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Original Research Article

Study on Pathogenicity and Effect of Different Inoculum Levels of Potato Cyst Nematode (*Globodera rostochiensis*) on Potato (*Solanum tuberosum* L.)

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A B S T R A C T

Potato cyst nematodes (PCN) (*Globodera* spp.) are serious potential pest of potato, leading to severe economic consequences, and are thereby regulated globally to restrict their movement along with potato seed tubers. Recent new detections from India in Uttarakhand, Jammu, Kashmir, and Himanchal Pradesh have focussed attention on the potential spread and economic justifications. Considering the impact of PCN, a pot culture experiment was conducted under polyhouse condition to study the pathogenic potential of potato cyst nematode, *Globodera rostochiensis* on potato cv. Kufri Jyoti at different inoculum levels of 0, 10, 100, 1000 and 10,000 Juveniles/pot. The results revealed that, the PCN Index was 4.0 at inoculum level of 10,000 J2/pot and 1.66 at 10 J2/pot. However, the reproduction factor was found to be highest (268.96) in the lowest inoculum level of 10 J2/ pot; followed by 121.29, 25.006 and 5.039 with initial inoculum levels of 100, 1000 and 10,000 J2/pot, respectively. Gradual increase in the nematode inoculum levels was reported to be declining the plant growth parameters, progressively from 100 to 10,000 J2/pot. The maximum reduction in yield over uninoculated control was recorded at inoculum level of 10,000 J2/pot (86.95%), followed by 62.97% and 38.31% at 1000 and 100 J2/pot, respectively.

Introduction

Potato cyst nematodes have been of great economic importance to potato (*Solanum tuberosum* L.) production, since their introduction to Europe in the mid of 1880's. They originated from the Andean region in South America (Grenier *et al*., 2010) but have subsequently spread to other potato-growing regions of the world (Hockland *et al*., 2012). The Potato Cyst Nematode species *Globodera rostochiensis* and *G. pallida* are among the most regulated quarantine pests globally, with a potential to cause potato yield losses of up to 70% (varying between 10-75%) as the demand of nutrition increases with the reproductive phase of nematode (Turner and Subbotin, 2013). Potato is one of the most important staple crops in the hilly states of India. Several studies have shown the presence of PCN in soil from potato cultivated fields around the Nilgiri hills and Kodaikanal hills of Tamil Nadu, India (Jones 1961; Thangaraju, 1983; Seenivasan *et al*., 2017). Among North Indian states, the presence of potato cyst nematodes was reported first time by Singh *et al*., (2010) in Himachal Pradesh. Later on, 12th October, 2018 Government of India imposed a domestic quarantine and restricted sale of seed potatoes outside Himachal Pradesh, Uttarakhand, Jammu and Kashmir along with

Tamil Nadu due to presence of potato cyst nematodes (Chandel *et al.*, 2020). It has now become an important biotic constraint in sustainable production of potato in the hilly regions (Mhatre *et al*., 2019).

Plant infected with PCN results in diversion of the nutrients of host plant to the nematode and causes physical damage to root tissue as the nematodes migrate through the root cortex, leading to potential secondary infections by opportunistic plant pathogens (Jones *et al*., 2013). This results in reduced water and nutrient uptake, leading to decreased crop yield. To ensure effective penetration of root tissue, the nematodes secrete effectors, comprised of proteins, that can alter the host cell structure. Additionally, they also suppress host plant defenses (Haegeman *et al*., 2012; Yang *et al*., 2019), thereby increasing the susceptibility of the plant to attack by other secondary infection by pests and pathogens. Due to their adverse effect on the quality and quantity of tuber yield, easy transmission and difficulties in management & eradication has made this pest as the big constraint in potato cultivation throughout the world. Thus, PCN are therefore included in the list of International as well as domestic quarantine pathogens of many countries including India (Mhatre *et al*., 2019).

The parasitic association of potato cyst nematodes in potato field usually shows chlorosis of leaves, stunting of plants, poorly developed root system and small sized tubers. PCN cysts are present on the root surface as well as in soil.

Hence, the present investigation was conducted to study the effect of different inoculum levels of potato cyst nematodes on plant growth parameters of potato under polyhouse condition.

Materials and Methods

Collection of root exudates

The potato tubers susceptible to Potato Cyst Nematode of potato cv. Kufri Jyoti was sown in pots of 2 kg capacity filled with sterilized pot mixture soil (Vermicompost: Sand: Soil @ 1:1:2 ratio) in a glasshouse.

Three weeks after sowing, the plants were uprooted and cleaned thoroughly under running water to remove adhering soil and then transferred into 1litre beaker containing 250 ml of tap water. Plants roots were then suspended in beaker and kept for overnight for incubation at 18-24℃ in the dark (Only the root zone was immersed in water). Then the root exudates were filtered through a Whatman No.1 filter paper for getting a clear suspension, then stored at 4 ℃ for short period prior to use as hatching agent as described by European and Mediterranean Plant Protection Organization (EPPO) (2013).

Collection of second stage juveniles from cysts

For extraction of juveniles from cysts, the soil sample was collected from potato fields having previous history of potato cyst nematode (*G. rostochiensis*) infestation. Then the soil samples were air dried and cysts were extracted by floatation using Fenwick can method (EPPO, 2013). For hatching, 5 days presoaked hydrated cysts of PCN were placed in collected root exudates from 3 weeks old potato plants of cv. Kufri Jyoti.

Freshly formed cysts of same reproduction cycle were collected on the basis of colour to avoid chances of diapause phase of PCN (Twomey *et al*., 1995). The freshly harvested juveniles were counted and collected for further studies.

Pathogenicity of *Globodera* spp. on potato cv. Kufri Jyoti

The experiment was conducted in Polyhouse facility at Tixen (Munsiyari), Potato Seed Multiplication Farm, Department of Horticulture, Pithoragarh (State Government of Uttarakhand). During the experiment, April-July 2020, the temperature ranged from 14-26ºC. The experimental site is located at an elevation of 2567 MSL at 30.0623 °N latitude and longitude 80.2370 °E. The pot culture experiments were conducted under polyhouse condition with five treatments and three replications in a Completely Randomized Design. Plastic pots of 5 kg capacity were selected and filled with sterilized (autoclaved at 121ºC for 40 minutes) pot mixture soil (1:1:2; Vermicompost: Sand: Soil). The equal size of healthy tubers was obtained from Potato Seed Federation Madkot, Munsiyari, Uttarakhand. Healthy tubers of susceptible cv. Kufri Jyoti were sown in pots. After emergence of the seedlings at 25 days after sowing different populations (0, 10, 100, 1000 and 10,000) of freshly hatched second stage juveniles (J2 of *Globodera rostochiensis*) per plant were inoculated by making four holes near the rhizosphere of each seedling. After inoculation, the holes were covered followed by light irrigation and uninoculated plants were treated as control. The plants were regularly watered until their harvesting. The experiment was terminated after 100 days of inoculation and observations of plant growth parameters, tuber yield, reproduction factor and potato cyst nematode index were recorded. Potato cyst nematodes index was determined using 1-4 grades, grade 1 = no females/inch root, 2 =1 females/inch root, 3 = 5 females/inch root and 4 = > 10 females/inch root was given as per Grainger's test (Grainger, 1952).

Potato cyst nematodes were extracted using Fenwick can method (EPPO, 2013) whereas numbers of juveniles were extracted from soil using Cobb's sieving and Decanting method followed by Modified Baermann's funnel technique. Final population (Pf) of nematodes was calculated by multiplying number of mature cysts found with average number of eggs found per cyst (average number of eggs/cysts was calculated by quantifying eggs found in 10 cyst) and adding it to the total number of Juveniles found per 5 Kg of soil [a+(bxc)]. The multiplication rate of nematode was determined by calculating the reproduction factor (Rf= Pf/Pi) where Pf represented as the final population and Pi is the initial population of nematode inoculated. The data was analyzed statistically by applying the technique of analysis of variance (ANOVA). Mean values were calculated at 5% significance level.

Results and Discussions

After sixty days of inoculation, PCN exhibited some visible symptoms in infested plants that were inoculated with 1000 and 10,000 Juveniles/plant. The results showed that, the PCN Index was highest 4.0 at inoculum level of 10,000 juveniles and 1.66 at 10 juveniles per plant. The results of host pathogenicity were recorded in terms of potato cyst nematode index (PCN Index) which was found to be directly proportional to the level of inoculum. The final root population of PCN was highest i.e. 50396.467 nematodes/plant at the highest inoculum level of 10,000 Juveniles/pot. However, the reproduction factor was highest 268.96 with the lowest inoculum level of 10 Juveniles/pot. The reproduction factor was 121.29, 25.006 and 5.039 respectively with the initial inoculum levels of 100, 1000 and 10,000 Juveniles per pot. Our findings confirm earlier results reported by Vanitha *et al*., 2019 who showed that a heavily infested plant with PCN affect the growth and yield parameter directly.

Table.1 Effect of different inoculum levels of *G. rostochiensis* on plant growth parameters

Treatment (J2/pot)	Shoot Length	Root Length	Vigour index	Fresh Shoot Weight	Fresh Root Weight	Dry Shoot Weight	Dry Root Weight	Yield
10	58.12 (4.48)	27.57 (11.09)	85.683 (6.72)	187.997 (14.34)	23.597 (18.85)	20.500 (14.41)	5.603 (7.69)	468.460 (19.36)
100	50.87 (16.40)	25.51 (17.73)	76.377 (16.85)	166.103 (24.32)	18.443 (36.57)	18.690 (24.81)	5.460 (10.04)	358.403 (38.31)
1000	40.27 (33.82)	19.23 (37.98)	59.500 (35.22)	135.290 (38.36)	12.700 (56.32)	15.130 (36.83)	3.283 (45.91)	215.093 (62.97)
10000	27.47 (54.85)	9.92 (68.01)	37.390 (59.29)	88.230 (59.80)	9.080 (68.77)	6.793 (71.64)	1.940 (68.03)	75.803 (86.95)
Control	60.85	31.01	91.863	219.493	29.080	23.953	6.070	580.987
SE(d)	0.106	0.149	0.156	0.270	0.131	0.702	0.387	0.704
CD (p=0.05)	0.238	0.332	0.348	0.602	0.293	1.566	0.862	1.569

(*Figures in parentheses are per cent decrease over uninoculated control)

Table.2 Effect of different inoculum levels of *G. rostochiensis* on nematode multiplication

Treatment (Pi)	Juvenile/ 5kg soil	Cyst/ 5kg soil	Average number of eggs/ Cyst	Final Population (Pf)	Reproduction Factor	PCN Index
10	17.333	14.667	182.2	2689.6	268.96	1.667
100	44.000	66.333	182.2	12129.933	121.29	2.667
1000	105.333	136.667	182.2	25006	25.006	3.667
10000	170.000	275.667	182.2	50396.467	5.039	4.000
Control	0.000	0.000	0	0	0	1.000
SE (d)	0.408	0.437	-	-	-	0.365
CD (p=0.05)	0.942	1.009	-	-	-	0.813

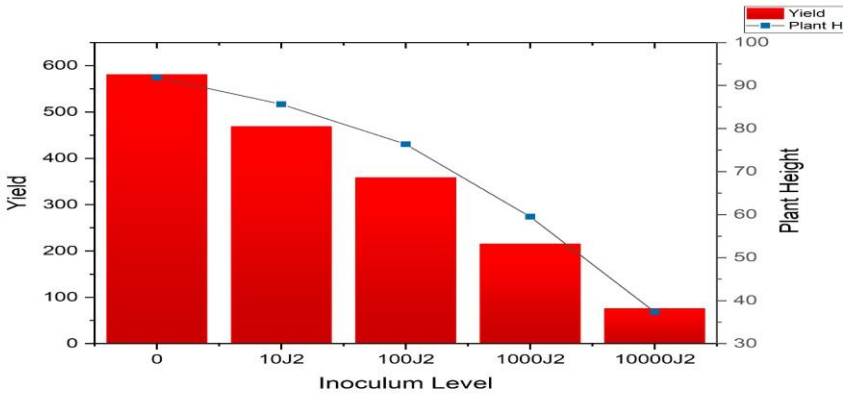

Figure.1 Effect of different inoculum levels of *Globodera* spp. on the plant height and yield

Figure.2 Effect of different inoculum levels (viz. T1- 10 juvenile, T2- 100 juvenile, T3- 1000 juvenile, T4- 10000 juvenile of PCN per pot and T5 is uninoculated control) of PCN on plant growth

Differential environmental conditions, duration of crop growth cycle and differences in the susceptibility of cultivars (use of resistant crop) may reflect differences in the reproduction rates of the nematode. Our results of present study revealed that, the total population (Pf) was highest in the plants inoculated with 10,000 J2/plant and lowest in those inoculated with 10 Juveniles per pot Table- 2. A significant linear relationship was found between the initial (Pi) and final population (Pf) of *Globodera rostochiensis*. Whereas, reproduction factor was significantly reduced with an increase in inoculum levels. This decline trend of reproduction factor suggested, a density dependent phenomenon.

The data presented in Table 1 clearly indicated that with an increase in inoculum levels (except Uninoculated Control and 10 Juveniles/pot) of PCN, there was a relative reduction in all the plant growth parameters viz. plant height, vigour index, fresh weight, dry weight and yield. However, the inoculum level of 10 Juveniles/pot did not show any significant variation in plant growth parameters and all the parameter were found at par with uninoculated plants. The highest reduction in plant growth was recorded at a level of 10,000 Juveniles/pot and the minimum was recorded at a level of 100 Juveniles/pot whereas 1000 Juveniles/pot recorded as an Economic Threshold Level (ETL). Inoculation of 1000 Juveniles/pot showed 35.23, 40.46, 38.67, 35.22 and 62.97 per cent reduction in plant height, fresh weight, dry weight, vigour index and yield per plant respectively compared to uninoculated control.

In our study the plant growth was significantly different between 100 and 1000 J2 inoculated/ pot. The maximum of 86.95 per cent yield reduction was recorded at an inoculum level of 10,000 Juveniles/pot followed by 62.97 and 38.31 per cent at a level of 1000 and 100 Juveniles/pot respectively (Figure 1). The nematode infected plants above inoculum level of 1000 J2/pot were poor in vigour and showed stunted growth. Infected plants also exhibited chlorosis, premature drying, with reduced number and size of tubers. The root biomass remains less developed and a greater number of lateral roots arises resulting in overall decreased plant growth and premature death. The presence of globose shaped females of PCN was also noticed on the surface of root hairs. The progressive decrease in plant growth with increasing inoculum levels of nematodes has also been reported by Trudgill *et al*., (1975)

and Vanitha *et al*., (2019). The results of our study were in agreement with Grainger (1951) who observed that potato cyst nematode decreases the growth and weight of new tubers produced by potato by more than 70% and infested plants are smaller and senesced earlier than uninoculated plant. Several studies report losses ranging from 25 to >50% at Pi of 40 eggs/g soil and from 35 to 75% at Pi of 80 eggs/g soil, nematode densities similar to those tested in our study (Seinhorst, 1982; Elston *et al.*, 1991; Greco and Moreno, 1992; Van den Berg *et al.*, 2006).

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