International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(3): 2501-2504

Received: 10-03-2020
Accepted: 12-04-2020

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Effect of different inoculum levels of root knot nematode (*Meloidogyne enterolobii*) on various growth parameters of tomato plant CV- Bhagya and Kashi Anupam

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DOI: https://doi.org/10.22271/chemi.2020.v8.i3aj.9582

Abstract
A glass house pot experiment was conducted to study the effect of different inoculum levels viz.,0, 100, 250, 500, 1000, 2000, 4000 and 8000 second stage juveniles of *Meloidogyne enterolobii* (J_2)/plant for the *Lycopersicon esculentum* (tomato) plants. grown in sterilised pots. The results indicated that an inoculum level of 500 and above J_2/plant significantly reduced the various plant growth parameters (shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, number of gall formation and final population of nematodes. An inoculum level of 500 J2 per kg soil and above significantly reduced the various plant growth parameters over uninoculated control and the maximum reduction was obtained at the inoculums level of 8000 larvae of nematode per kg soil. Increase in the inoculum levels of larvae per kg soil decrease the growth parameter of tomato.

Keywords: Root knot nematode, *Meloidogyne enterolobii*, tomato

Introduction
Tomato (*Lycopersicon esculentum* Mill.), is one of the most important vegetable crop. In India it occupy 2nd rank among all vegetable crops covering an area 8.09 lakh hectare total production of 19.69 metric tons. Average yield of tomato in the world is 37.5 MT/Ha, while it is 24.4 t/ha in India (NHB, 2016) [12].
The Root-knot nematodes of genus *Meloidogyne* are the main pathogens of tomato plants all over the world (Sasser and Carter, 1985; Sikora and Greco, 1993; Jacquet *et al.* 2005 [17, 19, 7]. The root-knot nematode *Meloidogyne enterolobii* is polyphagous and has many host plants including cultivated plants and weeds. It attacks woody as well as herbaceous plants. Root knot nematode infestation cause yield loss of 34.5 - 39.6 per cent throughout the world was reported by (Reddy, 1985 and Jonathan *et al.*, 2001) [15, 9]. Jain *et al.*, (2007) [8] stated that the crop losses due to root-knot nematode about Rs. 215 crores annually in India. In economical terms, the nematodes cause a loss of US$ 159 billion annually to the agriculture production globally (Abad *et al.*, 2008) [1].
The species *M. enterolobii* is considered as one of the most damaging species and to be of great importance due to its ability to develop and reproduce on several crops carrying resistance genes (Castagnone-Sereno, 2012) [4]. *Meloidogyne enterolobii* (Yang and Eisenback, 1983) [23] is one of the most destructive species of root-knot nematodes (RKN).
Furthermore, a higher pathogenicity and reproductive potential was found for *M. enterolobii* when compared with other root-knot nematode species such as *M. incognita or M. arenaria* (Kiewnick *et al.*, 2009) [10].

Material and Methods
The experiments were conducted at Departmental Glasshouse of Plant Pathology G. B. Pant University of Agriculture and Technology, Pantnagar Uttarakhand.
The two experiments had been carried out in steam-sterilized soil of one kg plastic pots in glasshouse. The pot soil was prepared by mixing sandy loam soil, river sand and vermicompost in 2:1:1 ratio. The thoroughly mixed soil was steam sterilized at 120°C for 120 minutes.

The soil was filled in 15 cm diameter 48 plastic pots (one kg capacity) which were previously sterilized by formalin. Five tomato seeds of varieties Kashi Anupam and Bhagya each were sown in pots. After germination of seed only one plant per pot were allowed to reach 4 leaf stage i.e. 15 days after seedling emergence and then the second stage larvae of *M. enterolobii* were inoculated @ 100, 250, 500, 1000, 2000, 4000 and 8000 larvae per kg soil by removing upper soil. For control un-inoculated pots were treated with distill water with no larvae in it. Each treatment were replicated three times. Under the glass house condition proper nutrition and water were given as when required. The freshly hatched second stage larvae of root-knot nematode, *M. enterolobii* were obtained from the egg masses taken from 8 week old infected plants of tomato (Hussy and Barker, 1973) [6]. After 60 days of nematode inoculation, the observations were recorded on root length, shoot length, fresh root weight, fresh shoot weight, dry weight of root and shoot, number of knots and final population of nematodes. With the help of meter scale root and shoot length of plants were measured. After taking fresh weight of the roots and shoots of plants, they were kept in bamboo wrapper and placed in hot air oven for 48 hrs at 60^{o}C to obtain dry weight. Number of galls were counted. The observation of final population of nematode, were taken 200 cc soil from each pot and kept in polythene bags. The processing of soil samples performed according to Cobb's Modified Decanting and Sieving Method for nematode recovery. Counting number of nematodes in 1 ml then for calculating final population multiplied with the total volume (in ml) of suspension of nematode obtained from each pot.

Results and Discussions

Two cultivars of tomato namely Bhagya and Kashi Anupam were selected to study the pathogenicity of *M. enterolobii*. The effect of seven inoculum levels (100, 250, 500, 1000, 2000, 4000 and 8000 larvae per kg soil) along with the check (uninoculated) on plant growth characters; gall formation and final population were observed. Observations were recorded after 60 days of inoculation and are being presented in Tables-1 and 2.

It is evident from the data summarized in Table- 1 and Table-2 that shoot length, root length, fresh and dry shoot and root weights of the inoculated plants decreased markedly with the increase in inoculum levels, though the reduction was not proportionate to the increasing level of inoculum. Significant reduction in shoot length was observed in all inoculum levels as compared with control except in 100 inoculum level, least reduction was observed in case of Bhagya (Fig- 2). However, in variety Bhagya 84.23cm shoot length, 133.66g fresh shoot weight and 26.87g dry shoot weight was observed in highest inoculum level of 8000 larvae per kg soil (Table- 1). Likewise in Kashi Anupam (Fig- 1) the reduction was 80.70cm, 127.40g and 19.54g respectively (Table- 2). Regarding the root weight it was observed that the two cultivars behaved differently. In variety Bhagya the root weight decreased slowly up to 2000 larval inoculum level and then dropped sharply at 4000 and 8000 levels, respectively (Table- 1), whereas in variety Kashi Anupam the root weight was observed almost similar to check at inoculum level of 100 larvae per kg soil (Fig- 3). It decreased slightly up to 250 larval level and then decreased markedly with the increase in inoculum level (Table 2). However, the significant reduction in fresh root weight over the check was observed in both cultivars at the highest inoculum level.

The data on the effect of inoculum levels of *M. enterolobii* on gall formation and final population are presented in Table 1 and 2, respectively. It was revealed that number of galls per plant increased with the increasing inoculum level up to 2000 larvae per kg soil in both cultivars of tomato plant. Drastic and significant reduction in number of galls in both the cultivars was observed at 4000 and 8000 inoculum level. In general, the cv. Kashi Anupam was comparatively more susceptible to root-knot nematode *M. enterolobii* (Fig- 1, 2 & 3).

Nematode population in soil was observed to increase with an increase in inoculum levels up to 2000 larval levels in both the cultivars. With increase in inoculum level (8000 larvae) there was a sharp decline in total nematode population. However, the maximum rate of nematode multiplication was observed in Kashi Anupam at 100 inoculum level while it was less in Bhagya at 100 inoculum level. Least multiplication rate of root knot nematode was observed in highest inoculum level in both the cultivars. The plant inoculated with second stage juveniles of *M. enterolobii* significantly affected both root gall and final total population of nematode after harvest (Table- 1). However, remarkable increase in number of galls was observed at 500 J2 per kg soil. The J2 population of 100 and 250 per kg soil were at par with each other. Maximum galls were produced at 2000 J2 per kg soil, while the galls were minimum in plants inoculated with 100 J2 per kg soil. In uninoculated control no root galls were found.

cv- Bhagya cv- Bhagya

Fig 1-2: Effect of different inoculum levels of second stage larvae of *M. enterolobii* on growth parameters of tomato plants and Kashi Anupam (Fig- 1 & 2).

Fig 3: Effect of different inoculum levels on gall formation on roots of tomato

Table 1: Effect of different inoculum levels of second stage larvae of *M. enterolobii* on various growth parameters of tomato plants cv- Bhagya

S. No.	J2/kg soil	Root Length (cm)	Shoot Length (cm)	Fresh Root Weight (gm)	Dry Root Weight (gm)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	No. of knots	Final Population Per kg soil
1	100	28.30	135.40	14.30	2.80	191.43	69.26	93.00	2506.00
2	250	26.60	129.06	13.16	2.50	188.06	61.37	98.00	2805.00
3	500	25.13	121.30	10.80	2.20	164.33	49.23	171.00	7646.00
4	1000	21.30	107.70	9.59	1.80	152.33	46.98	251.00	14721.00
5	2000	19.60	98.43	8.46	1.50	148.33	38.56	368.00	21747.00
6	4000	15.13	87.13	7.92	1.30	145.03	34.80	290.00	18702.00
7	8000	14.83	84.23	7.16	0.93	133.66	26.87	225.00	15604.00
8	Control	31.60	146.06	17.33	3.10	226.20	80.80	0.00	0.00
	SEm±	0.75	0.63	0.18	0.55	0.78	0.39	0.54	2.30
	CD at 5%	0.22	1.91	0.55	0.65	2.35	1.16	9.21	6.92
	CV	0.57	0.97	2.87	4.74	0.80	1.32	0.50	0.38

Table 2: Effect of different inoculum levels of second stage larvae of *M. enterolobii* on various growth parameters of tomato plants cv- Kashi Anupam

S. No.	J2/kg Soil	Root Length (cm)	Shoot Length (cm)	Fresh Root Weight (gm)	Dry Root Weight (gm)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	No. of knots	Final Population Per kg soil
1	100	25.33	127.00	13.20	2.50	184.20	65.60	97.66	2596.67
2	250	23.33	122.33	12.97	2.20	177.00	56.76	106.00	2985.67
3	500	21.20	118.03	10.20	1.80	154.00	46.56	180.00	7797.00
4	1000	18.70	106.20	9.30	1.60	148.40	39.03	261.00	14976.00
5	2000	14.80	96.16	8.16	1.40	143.00	32.00	377.00	21976.33
6	4000	13.20	85.30	7.60	1.20	138.83	15.93	301.00	18844.67
7	8000	12.80	80.70	6.90	1.10	127.40	19.54	239.00	15874.00
8	Control	29.77	134.73	15.86	2.80	202.00	71.00	0.00	0.00
	SEm±	0.16	0.24	0.44	0.57	0.41	0.21	0.51	1.63
	CD at 5%	0.50	0.74	0.13	0.17	1.23	0.64	7.95	4.89
	CV	1.46	0.39	0.73	5.47	0.44	0.83	0.45	0.26

The similar results were also observed by Silva and Oliveira (2010) [20] who reported that as root galling of tomato plant and nematode juveniles increases, plant height and number of leaves are negatively affected. Similar results have been reported by Ploeg (2001) [13] he observed that the nematode affect crop productivity when they attack the roots of seedling immediately after seed germination. Cetintas *et al.*, (2007) [5] observed plants attacked by *M. enterolobii* affect the growth, yield, and lifespan of infested plants, which causes stunted growth, wilting and leaf yellowing. It is considered as very damaging among other *Meloidogyne* spp. due to its wide host range, high reproduction rate and induction of large galls (Castagnone-Sereno, 2012) [4]. *Lycopersicon esculentum* (tomato) and *Citrullis lanatus*

(water melon; Cetintas *et al.*, 2007; Kiewnick *et al.*, 2009; Ramirez-Suarez *et al.*, 2014) [5, 10, 14] They also observed that the shoot and root weight of the plants were significantly affected by the nematode. Study conducted by Sassanarukkit *et al.* (2010) **[16]** drew correlation between the number of infective juveniles of root knot nematode, root galls and yield decline**.** In comparative population build up studies conducted on four guava cultivars, maximum juveniles (250/100 g soil) were recorded in cv. Pantritong whereas cv. Pantyoddang could support very low number of juveniles (19/100g soil).

Conclusion

In the glass house experiment, the growth parameters (shoot length, root length, fresh shoot weight, fresh root weight, number of gall formation and final population) were studied on tomato plant variety Kashi Anupam by using different inoculum levels i.e. 100, 250, 500, 1000, 2000, 4000 and 8000 J2 larvae of nematode per kg soil in pot experiment, respectively. Results showed that an inoculum level of 500 J2 per kg soil and above significantly reduced the various plant growth parameters over uninoculated control and the maximum reduction was obtained at the inoculum level of 8000 larvae of nematode/kg soil. Similar results were obtained in other cultivar Bhagya under the same glass house condition.

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