm diameter earthen pots filled with sterilized soil and sand mixture (in 2 : 1 proportion) and one seedling was maintained in each pot after germination. Each seedling at its two-leaf stage was inoculated with uniform quantity of the suspensions containing 100 reniform infective juveniles. Two replications were maintained for each treatment. Various cultivars used for the varietal evaluation study are given in Table 2. Thirty days after the date of inoculation, plants were uprooted carefully and freed of soil. The roots were retrieved carefully as described earlier. For

Table 3. Plant pathogenic nematodes encountered in the locations surveyed and their absolute densities

Location	*Aphelenchus* sp.	*Helicotylenchus* sp.	*Hoplolaimus* sp.	*Pratylenchus* sp.	*Rotylenchulus reniformis*	*Tylenchorhynchus* sp.	Dorylamid–PPN	*Tylenchus*-like PPN	Free-living nematodes
Belgaum									
Angadi	–	400.0	–	66.0	330.0	–	166.0	–	100.00
Budarkatti	133.00	360.0	–	66.0	533.0	–	260.0	460.0	300.00
Dushnur	33.00	–	–	133.0	466.0	–	666.0	–	200.00
Nayanagar	–	–	–	–	200.0	–	166.0	–	33.00
Sampnur	100.00	66.0	–	233.0	1233.0	–	700.0	33.0	133.00
Sutalgatti	133.00	–	–	166.0	900.0	–	533.0	–	400.00
Dharwad									
Agricultural Research Station, Dharwad	–	–	–	66.0	733.0	–	200.0	100.0	233.00
Cotton Research Station, Hebballi	–	–	–	33.0	33.0	–	200.0	–	200.00
Hangarki	100.00	–	–	100.0	766.0	1966.40	300.0	–	200.00
Garag	66.66	–	–	–	1500.0	333.31	300.0	–	233.31
Jeergewad	500.00	–	100.0	400.0	1000.0	–	733.3	–	733.33
Shirkol	160.00	–	–	330.0	242.7	–	430.0	–	560.00
Tadakod	333.31	–	–	100.0	2066.0	133.31	433.3	–	333.31
Gadag									
Doddur	–	–	–	33.0	–	4.58	333.3	–	166.67
Govnal	–	–	100.0	200.0	1633.3	33.32	400.0	66.7	200.00
Kotaba	100.00	–	–	66.6	66.6	33.00	133.3	33.0	100.00
Nelluri	33.00	–	33.0	266.0	500.0	–	204.8	100.0	33.00
Haveri									
Devgiri	166.67	–	–	–	300.0	–	200.0	–	233.33
Hosaritti	–	–	–	166.7	266.7	4.43	266.7	233.3	33.00
Kakoal	33.00	–	33.0	33.0	666.0	–	133.3	33.0	–
Shiggoan	–	–	400.0	–	4500.0	33.33	300.0	433.3	–
Yalavigi	66.67	–	233.3	366.7	1000.0	–	300.0	33.0	200.00

PPN, Plant parasitic nematodes.

Table 4. Community analysis of plant parasitic nematodes associated with *Bt* cotton

Nematode species	Absolute frequency	Absolute density	Relative frequency	Relative density	Prominence value
Aphelenchus sp.	63.6	66.4	11.6	4.0	5.3
Helicotylenchus sp.	13.6	40.3	2.5	2.4	1.5
Hoplolaimus sp.	27.3	41.8	5.0	2.5	2.2
Pratylenchus sp.	81.8	119.3	14.9	7.2	10.8
Rotylenchulus reniformis	95.5	757.4	17.5	45.9	73.9
Tylenchohrynchus sp.	27.3	51.8	8.33	3.1	2.7
Dorylamid PPN	100.0	312.6	18.3	18.9	31.3
Tylenchus-like PPN	45.6	74.0	8.3	4.5	5.0
Free-living nematodes	90.9	184.1	16.6	11.1	17.6

PPN, Plant parasitic nematodes.

Table 5. Effect of different inoculum levels of *R. reniformis* on growth parameters of *Bt* cotton and reproduction of nematode

Initial inoculum level	Plant height (cm)	Number of squares	Shoot weight (g)		Root weight (g)		Total biomass (g)		Number of juveniles per 200 cm^3 of soil	Number of females or egg sacs per gram of root
			Fresh	Dry	Fresh	Dry	Fresh	Dry		
T_1 (check)	22.4	2.2	17.2	7.8	2.6	0.8	19.8	8.6	–	–
T_2 (associated check)	22.1	1.7	17.2	7.7	2.5	0.8	19.7	8.5	–	–
T_3 (10)	21.0	1.5	10.7	6.9	2.00	0.7	12.7	7.5	372.66	0.25
T_4 (50)	20.7	1.5	9.7	6.5	1.8	0.6	11.5	7.2	447.5	3
T_5 (100)	17.6	0.7	6.3	6.4	1.6	0.6	8.0	7.1	588.26	6.25
T_6 (500)	17.0	0.3	6.1	5.5	1.7	0.6	7.8	6.1	647.11	19.75
T_7 (1000)	15.0	0.0	5.5	1.3	1.2	0.5	6.8	1.8	844.64	26
SEm ±	1.5	0.4	1.3	0.6	0.1	0.1	1.4	0.6	47.78	1.38
CD @ 5%	2.3	1.2	3.9	1.7	0.4	0.2	4.2	1.8	140.54	2.89

observation, the number of obese females or the number of egg sacs developed was counted. Prior to taking observations, the roots were stained by dipping them in 0.25% trypan blue for 3 min to facilitate easy identification and counting of the number of nematodes developed on the root system of each cultivar[9]. Based on the number of young females or egg sacs developed, the cultivars were classified as follows[10]:

(1) Immune: No females or eggmass per plant.
(2) Resistant: 1–10 females or eggmasses per plant.
(3) Moderately resistant: 11–20 females or eggmasses per plant.
(4) Susceptible: 21–30 females or eggmasses per plant.
(5) Highly susceptible: > 30 females or eggmasses per plant.

Data obtained in the present study on parameters such as plant height (cm), fresh and dry weight (g) of shoot and roots, number of egg sacs per gram of root and final nematode population in 200 cm^3 of the soil sample collected from the root zone of cotton plants were subjected to ANOVA for a completely randomized design, according to the procedure given by Snedecor and Cochran[11].

Table 3 shows distribution of different nematodes and their absolute densities observed in samples collected from 22 different locations. *R. reniformis* and dorylaimids occurred more frequently followed by *Pratylenchus* sp., and *Aphelenchus* sp. with absolute frequencies of 95.4, 100, 81.8 and 63.6 respectively. Community analysis of different plant parasitic nematodes associated (as also, non-parasitic, i.e. free-living) with *Bt* cotton is given in Table 4. *Hoplolaimus* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. were less frequently encountered in the samples with respective absolute frequencies of 27.3, 27.3 and 11.6. Based on the prominence values, it can be seen that in the areas surveyed, *R. reniformis* was the most prominent nematode associated with *Bt* cotton crop rhizospheres followed by dorylaimid plant parasitic nematodes, *Pratylenchus* sp., *Aphelenchus* sp., *Hoplolaimus* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. Based on the results pertaining to prominence values of different plant parasitic nematodes, *R. reniformis* was chosen (dorylaimids represent a mixture of several taxa) to study its pathogenic effects on *Bt* cotton, nematode biology and also the varietal reaction studies.

Higher inoculum levels (100, 500 or 1000 per rhizosphere) reduced plant height. These treatments were significantly different from others. They also produced less number of squares apart from total biomass. The number of egg sacs per gram of root and final nematode population per 200 cm^3 soil increased with the level of inocula used (Table 5).

Apart from these quantifications of different plant growth parameters vis-á-vis nematode inoculation, it was observed that the roots showed brownish discolouration,

especially at the point of infection. These roots were thin and dry with brown lesions at the point of infection. Nematode infection of cotton root system could be visible to the unaided eye when the root system was dipped in 0.25% trypan blue or in water to which few drops of fountain-pen ink was added. The latter substance was evaluated for its efficiency to stain the egg sacs of the reniform nematode pathogen as a substitute/alternative for a laboratory-grade stain/chemical like trypan blue.

The experiment conducted to study the biology of this nematode revealed that the nematode could complete its life cycle within 27 days after inoculation to *Bt* cotton rhizospheres. Observations recorded on various developmental stages are depicted in Figure 1. Infective juveniles penetrated the roots within three days after inoculation and were found to remain vermiform up to six days after inoculation. Slightly swollen to fully obese females of the reniform nematode were encountered between 12 and 18 days after nematode inoculation and obese females with gelatinous matrix were observed on the 21st day after inoculation. Eggmasses in the gelatinous matrix were observed on the 24th day after inoculation (Table 6).

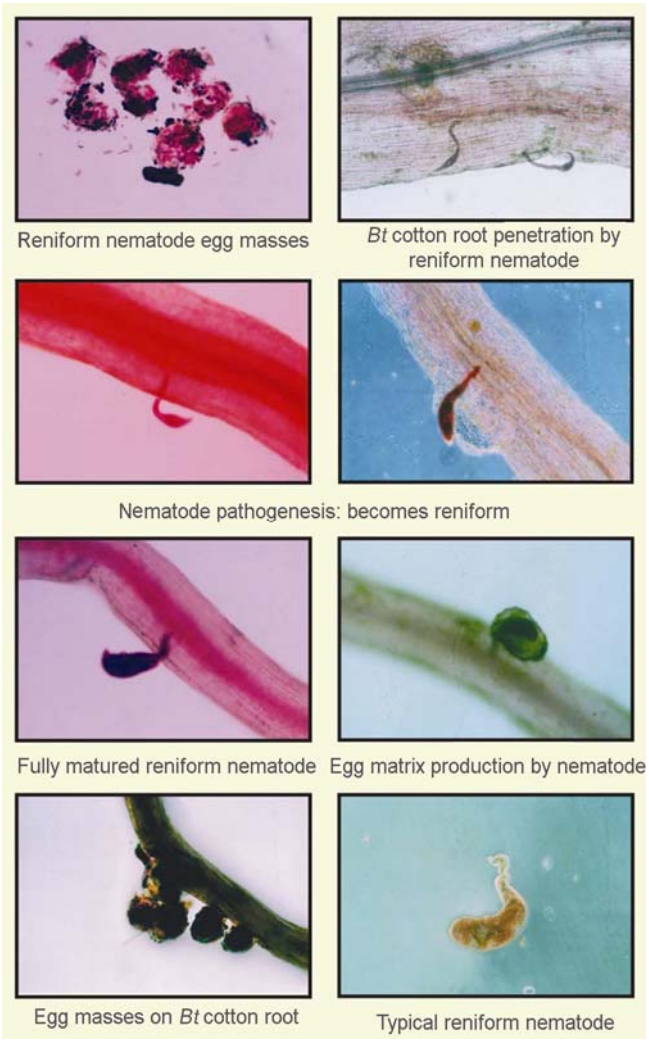

Figure 1. Life cycle stages of *Rotylenchulus reniformis* observed on *Bt* cotton.

Table 2 shows the reaction of different inter- and intraspecific hybrids drawn from *G. hirsutum* and *G. barbadense* to the reniform nematode inoculation, and the reaction of the cultivar was evaluated based on the number of females or egg sacs of the nematode which developed on the roots. Out of 25 germplasm tested, three hybrids showed a disease index of 1 – Chiranjeevi, Tulsi-4 and RCH-2. Twenty cultivars showed a disease index of 2 (resistant) and two, MRCH-7918 and Tulsi-117 were moderately resistant/susceptible. The check, CPD 8-1 (cv. *Sharada*) showed a susceptible reaction.

The reniform nematode was found to be the most predominant species in the soil samples of *Bt* cotton rhizospheres collected from different varieties. This was followed by the lesion nematode, *Pratylenchus* sp. and *Aphelenchus* sp. The reniform nematode is prevalent in higher densities in cotton-growing areas of Punjab, Haryana and Uttar Pradesh[12–14]. These studies reported similar nematode genera in their survey of nematode parasites associated with (non-*Bt*) cotton. *Bt* genes (*Cry 1Ac*, *Cry 2Ab* and their combinations) deployed for bollworms–complex management do not seem to have any effect on plant parasitic nematodes. The pathogenic nematode scenario is the same whether the cotton crop involved is *Bt* or non-*Bt*. The reniform nematode, *R. reniformis* is one of the most widely distributed species in South India, infecting a variety of crops like bhendi, brinjal, tomato, other vegetables, sorghum, pearl-millet (*Pennisetum typhoides*), ragi (*Elusine coracana*) and other millets, castor (*Ricinus communis*), chillies (*Capsicum annuum*) and pulses besides cotton (*G. hirsutum*). Studies indicate that the reniform nematode is highly pathogenic to its hosts in black clay loams apart from the red and sandy loams[15]. And the frequent occurrence of *R. reniformis* in this study may be due to the fact that the soil samples were mainly collected from parts of North Karnataka where cotton is predominantly grown in the black cotton soils.

Table 6. Biology and development of *R. reniformis* on *Bt* cotton (cultivar MRC-7918) under Dharwad conditions

Days after inoculation	Development stage
3	Penetrated infective stage juveniles
6	Penetrated and vermiform juveniles
9	Vermiform females slightly swollen at the tail region
12	Slightly swollen females
15	Swollen females
18	Fully developed obese females
21	Females with gelatinous matrix
24	Females with eggmasses in gelatinous matrix
27	Females with brown egg sacs

Data from the pathogenicity experiment confirm that an initial population of 100 and above juveniles per plant was responsible for the reduction of plant growth parameters of *Bt* cotton and that these inoculum densities can also support a higher population of the nematode without any adverse effect on its reproduction and development. Pathogenic effects in cotton and tomato have been observed at such populations in the past[16–18]. On the whole, the reniform nematode, *R. reniformis* was found to be pathogenic to *Bt* cotton even at a minimum initial inoculum level of 100 infective juveniles per plant and the egg sacs of this nematode were easily detected by dipping the infected root system in water to which few drops of trypan blue/fountain-pen ink are added.

Biology studies indicated that fully developed, pale brown egg sacs were found on the 27th day after inoculation. Some juveniles were found emerging from the egg sacs on the 27th day after inoculation. Therefore, the life cycle on cotton from egg to egg-producing females was 27–29 days under Dharwad conditions. This result therefore supports the findings of Birchfield[19], who reported that the reniform nematode took 27–29 days to complete its life cycle.

Among various *Bt* hybrids (*Gossypium* spp.) which were screened to know their reaction to the reniform nematode, none of the varieties and hybrids exhibited susceptible reaction. The varieties and hybrids evaluated were found to be immune (3), resistant (20) or moderately resistant (2). Rashmi and Lingaraju[4] reported a similar behaviour in respect of non-*Bt* genotypes (cultivars, inter- and intra-specific hybrids). Hence, it was not possible to broadly distinguish species with respect to their reaction. The difference in the results obtained in the present studies may be attributed to variations in the pathogenic population of *R. reniformis*, as occurrence of races within *R. reniformis* has been reported to be present in our country[20] as also reniform nematode exhibits geographical variation[21]. Till date, the varietal reaction of different cultivars against reniform nematode has not been studied in respect of *Bt* cotton; hence the results can be used for breeding efforts for resistance against reniform nematode.

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