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Pathogenicity of Root-knot nematode (*Meloidogyne incognita*) and Determination Threshold Level in Potato (*Solanum tuberosum* L.) crop cv. Lady Rosseta

Bhumika Patel, D. B. Patel, Poonam V. Tapre*, N. K. Singh and Rushikesh Patel

Dept. of Plant Nematology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar (385 506), India

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Corresponding Author

Poonam V. Tapre

e-mail: taprepoonam@gmail.com

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Abstract

Pot studies were undertaken to prove the pathogenicity of root-knot nematode (*Meloidogyne incognita*) and to determine the threshold level in potato cv. Lady Rosseta arranged with inoculum levels *viz.,* 0 (uninoculated check), 10 (J_2 plant^{-1} pot^{-1}), 100 (J_2 plant^{-1} pot^{-1}), 1,000 (J_2 plant^{-1} pot^{-1}) and 10,000 (J_2 plant^{-1} pot^{-1}). Sixty days after inoculation, recorded data revealed that initial inoculum level of 100 Juveniles/plant/pot (10 kg soil) significantly reduced the plant growth parameters *viz.,* plant height (cm), tuber weight (g), fresh shoot and root weight (g) and dry shoot and root weight (g) and were pathogenic to potato cv. Lady Rosseta. No significant reduction in plant growth parameters was observed between uninoculated check and initial inoculum level of 10 (J_2 plant^{-1} pot^{-1}). Maximum reduction in plant growth parameters were observed at inoculum level of 10,000 J_2 plant^{-1} pot^{-1}. Plant growth parameters in uninoculated check was higher than other treatments. Increase in root-knot index and number of root galls plant root^{1} (1 g) were noticed with increase in inoculum levels. Reproduction of root-knot nematode (*M. incognita*) in potato cv. Lady Rosseta revealed significant increase in nematode population parameters *viz.,* different stages of embedded females and egg masses plant root^{1} (1 g), soil nematode population pot^{1} and total nematode population build-up with increase in inoculum level. Minimum nematode population parameters were recorded at inoculum level of 10 J_2 plant^{-1} pot^{-1} and maximum nematode population parameters were recorded at inoculum level of 10,000 J_2 plant^{-1} pot^{-1}. Reproduction rate (Pf/Pi) decreased with increase in inoculum levels. It was maximum (404.90) at the inoculum level of 10 J_2 plant^{-1} pot^{-1} and minimum (4.03) at the inoculum level of 10,000 J_2 plant^{-1} pot^{-1}.

Keywords: *Meloidogyne incognita*, *Solanum tuberosum* L., inoculums level, pathogenicity

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important staple food crop. Potato originated in the high hills of Peru and Bolivia of South Africa, where it was domesticated approximately 7,000 to 10,000 years ago. Presently, India ranks third in area and production in the world. The area under crop during 2017-18 was 21,76,000 ha with annual production of 49,344 t (Anonymous, 2018). Post harvest improvement such as fast and cheap transportation, storage and processing will help to make potato production more profitable for farmers by improving their access to markets, raising local value addition. But due to number

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of production problems that accounts for low regional as well as national yield have been identified. The growers of are also suffering up to a great extent not only due to low production, poor keeping quality of tubers but also biotic stress such as late blight, common scab, and mosaic virus (Agrawal et al., 2016). However, the pests and diseases are becoming a major setback in its production. Potato suffers from infestation of a number of subterranean insect pests, viz. red ant, *Dorylus orientalis* Westwood; cutworm, *Agrotis ipsilon* Hufnagel and wireworm, *Agrotis* sp. which directly affect its quality as well as quantity (Lanunochetla and Neog., 2012). Root-knot nematodes (*Meloidogyne* spp.) are one of the most important polyphagous pests in agriculture. Among the top five plant pathogens affecting world's food production, root-knot nematodes are one of the most devastating pathogen of crops. The genus *Meloidogyne* includes about 100 species. Some species are considered globally important and can cause severe damage on economic crops (Perry and Moens, 2006). Several weed species (226 species belonging to 43 families) are known to act as hosts of root-knot nematodes worldwide (Rich et al., 2008). The effect of nematode infection on plant root induce typical symptoms, popularly known as 'root-knot' or 'root gall' of varying sizes depending on the species of root-knot nematode and the host. The root-knot nematodes (*Meloidogyne species*) are sedentary endoparasites and most damaging agricultural nematode pest, attacking a wide range of crops. Second stage juveniles reach the plant root, J_2 penetrate the nearest root tips in the elongation zone (Lohar and Bird, 2003). Second stage juveniles hatched from the eggs in egg masses laid by females on the infected roots initiate the penetration causing the infection (Kumar et al., 2015). Root-knot nematodes are adapted to parasitize on large number of plants and over 3000 wild and cultivated plant species are reported to be affected (Hussey and Janssen, 2002). Potato cultivars can behave differently to challenge by *Meloidogyne* spp. (Charchar, 2009, Brown et al., 2009). The present investigation was undertaken to know the effect of different initial inoculum levels of *M. incognita* in potato (*Solanum tuberosum* L.) crop cv. Lady Rosetta to determine threshold level.

2. Materials and Methods

2.1. Isolation, maintenance and multiplication of pure culture of Meloidogyne incognita

The culture of root-knot nematodes *Meloidogyne incognita* was isolated from the potato crop field affected by root-knot nematodes (*Meloidogyne incognita*) from Horticulture Farm in Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The roots of potato plant were collected from the potato affected field and roots were washed free of soil. Fully developed egg masses of *M. incognita* were picked-up from the galled roots with the help of forceps and transferred on a Jackson make tissue paper supported by 25 mesh wire gauge over a Petridish containing sufficient amount of water to keep egg masses in contact with water in a Petridish assembly method (Chawla and Prasad, 1974). After 48 hours, nematode suspension from Petridish was carefully collected and for further multiplication, these nematodes were inoculated to susceptible tomato seedlings grown in 15 cm diameter earthen pots previously disinfected with 4 per cent formaldehyde (formalin 40 EC) and filled with steamed soil. After about 45 days, soil as well as root samples from these pots revealed the presence of large number of juveniles of *M. incognita* in the soil samples and mature females as well as egg masses adhering to the root surfaces. The culture was multiplied in the bigger earthen pots having 10 kg soil/ pot on tomato seedlings at the Department of Nematology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. This culture was used for conducting the present research trial on potato.

2.2. Soil sterilization

The sandy loam soil (coarse sand 1.3%; fine sand 63 per cent; silt 15 per cent and clay 20%) was collected from the nearby field and sieved through 25 mesh sieves to remove bigger size stones and other matters and filled in aluminum trays of 45×30×15 cm^3 size. Then the soil was mixed with fine decomposed Farm Yard Manure (FYM) in the proportion of 9 : 1 and the mixture was passed through two mesh sieve. The mixture was then steam sterilized in soil sterilizer (boiler) at the Department of Plant Nematology, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, for three hours at 1.4 kg cm^{-2} pressure at 121 °C temperature. Such steam sterilized soil was used for conducting research experiments in pots.

2.3. Disinfection of pots

Plastic pots of 24 cm diameter were washed with water and then disinfected with 4% formaldehyde (formalin 40 EC) solution. The pots were exposed for 30 minutes to evaporate formalin before they were used for various research studies.

2.4. Extraction of nematodes

To extract more number of *Meloidogyne incognita* J_2 (second stage juveniles), infected plants of brinjal from the respective pure culture micro plots were uprooted carefully with maximum root system and roots were washed free of soil. Thereafter, fully developed egg-sacs of *Meloidogyne incognita* were picked-up from the galled roots with the help of forceps in substantial numbers and transferred on a Jackson make tissue paper supported by 25 mesh wire gauge over a Petridish containing sufficient amount of water to keep egg-sacs in contact with water in a Petridish assembly method (Chawla and Prasad, 1974). After 48 hours, nematode suspension from Petridish was carefully collected and J_2 count/ml water suspension was determined by counting nematode under stereo zoom binocular microscope. Such five counts were taken from the same collection and average J_2 count/ml suspension for each population was worked out. Nematodes, thus obtained were used for inoculation purpose in further investigation.

2.5. Experimentation

An experiment was conducted to prove the pathogenicity and to determine the threshold level of root-knot nematode (*Meloidogyne incognita*) in potato cv. Lady Rosetta in plastic pots of 24 cm diameter using completely randomized design with six replications. *Meloidogyne incognita* J_2 was inoculated to potato cv. Lady Rosetta. The treatments are uninoculated check, 10 (inoculation of *Meloidogyne incognita* J_2 plant^{-1} pot^{-1}), 100 (inoculation of *Meloidogyne incognita* J_2 plant^{-1} pot^{-1}), 1000 (inoculation of *Meloidogyne incognita* J_2 plant^{-1} pot^{-1}) and 10000 (inoculation of *Meloidogyne incognita* J_2 plant^{-1} pot^{-1}).

Plastic pots having 24 cm diameter were sterilised with 4 per cent formaldehyde (formalin 40 EC) and filled with steam sterilised soil mixture (Soil : FYM = 9 : 1). Nematode free tubers (weight ranging from 91-95 g) of potato crop Lady Rosetta were sown at the rate of one tuber per pot. After germination, J_2 of *Meloidogyne incognita* was inoculated in the rhizosphere of each plant after making a ring around plant stem in each case as per the treatment. Plants were watered regularly and protected from the insect-pests damage by spraying recommended pesticides. Sixty days after inoculation, plants were uprooted carefully. Roots and tubers were washed with water and data were recorded for each treatment. Finally, the data were statistically analysed using Duncan's New Multiple Range Test (DNMRT).

3. Results and Discussion

The data indicated that the plant height of potato significantly reduced with inoculum level of 100 J_2 plant^{-1} pot^{-1} and above. However, inoculum levels of 0 and 10 J_2 plant^{-1} pot^{-1} observed no significant reduction in plant height. Maximum reduction of plant height was observed in the treatment with 10,000 J_2 plant^{-1} pot^{-1} (19.75 cm). The plant height in uninoculated check was 36.17 cm; it reduced to 19.75 cm at inoculum level of 10,000 J_2 plant^{-1} pot^{-1} (Table 1).

The results revealed that tuber weight (g) was reduced with increase in inoculum level (Table 1). Insignificant difference was observed between 0 and 10 inoculum levels which showed tuber weight 137.2 g and 135.82 g, respectively. Tuber weight was significantly reduced with inoculum levels of 100 and above. However, maximum reduction in tuber weight (102.23 g) was recorded at inoculum level of 10,000 J_2 plant^{-1} Pot^{-1} (Table 1).

Table 1: Effect of different inoculum levels of root-knot nematode (*Meloidogyne incognita*) on growth and development of potato cv. Lady Rosetta

Inoculum levels of J_2 plant^{-1} Pot^{-1}	Plant height (cm)	Tuber weight (g)	Fresh weight (g)		Dry weight (g)		Root-knot index (0-5)*	
			Shoot	Root	Shoot	Root	Root	Tuber
0	36.17^{a}	137.25^{a}	130.74^{a}	18.58^{a}	13.08^{a}	1.48^{a}	0.00	0.00
10	35.33^{a}	135.82^{a}	128.28^{a}	18.41^{a}	12.58^{a}	1.43^{a}	0.12	0.10
100	31.75^{b}	127.73^{b}	116.83^{b}	15.29^{b}	10.51^{b}	1.08^{b}	0.43	0.60
1,000	26.58^{c}	113.81^{c}	101.65^{c}	12.70^{c}	10.14^{b}	0.97^{c}	1.53	1.00
10,000	19.75^{d}	102.23^{d}	86.63^{d}	9.61^{d}	8.85c	0.77^{d}	4.25	2.25
SEm±	0.65	2.22	2.55	0.68	0.23	0.02	-	-
C.V. %	5.29	4.40	5.53	11.19	5.07	5.12	-	-
CD (*p*=0.05)	1.882	6.452	7.414	1.986	0.665	0.070	0.152	0.167

Figures indicating common alphabets in superscript do not differ significantly at 5 % level of significance according to DNMRT; *0 = Free; 5 = Maximum disease index

Fresh shoot and root weight (g) got reduced with increase in inoculum level in potato. Insignificant difference was observed between 0 and 10 inoculum levels in both the parameters *i.e.,* fresh shoot weight (g) and fresh root weight (g). The fresh shoot and root weight (g) were significantly reduced with inoculum levels of 100 and above. However, maximum reduction in fresh shoot and root weight were recorded in inoculum level of 10,000 J_2 plant^{-1} pot^{-1} (Table 1).

With increase in nematode inoculum levels, progressive decline in dry shoot and root weight (g) was observed. Insignificant difference was observed between 0 and 10 inoculum levels. The dry shoot and root weight (g) were significantly reduced with inoculum levels of 100 and above. Maximum dry shoot and root weight of 13.08 (g) and 1.48 (g) respectively were recorded in uninoculated check and minimum dry shoot and root weight of 8.85 (g) and 0.77 (g) respectively were recorded at inoculum level of 10,000 followed by 1,000.

With increase in nematode inoculum levels, progressive increase in root-knot index (RKI) observed on root as well as tuber. Maximum root-knot index on root and tuber was recorded were 4.25 and 2.25, respectively with 10,000 inoculum level (Table 1).

With regards to nematode reproduction, significant differences were observed for root, soil and total nematode population build up per plant at different nematode inoculum levels. In general, the nematode population increased progressively with an increase in inoculum levels from 10 to 10,000 J_2 plant^{-1}. Nematode population in root and soil

were increased with inoculum level of 100 and above J_2 of *M. Incognita* pot^{-1}. It was maximum in inoculation with 10,000 J_2 pot^{-1} followed by 1,000 and 100 J_2 pot^{-1} (Table 2).

With increase in nematode inoculum level, progressive increase in number of root galls plant root^{-1} (1 g) was recorded. Maximum root galls observed with inoculation of 10,000 J_2 plant^{-1} pot^{-1} compared to other inoculum level may be due to high initial inoculum level (Table 2).

The nematode population in roots revealed considerable increase in different stages of embedded females and egg masses plant roots^{-1} (1 g) and significantly increased with increase in inoculum levels. Inoculum levels 10 and above significantly increased the different stages of embedded females and egg masses plant root^{-1} (1 g) (Table 2).

Soil nematode population/pot significantly increased with an increase in inoculum levels. A significant increase in soil nematode population was recorded at 10 and above inoculum levels. Maximum nematode population was recorded in 10,000 J_2 inoculated plant^1 and minimum soil nematode population was recorded in 10 J_2 inoculated plant^{-1} (Table 2).

Total nematode population build-up and reproduction rate (P_f/P_i) of *M. incognita* in potato cv. Lady Rosetta, proportional decrease in total nematode population build-up and reproduction rate (P_f/P_i) was recorded. A significant increase in total nematode population was build-up at 10 and above inoculum levels (Table 2). Reproduction rate (P_f/P_i) decreased with increase in inoculum levels, which may be attributed to the nematode competition for feeding on

Table 2: Reproduction of root-knot nematode (*Meloidogyne incognita*) at different inoculum levels in potato cv. Lady Rosetta

Inoculum levels of J_2 plant^{-1} Pot^{-1}	No. of galls/ 1 g root	Nematode population/1 g root		Soil nematode population pot^{-1}	Total nematode population buildup plant^{-1}	Reproduction rate (Pf/Pi)
		Females	Egg masses			
0	0.71e (0.50)	0.71e (0.50)	0.71e (0.50)	0.71e (0.50)	0.71e (0.50)	0.00
10	1.28d (1.64)	1.39d (1.93)	1.28d (1.64)	63.08d (3979.08)	63.47d (4028.44)	404.90
100	4.74^c (22.47)	3.51^c (12.32)	3.34^c (11.16)	96.53^c (9318.04)	98.47^c (9696.34)	97.10
1,000	7.39^b (54.61)	6.11^b (37.33)	6.22^b (38.69)	131.54^b (17302.77)	135.11^b (18254.71)	18.28
10,000	11.55^a (133.40)	9.06^a (82.08)	8.70^a (75.69)	197.43^a (38978.60)	200.78^a (40312.61)	4.03
SEm±	0.11	0.07	0.06	1.91	1.99	-
C.V. %	5.18	4.25	3.85	4.79	4.90	-
CD (*p*=0.05)	0.316	0.21	0.185	5.57	5.809	

Figures in parentheses are re-transformed values (square-root transformation). Figures indicating common alphabets in superscript do not differ significantly at 5 % level of significance according to DNMRT

the limited plant root system. Maximum reproduction rate 404.90 was recorded in 10 J_2 inoculation plant^{-1} and minimum reproduction rate 4.03 was recorded in10,000 J_2 inoculation plant^{-1} (Table 2).

The results obtained under the study are in confirmation with those reported by in the screen house, tomato (cv. DT 69/257) seedlings grown in steam sterilized soil were inoculated with graded inoculum of 5000, 10000, 15000, 20000 and 25000 eggs of *Meloidogyne incognita*. At inoculum levels of 15000, 20000 and 25000 eggs of *M. incognita*, the number of leaves per plant, plant height, fruit yield and root-galls were significantly reduced (Olabiyi, 2006). Khan *et al.* (2012) studied pathogenic potential of *M. arenaria* race-1, *M. incognita* race-1 and *M. javanica* race-2 on mungbean at different inoculum levels *i.e.* 250, 500, 1000, 2000, 4000 and 8000 J_2/kg soil/plant and recorded significant reduction in plant growth parameters (Plant length, fresh and dry weight, seed weight, and number of nodules/root) at inoculum level of 1000 J_2 of *M. incognita*, 2000 J_2 of *M. javanica* and 4000 J_2 of *M. arenaria*/kg soil/plant. Results indicated pathogenic level of 4000, 2000 and 1000 J_2/kg soil/plant of *M. arenaria*, *M. javanica* and *M. incognita* on mungbean, respectively. Bawa *et al*. (2014) studied on pathogenicity of southern root knot nematodes (*Meloidogyne incognita,* Chitwood) on Roma King tomato cultivar (cv.) under screen house temperature 27 ± 2°C and reported that at high inoculum levels of 6000, 8000 and 10000 eggs; flowering, number of leaf, plant height and fruit yield were significantly ($P > 0.05$) reduced. 100 per cent loss occurred on number of fruit and fruit weight of tomato plants inoculated with 10,000 eggs. The root knot nematodes (*Meloidogyne* spp.) are important group of plant parasitic nematode which possesses threat to tomato (*Solanum lycopersicum* L.) production. The inoculum levels were : 0, 500, 1000 and 2000 freshly hatched second stage juveniles (J_2) of root-knot nematodes/kg soil/pot. All pots were inoculated with root-knot nematode J_2 a week after transplanting of the tomato seedlings. From the results obtained, it was observed that all the inoculum levels reduced the stem girth, plant height, number of leaves, and fresh and dry root weights. With increasing the nematode inoculums level resulted in corresponding increase in number of galls and nematode population build-up. The reduction in growth parameters and nematode infestations were found to be proportional to the inoculum level (Kankam and Adomako, 2014). Kshetrimayum (2014) investigated pathogenic level of

M. incognita in green gram. He revealed that plant growth parameters were inversely proportional to the inoculum levels of root-knot nematode, except for fresh and dry root weights. An inoculum level of 100 nematodes/kg pot soil plant^{-1} caused significant reduction in plant growth parameters and proved pathogenic to the green gram plants. Osunlola and Fawole (2015) who experimented the pathogenicity of *M. incognita* on three sweet potato cultivars : Kayode, TIS 4400-2 and TIS 70357-OP-1-79 in a screen house experiment in Nigeria and reported that *M. incognita* significantly ($p < 0.05$) reduced the fresh shoot weight by 16.3-23.6%, fresh root weight by 28.3-62.3 %, number of tubers by 63.2-69.2% and tuber yield by 72.3-83.2%. The gall index and the final nematode population increased with increase in inoculum density. The result showed that *M*. *incognita* caused growth, yield and quality reduction in sweet potato. Anamika (2015) studied on pathogenicity test of *Meloidogyne incognita* on different vegetable crops (tomato, brinjal, spinach and beet root) at different inoculum levels of 500, 1000, 2000 and 3,000 larvae per plant in pot culture. The influence of four initial inoculum densities of root-knot nematode, *Meloidogyne incognita* and plant age on root-knot disease of test crop showed significant reduction in the plant growth parameters. Increase of initial inoculum densities resulted in increased in root-knot there by increasing nematode population, but this increase was inversely proportional with the nematode population which was density dependent which causes competition for nutrition among the developing nematodes within available root system and also due to inability of juveniles of subsequent generation to find infection sites.

4. Conclusion

Experiment pertaining to prove the pathogenicity revealed that sixty days after initial inoculum level of 100 J_2 plant^{-1} pot^{-1} (10 kg soil) significantly reduced the plant growth parameters *viz.,* plant height (cm), tuber weight (g), fresh shoot and root weights (g) and dry shoot and root weights (g) whereas, maximum reduction were observed at inoculum level of 10,000 J_2 plant^{-1} pot^{-1} as compared to uninoculated check. No significant reduction in plant growth parameters was observed between uninoculated check and initial inoculum level of 10 (J_2 plant^{-1} pot^{-1}).

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