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Pathogenicity of Root-knot Nematode, *Meloidogyne incognita* Infecting Cucumber under Protected Cultivation

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ABSTRACT: In a study on pathogenicity of *Meloidogyne incognita* an inoculum level of 10 juveniles (J_2)/plant/pot was detrimental to the growth and development of cucumber, indicating it as a pathogenic threshold level of *M. incognita* during 2022 and 2023. Root, soil and total nematode population of the cucumber increased progressively with an increase in inoculum levels from 10 to 10,000 J_2/plant. Nematode reproduction rate decreased with an increase in inoculum levels and it was maximum in the level of 10 J_2/plant and minimum in 10,000 J_2/plant against root-knot nematode in cucumber.

Keywords: Pathogenicity, cucumber, *M. incognita*, inoculum level.

INTRODUCTION

Cucumber is a semi-tropical vegetable and grows best under polyhouse conditions. In polyhouse under stable environmental, with favourable conditions, optimum nutrients, less pest-disease infestation and the cucumber plant grows speedily. The optimum temperature for better development of fruits is 14-20°C. It requires mild climate and it does well under polyhouse conditions. In India protected cultivation area is around 30000 ha which contributes 0.23 per cent of horticulture cropped area (Pal *et al.*, 2020). In India, cultivation of cucumber is noted at least 3000 years ago and during 100 B.C. in China and remained the largest cucumber producer in the world with 61,949 tonnes/ha production volume per year and the major cucumber cultivating states in India are Haryana, Karnataka, Madhya Pradesh, Tamil Nadu, Andhra Pradesh, Telangana, Assam, Uttar Pradesh, Bihar, Jammu & Kashmir (Anonymous, 2021). India contributes an average annual production of 1922.3 million tonnes from an area of 139.7 hectares (Anonymous, 2024). In Gujarat annual production of cucumber is 177.67 million tonnes from an area of 12.28 hectares (Anonymous, 2024).

Like other field and polyhouse crops, cucumber is also prone to attack by several biotic and abiotic stresses. Among the different biotic stresses, diseases incited by plant parasitic nematodes *viz.*, *Meloidogyne* spp., *Rotylenchulus reniformis*, *Heterodera* spp., *Pratylenchus* spp., *Hoplolaimus* spp. and *Helicotylenchus* spp. are conjoined with vegetable crops in India. Nematodes being ubiquitous browsers and hidden enemy cause discernable damage to the crop because of longer duration with deeper root system. Moreover, in addition to their own pathogenetic effects, they also play a crucial role in predisposing the host to attack by other pathogenic microorganisms like fungi, bacteria, viruses *etc.* which in turn create more aggravated situation (Sasser and Jenkins 1975). Plant parasitic nematode causes approximately 21.00 per cent losses in crops, it's amounting to Rs.102,039.79 million (1.58 billion USD) annually in India. Due to favourable environmental conditions in field and polyhouse, multiplication of insect pests and pathogens including plant parasitic nematodes are very high which leads to significant reduction in the quantity and quality of cucumber fruit production (Ismail *et al.*, 2012; Bhati and Baheti, 2020a).

Out of these nematodes, root-knot nematodes are sedentary endoparasitic nematodes which complete their life cycle inside the plant tissues. Otherwise, the common symptoms of the infestation with root-knot nematodes are stunting, yellowing and wilting, but the major symptom is the gall formation in plant roots (Khalil, 2013). It is the most important and causing great damage to crops grown under polyhouses including cucumber (Rao *et al*., 2015; Bhati and Baheti 2020b). Root-knot nematodes, *Meloidogyne* spp. is responsible for 12.00 per cent estimated losses in cucumber crop with monetary loss of Rs. 110.46 million per annum in open field (Kumar *et al*., 2020). This dreadful pest poses a serious problem because of its higher reproductive potential and subsequent number of generations. The information on the losses caused by the root-knot nematodes in cucumber crop under protected conditions is still limited and their management strategy should be taken into account.

The pathogenicity of *Meloidogyne* spp. in which inoculated cucumber plants with known population of root-knot nematodes, with a new to assess the extent of damage and disease symptoms through quantifiable parameters, such as gall formation, root weight, plant height and overall yield. The *Meloidogyne incognita* has detrimental effect on the sustainable production of cucumber production. It is a significant pest that significantly reduces cucumber plant growth and causes yield losses of 66.23 percent (Tamireddy *et al*., 2022). This investigation will provide critical insights into the nematode-host interaction dynamics, contributing to the development of effective management strategies in cucumber production within protected environments. So, it is crucial to conduct a study on the pathogen density levels against a susceptible host under controlled conditions and it helps in the making proper decisions for the integrated management of pathogen (Lalhruaitluanga and Hazarika 2020).

MATERIALS AND METHODS

Pathogenicity of *Meloidogyne incognita* on cucumber to determine threshold level in pots were carried out at Department of Nematology, B. A. College of Agriculture, Anand Gujarat Agricultural University, Anand, Gujarat during 2022 and 2023. Earthen pots of 15 cm diameter were washed and disinfected using 4% formaldehyde (Formalin 40 EC) solution. After drying, the pots were filled with steam sterilized soil (1.5 kg/pot). Three seeds of root-knot susceptible cucumber variety were sown in each pot. On germination, plants were thinned down to one plant/pot. Second stage juveniles (J_2) were extracted from the respective egg masses of *Meloidogyne incognita* inoculated on artificially in the rhizosphere of each plant by removing soil around plant stem with the help of forceps. The inoculum levels were tested 0, 10, 100, 1000 and 10,000 J_2 of *M. incognita* on cucumber plant. The uninoculated plants served as untreated control. Each treatment was repeated four times in Completely Randomized Design. Plants were watered regularly. Plants were uprooted carefully (After 45 days of inoculation). The roots were washed with water, observations on plant growth characters and nematode multiplication were recorded and analyzed.

RESULTS AND DISCUSSION

Significant differences in vine length, fresh shoot and root weights, root-knot index (RKI) and root nematode population as well as soil and total nematode population were observed for various inoculum levels of *M. incognita* (Table 1-3).

The data presented in Table 1 revealed significant differences among the treatments during the year 2022, 2023 and in pooled analysis for vine length. The year were significant and T×Y interaction were non significant. The results revealed that with increase in levels of inoculum, there was progressive decrease in vine length.

During 2022, significant reduction in vine length (58.82) of cucumber was observed at initial inoculum level of 10 Juveniles (J_2) per plant. The lowest vine length (26.08) was recorded in the level of 10,000 J_2 per plant. The uninoculated plants (control) showed highest vine length (166.09). This was significantly different from 10 J_2 per plant (142.98). Maximum vine length (177.20) was recorded in uninoculated (control) and it was statistically at par with 10 J_2 per plant (166.40). Inoculum level of 100 J_2 per plant (62.20) and 1000 J_2 per plant (49.20) statistically at par with each other in the year of 2023.Similar trends observed in pooled over years. There was significant reduction in vine length at an inoculum level of 10 J_2 per plant and above.

Maximum fresh shoot weight (93.90) was observed in uninoculated plants (control) and it is differed from 10 J_2 per plant (80.80). Initial inoculum level of 10 J_2 of *M. incognita* per plant significantly reduced the fresh shoot weight as compared to its lower inoculum levels. The inoculum level 100 J_2 per plant (69.60) and 1000 J_2 per plant (61.90) were significantly at par with each other. Minimum fresh shoot weight was recorded in 10,000 J_2 per plant during the year 2022. Same trend recorded in 2023 and in pooled analysis. The T×Y interaction was non significant indicating there was no environmental effect on treatment during both the years.

The data on fresh root weight Table 2 indicated exactly the same trend as observed in fresh shoot weight per plant during both the years and in pooled analysis indicating initial inoculum of 10 J_2/plant as damaging to fresh root weight in individual as well as in pooled analysis. The inoculum level 100 J_2/plant and 1000 J_2/plant were found at par with each other. There was significant reduction in fresh root weight which was observed from an inoculum of 10,000 J_2/plant and above as compared to control in 2022, 2023 and pooled analysis.

With regard to root-knot index (Table 2) all the treatments significantly differed from each other. It increased with an increase in inoculum levels. The data on root-knot index (5.00) revealed significant differences among the treatments during 2022, 2023 and pooled over years. The year effect was significant while T×Y interaction was non significant. Root-knot index was found to increase with increase in initial inoculum level in both the years and pooled over years. Significantly highest root-knot index was observed in 10,000 J_2 per plant as compared to their respective lower levels during 2022 and 2023. The inoculum level 100 and 1000 J_2 per plant was differed from each other during both the years and pooled over years.

Regarding reproduction of *M. incognita*, there was increase in number of females, number of egg masses and number of eggs per egg mass with increase in inoculum levels (Table 3). All the three attributes were found maximum in the inoculum level of 10,000 J_2/plant. Differences among the treatments were found significant in 2022, 2023 and pooled over years. As expected, there was no females in control plants as nematodes were not inoculated. Similarly, during 2022, 2023 and in pooled analysis, the differences among the treatments were significant for number of eggs per 5 g roots.

All the inoculum levels were significantly differed from each other for number of eggs/egg mass for both the years as well as in pooled number of eggs/egg mass were increased with increase in inoculum level. Maximum eggs/egg mass was recorded in inoculum level of 10,000 J_2 per plant.

With respect to soil nematode population, it increased correspondingly with an increase in the levels of nematode inoculum. All inoculum levels were differed significantly from each other. Maximum soil nematode population (15488) was recorded in the level of 10,000 J_2/plant. It was minimum in the level of 10 J_2/plant (37) followed by the level of 100 J_2/plant (124) during 2022 (Table 3). Same trend observed in 2022 was reported for 2023 and pooled. The year effect was significant, while the Y × T interaction was non significant.

Data on total population (Table 3) exhibited exactly the same trend as noticed for soil population, with maximum reproduction rate in initial inoculum level of 10 J_2 per plant followed by 100, 1000 and 10,000 J_2 per plant (Table 3). Total nematode population increased with an increase in the levels of nematode inoculum. Control plants which were not inoculated with nematodes had obviously no nematodes recorded during 2022, 2023 and pooled over years. Hence, reproduction rate progressively increases with decrease in nematode inoculum levels. Reproduction rate was maximum (74.1 times) in the level of 10 while it was minimum (2.81 times) in the highest level of 10,000 J_2 per plant. This might be due to the destruction of root system by the parasitism of root-knot nematodes which led the competition for food and nutrition among the developing nematode within the root system and also due to inability of juveniles to find out new infection sites subsequent generation. The high rate of multiplication at low levels of inoculum, on the other hand, could possibly be due to the positive factors like abundance of food, lack of competition and the ability of host to support their levels of population. Thus, it is concluded that an inoculum level of 10 and above levels of *M. incognita* J_2/plant proved detrimental to the growth and development of cucumber (Table 3). An initial inoculum level of 10 J_2 of *M. incognita* per plant was found damaging to the cucumber as it significantly reduced the plant growth characters *viz.*, vine length, fresh shoot and root weight.

The foregoing results revealed that in most of the plant growth characters, an inoculum level of 10 J_2/plant and above of *M. incognita* was found to be damaging to cucumber. No reports are available in the literature about the effect of *M. incognita* on cucumber. The results reported by Lalhruaitluanga and Hazarika (2020); Pankaj and Siyanand (1990); Venkatesan (2009). They found 100 J_2/plant inoculum level was damaging in pumpkin, round melon and bittergourd, respectively. Reduction in rate of reproduction with increase in initial inoculum at 1000 J_2/plant inoculum level in bitter gourd and smooth gourd was observed and similar results as reported by Gupta *et al.* (1995). Singh and Patel (2012); Giribabu *et al.* (2008) reported reduction in growth and development of bottle gourd at 100 and above inoculum levels due to *M. incognita.* They observed such reduction at 45 days after inoculation (DAI) in case of vine length as well as fresh shoot and root weights. Similar result with respect to vine length as well as fresh shoot and root weights per plant and nematode multiplication were akin to above results.

Table 1: Pathogenicity of root-knot nematodes, *M. incognita* in cucumber: Effect on vine length, fresh shoot weight and fresh root weight and root-knot index during 2022 and 2023.

Treatments		Vine length (cm) at 45 DAS			Fresh Weight (g) at 45 DAS						Root-knot index (0-5 scale) at 45 DAS		
					Shoot			Root					
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
0		166.09	177.20	171.65	93.90	88.50	91.20	2.80	5.78	4.29	0.00 (0.5)	0.00 (0.5)	0.00 (0.5)
10		142.98	166.40	154.69	80.80	76.22	78.51	4.40	7.06	5.73	1.24 (1.54)	1.22 (1.50)	1.23 (1.51)
100		58.82	62.20	60.51	69.60	55.32	62.46	5.10	7.56	6.33	1.87 (3.50)	1.99 (3.98)	1.93 (3.72)
1000		46.10	49.20	47.65	61.90	51.90	56.90	6.70	10.00	8.35	2.11 (4.46)	2.10 (4.41)	2.11 (4.45)
10,000		26.08	30.00	28.04	38.70	33.60	36.15	7.48	11.60	9.54	2.35 (5.00)	2.35 (5.00)	2.35 (5.00)
S.Em.±	T	6.39	5.91	4.81	3.57	2.76	2.48	0.35	0.35	0.28	0.04	0.03	0.03
	Y	-	-	2.75	-	-	1.43	-	-	0.16	-	-	0.02
	T × Y	-	-	6.16	-	-	3.19	-	-	0.35	-	-	0.03
CD at 5%	T	18.86	17.44	13.81	10.53	8.14	7.11	1.05	1.03	0.81	0.11	0.08	0.07
	Y	-	-	Sig.	-	-	Sig.	-	-	Sig.	-	-	Sig.
	T × Y	-	-	NS	-	-	NS	-	-	NS	-	-	NS
CV (%)		16.24	13.63	14.88	11.57	10.09	10.97	14.96	9.26	11.47	5.22	3.75	4.54

Note: 1. DAS= Days after sowing, NS= Non-significant, S= significant; 2. Figures in parentheses are re-transformed values of ($\sqrt{x+0.5}$)

Table 2: Pathogenicity of root-knot nematodes (*M. incognita*) in cucumber: Effect on number of females, egg masses and eggs/egg mass during 2022 and 2023.

Treatments		Nematode population/5 g roots						Number of eggs/egg mass		
		Number of females			Number of egg masses					
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
0		0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
10		1.33 (21)	1.43 (27)	1.38 (24)	1.09 (12)	1.15 (14)	1.12 (13)	2.83 (681)	2.82 (656)	2.82 (661)
100		1.83 (68)	1.86 (72)	1.84 (69)	1.55 (35)	1.60 (40)	1.58 (38)	3.57 (3750)	3.56 (3656)	3.57 (3715)
1,000		2.49 (309)	2.60 (398)	2.54 (347)	2.15 (141)	2.20 (157)	2.17 (148)	3.90 (7889)	3.90 (8017)	3.90 (7943)
10,000		2.66 (457)	2.69 (490)	2.68 (479)	2.31 (202)	2.33 (212)	2.32 (209)	4.04 (10965)	4.03 (10641)	4.04 (10839)
S.Em.±	T	0.04	0.03	0.03	0.01	0.02	0.01	0.03	0.03	0.02
	Y	-	-	0.01	-	-	0.01	-	-	0.01
	T × Y	-	-	0.03	-	-	0.02	-	-	0.03
CD at 5%	T	0.11	0.08	0.07	0.04	0.06	0.04	0.09	0.07	0.06
	Y	-	-	Sig.	-	-	Sig.	-	-	Sig.
	T × Y	-	-	NS	-	-	NS	-	-	NS
CV(%)		5.13	3.41	4.33	1.87	3.03	2.54	2.46	1.93	2.21

Note: 1. 0 = Free; 5 = Maximum disease intensity, NS= Non-significant, S= significant; 2. Figures in parentheses are re-transformed values of log (X+1)

Table 3: Pathogenicity of root-knot nematode (*M. incognita*) on cucumber: Effect on soil, total population and reproduction rate during 2022 and 2023.

Treatments		Soil (200 cc) at 45 DAS			Total nematode population (soil + root)			Reproduction Rate (P_f/P_i)
		2022	2023	Pooled	2022	2023	Pooled	
0		0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00
10		1.57 (37)	1.66 (46)	1.61 (41)	2.88 (713)	2.85 (708)	2.87 (741)	74.1
100		2.09 (124)	2.12 (132)	2.11 (129)	3.60 (4055)	3.59 (3899)	3.60 (3981)	39.81
1000		3.41 (2570)	3.47 (2951)	3.44 (2754)	4.04 (10304)	4.07 (11614)	4.05 (11220)	11.22
10,000		4.19 (15488)	4.25 (17783)	4.22 (16596)	4.43 (30061)	4.46 (29107)	4.45 (28184)	2.81
S.Em.±	T	0.01	0.02	0.02	0.03	0.02	0.02	-
	Y	-	-	0.01	-	-	0.01	-
	T × Y	-	-	0.02	-	-	0.02	-
CD at 5%	T	0.03	0.06	0.07	0.08	0.07	0.06	-
	Y	-	-	Sig.	-	-	Sig.	-
	T × Y	-	-	NS	-	-	NS	-
CV (%)		1.14	2.05	1.67	2.11	1.64	1.88	-

Note: 1. 0 = Free; 5 = Maximum disease intensity, DAS= Days after sowing; 2. NS= Non significant, Sig. = Significant; 3. Figures in parentheses are re-transformed values of log (X+1)

CONCLUSIONS

In a study on pathogenicity of *Meloidogyne incognita*, an inoculum level of 10 juveniles (J_2)/plant/pot was found detrimental to the growth and development of cucumber. Root, soil and total nematode population of *M. incognita*/plant increased progressively with an increase in inoculum levels from 10 to 10,000 J_2/plant. Nematode reproduction rate decreased with an increase in inoculum levels and it was maximum in the level of 10 J_2/plant and minimum in 10,000 J_2/plant; while in the study on pathogenicity of *M. javanica* pt.1 an inoculum level of 10 (J_2)/plant/pot was detrimental to the growth and development of cucumber indicating the pathogenic threshold level of *M. javanica* pt.1. Root, soil and total nematode population of *M. javanica* pt.1/plant increased progressively with an increase in the inoculum levels from 10 to 10,000 J_2/plant. Nematode reproduction rate decreased with an increase in inoculum levels and it was maximum in the level of 10 J_2/plant and minimum in 10,000 J_2/plant.

FUTURE SCOPE

This study establishes the pathogenic threshold levels of *Meloidogyne incognita* and *M. javanica pt.1* in cucumber, highlighting their impact on plant growth and nematode reproduction. Future research should focus on determining the economic threshold level (ETL) through field trials, screening and developing nematode-resistant cucumber varieties, and exploring grafting techniques with resistant rootstocks. Integrated Nematode Management (INM) strategies, including biocontrol agents, organic amendments, and safe chemical nematicides, should be optimized for sustainable control. Molecular studies on virulence genes and nematode-host interactions will aid in early detection and resistance breeding. Additionally, AI-based decision-support tools and sensor-based monitoring in polyhouses can enhance precision nematode management in protected cultivation.

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