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Pathogenicity of Root Knot Nematode (*Meloidogyne incognita*) and its Effect on Yield of Cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

Background: Cowpea [*Vigna unguiculata* (L.) Walp.] is an important food legume in the drier region and an essential component of the tropics and subtropics for the cropping system. Heavy infestation of cowpea by root knot nematode *Meloidogyne incognita* led to early senescence of the crop. The experiment was carried out to determine the potential level and the extent of yield loss in cowpea due to root knot nematode.

Methods: A pot culture study and a field experiment were conducted to determine pathogenic potential level of root knot nematode and to determine the yield loss in cowpea with various treatment of 10, 100, 1000 and 10000 freshly hatched juveniles.

Result: The treatment with nematicide, Carbofuran 3G @ 3 kg a.i./ha, reduced the final nematode population *i.e.*, 50.94% in soil, 46.12% on root and 46.51% decrease in root knot index. The present study revealed that 1000 J2/pot was the ETL level that reduced all the growth parameters of cowpea and avoidable yield loss of 15.78% was recorded in Ccowpea due to M. *incognita*.

Key words: Cowpea, *Meloidogyne incognita*, Pathogenicity, Root knot nematode, Yield.

INTRODUCTION

Cowpea [*Vigna unguiculate* (L.) Walp.] is an important food legume in the drier region and an essential component of the tropics and subtropics for the cropping system (Singh *et al.* 2003). In India, it is used for grain and can also be consumed as vegetables and after harvest, the part can be used for animal fodder. Cowpea is available in many areas but now a days its cultivation is spreading throughout the tropical and subtropical regions for the importance and popularity of its nutritional value (Abayomi *et al.* 2008; Aikins and Afuakwa, 2008; Sahoo *et al.,* 2020). Globally the cultivation of cowpea is estimated at around 14.5 million hectares in area with a production of 4.5 million tons, which is quite good in case of India as 23012 ha, with a production rate of 133587 tons and productivity is 5.8 t/ha. The leading states in India are U.P, Bihar, Jharkhand, West Bengal and Odisha.

In Odisha, the total area is about 52.97 thousand hectares, producing 39.54 thousand tons (Agricultural Statistics, 2014-2015). In Odisha, cowpea is an important commercial vegetable crop grown mainly in almost all districts both during the *kharif* and *rabi* season. Root knot nematode, *Meloidogyne incognita* has become a major pest of nearly all crops and mainly for this cowpea it is a serious pest (Sikora *et al.,* 2005; Youssef *et al.*, 2021), impacting both quality and quantity of produce. A yield loss of 69% caused by root knot nematode was reported by (Bridge *et al.* 2005; Osipitan *et al.,* 2021). Keeping in view the importance of crop scanty information available on pathogenicity of root knot nematode on cowpea and its effect on yield, the experiment was carried out to determine the potential level and the extent of yield loss in cowpea due to root knot nematode.

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MATERIALS AND METHODS

Experimental site and Preparation of soil and pots

The experiment was conducted in College of Agriculture and Technology, OUAT, Bhubaneswar. Sterilization of the soil mixed with sand and FYM in a ratio of 2:1:1 at 1.1 kg/cm^2 pressure for one hour daily for two consecutive days and earthen pots of 15 cm diameter were filled up after surface sterilization in 4% formalin solution.

Maintenance, culturing, isolation of egg masses and juveniles of *Meloidogyne incognita*

Egg masses of *Meloidogyne incognita* was collected from infected roots and the population was multiplied on a susceptible variety (Pusa Ruby of Tomato) grown in pots. Galled roots of susceptible plants were collected and egg masses were picked up. The collection of juveniles was continued for 7 to 8 days and was used subsequently for experimental purposes. Sterilization by Mercurochrome (0.1%) for 30 minutes was done to avoid bacterial and fungal contamination. The nematode suspension containing the J2 was taken in a beaker after being washed 4-5 times in sterilized water and the standardization of nematodes number was done with a counting disc.

Inoculation of nematodes

10,100, 1000, 10,000 per pot were induced around the root zone by making small holes. The Treatments were as follows: T1= Check (No nematodes); T2= 10 nematodes (J2)/ pot; T3= 100 nematodes (J2)/ pot; T4= 1000 nematodes (J2)/ pot; T5= 10000 nematodes (J2)/ pot including an uninoculated check arranged in completely randomized design. At 45 days after inoculation, the experiment was terminated and observations were recorded on plant growth parameters.

Estimation of *Meloidogyne incognita* population in root and soil

Infected roots measuring 1g from each replication of different treatments are stained by Byrd method (Byrd *et al.*, 1983). Nematode population in soil was estimated by Cobbs' sieving technique (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1961). The ratio of nematode multiplication in different treatment was calculated by using the following formula:

$$\text{Reproduction factor (Rf value)} = \quad Rf = \frac{Rf}{Ri}$$

Where,
Rf= Rate of nematode multiplication.
Pf= Final nematode population.
P_i= Inoculated (initial) nematode population.

Assessment of yield loss due to root-knot nematode in cowpea

Pair plot technique was used *i.e.* two blocks treated and untreated each having 10 sub plots of size 3 m×2 m serving as replication. For treated plots, carbofuran (Furadan 3G) @ 3kg ai/ha was applied before planting cowpea seeds followed by light watering. 100 days after sowing, the experiment was terminated and observations were recorded.

Statistical analysis

Observation recorded subjected to statistical analysis in a completely randomized design. The comparison of the treatment means was done by calculating S.E. (M) and (C.D) in the following manner.

$$S.E\ (M) = \sqrt{\frac{2 \times \text{Error mean of square}}{\text{Number of replications}}}$$

$$C.D. = t \text{ at } 5\% \text{ for error d.f.} \times S.E\ (M)$$

Data obtained were analysed according to't' test for paired comparison at 5% level of probability.

$$\text{'t' error degree of freedom} = \frac{X_1 - X_2}{S_d}$$

Where,
X_1= Mean yield of the treated plot.
X_2= Mean yield of untreated plot.

$$Sd = \frac{s}{\sqrt{n}}$$

Where,

$$S = \text{Standard deviation} = \sqrt{\Sigma d2 - \frac{\Sigma d2}{n}}$$

n= Number of paired plots.
d= Differential value between two paired plots.

The avoidable yield loss and percent increase in yield over control (untreated) by following formulae (Pradhan, 1964).

Avoidable yield loss (%) =

$$\frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of treated plot}} \times 100$$

Increase in yield (%) =

$$\frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of untreated plot}} \times 100$$

RESULTS AND DISCUSSION

Fresh and dry weight of shoot and root

Table 1 shows, when there is an increase in the inoculums level of *Meloidogyne incognita*, there is a significant reduction in fresh and dry shoot weights observed which ranged from, 2.65 to 33.06 and 5.43 to 32.86% (Fig 1), respectively. A significant reduction was observed at 1000 J2 / kg soil inoculum level. There was 23.42 and 29.07% reduction in fresh and dry weight of shoots respectively. The reduction in fresh and dry root weights ranged from, 4.14 to 31.08 and 2.64 to 35.97% respectively. The significant reduction could be observed at and above 1000 J2/ kg of soil. At 1000 J2 / kg soil inoculum level, there was 25.24 and 25.92% reduction in fresh and dry weight of roots respectively.

The rate of nematode multiplication showed a declining trend with the increase in the initial inoculum level suggesting it to be a density depending phenomenon. Lesser is the nematode, lesser is the competition of nematode for host penetration, food and space, therefore greater nematode reproduction rate on cowpea (Haider *et al.*, 2003 and Kumar *et al.*, 2011). An inverse relationship was observed between the nematode build up and inoculum levels (Hussain *et al.* 2011). All these results represent the outcome of an interaction of plant growth rate with nematode attack and population increase (Haider *et al.*, 2009).

Plant height and root length

The plant height and root length (Fig 2) were progressively reduced with the increasing nematode population/inoculums

Fig 1: Effect of initial levels of *Meloidogyne incognita* on root growth of cowpea.
T1= No J2/pot, T2= 10 J2/pot, T3= 100 J2/pot, T4=1000 J2/pot, T5= 10000 J2/pot.

Fig 2: Effect of initial levels of *Meloidogyne incognita* on shoot growth of cowpea.
T1= No J2/pot, T2= 10 J2/pot, T3= 100 J2/pot, T4=1000 J2/pot, T5= 10000 J2/pot.

Table 1: Effect of different initial inoculums of *Meloidogyne incognita* on shoot growth and root growth parameters on cowpea.

Treatments	Shoot height (cm)	% decrease	Fresh shoot weight (g)	% decrease	Dry shoot weight (g)	% decrease	Root length (cm)	% decrease	Fresh root weight (g)	% decrease	Dry root weight (g)	% decrease
T1= Un-inoculated	38.10	-	23.35	-	4.23	-	26.53	-	17.63	-	3.78	-
T2= 10 J2/kg soil	37.85	0.65	22.73	2.65	4.00	5.43	26.03	1.88	16.90	4.14	3.68	2.64
T3= 100 J2/kg soil	37.25	2.23	22.25	4.71	3.90	7.80	25.18	5.08	16.00	9.24	3.53	6.61
T4= 1000 J2/kg soil	29.50	22.57	17.88	23.42	3.00	29.07	23.68	10.71	13.18	25.24	2.80	25.92
T5= 10000 J2/kg soil	26.80	29.65	15.63	33.06	2.84	32.86	23.23	12.43	12.15	31.08	2.45	35.97
SE(M)	0.42	-	0.55	-	0.23	-	0.66	-	0.96	-	0.28	-
CD (0.05)	0.92	-	1.18	-	0.51	-	1.43	-	2.07	-	0.61	-

(Mean of four replication)*.

levels as shown in Table 1. The data revealed that there were no significant differences in the shoot growth parameters with an initial inoculum level of 10 and 100 J2 per pot in comparison to un-inoculated treatment. The reduction in shoot length, ranged from 0.65 to 29.65% significant reduction could be observed at 1000J2 / kg soil inoculum level. There was 22.57% reduction in shoot length. Similarly, the reduction of root length ranged from 1.88 to 12.43%. But significant reduction was noticed at and above 1000J2/ kg of soil with a reduction percentile of 10.71% reduction in root length. The nematode root infection renders plants unfit to absorb water and nutrients and other essential elements from the soil leading to poor plant growth (Gowen *et al*, 2005). Progressive reduction in plant growth parameter *viz.*, height, fresh and dry weight of shoots and roots was noticed with the increase in the inoculum level of *M .incognita* (Fig 4).

Number of galls /egg mass, final nematode population and multiplication rate

Further increase to 10,000 juveniles in the inoculum resulted in more root knots but not significant with 1000 level. The number of egg masses / plant varied from 28.75 to 181.50 and number of galls /plant varied from 32.25 to 182.00 with the increase of inoculum level from 10 to 10,000 /juveniles per kg of soil. The nematode population per 200cc of soil was minimum (199.50) at 10 juveniles' level and maximum (7385.50) at 10,000 Juveniles level, when inoculated initially. The total population of nematodes both in soil and root varied

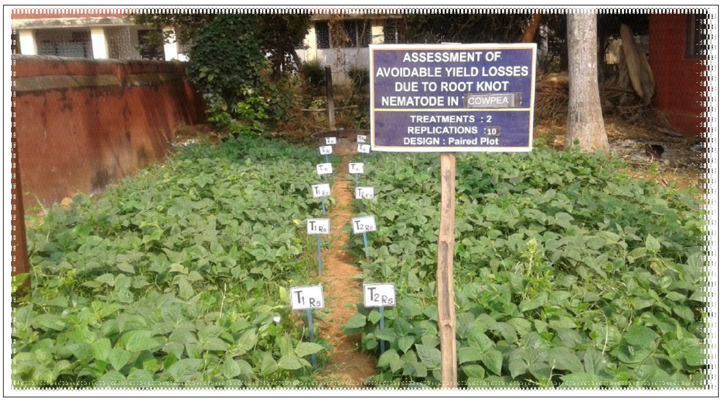

Fig 3: Field view of yield loss experiment of cowpea due to *Meloidogyne incognita.*

Table 2: Effect of different initial inoculums of *Meloidogyne incognita* on nematode multiplication in cowpea.

Treatments	*Number of galls per plant	*Number of egg masses per plant	**Nematode population (200cc soil)	**Final population	Multiplication factor R=Pf/Pi
T1= Un-inoculated	0 (1.0)	0(1.0)	0 (1)	0 (1)	0
T2= 10 J2 /kg soil	32.25 (5.76)	28.75(5.40)	199.50 (2.32)	1451.50 (3.15)	145.15
T3= 100 J2 /kg soil	45.25 (6.80)	43.50(6.66)	1603.75 (3.20)	8389.00 (3.92)	83.89
T4= 1000 J2 / kg Soil	172.50 (13.16)	168.50(13.00)	5611.00 (3.75)	28118.00 (4.44)	28.11
T5= 10000 J2 / kg Soil	182.00 (13.53)	181.50(13.50)	7385.50 (3.87)	36695.00 (4.56)	3.66
SE(M)	(0.21)	(0.26)	(0.06)	(0.07)	-
CD (0.05)	(0.47)	(0.56)	(0.18)	(0.15)	

(Mean of four replication). **Figure in parentheses are n+1 value. **Figure in parentheses are log (n+10) values.

Table 3: Effect of *Meloidogyne incognita* on vegetative growth of cowpea.

Treatments	Plant height (cm)	% increased over untreated	Fresh weight of shoot(g)	% increased over untreated	Dry weight of shoot (g)	% increase over untreated	Fresh weight of root (g)	% increased over untreated	Dry weight of root (g)	% increased over untreated
Treated(Carbofuran @3Kg a.i/ha)	116.45	54.64	34.58	26.94	4.10	25.38	15.31	42.15	2.78	41.11
		% decreased over		% decreased over		% decreased over		% decreased over		% decreased over
Untreated	75.30	treated 35.33	27.24	treated 21.22	3.27	treated 20.24	10.77	treated 29.65	1.97	treated 29.13
Cal. 't' Value	28.58	-	9.92	-	4.61	-	11.35	-	4.50	-

(Mean of ten replication)*

Table 4. Final Population of *Meloidogyne incognita* showing root knot index and yield in cowpea.

Treatments	Root knot nematode population						Yield	
	Per 200cc soil	% decreased over untreated	5g root	% decreased over untreated	Root gall index	% decreased over untreated	(Kg/plot)	% increase in yield over untreated
Treated (Carbofuran @3kg a.i/ha)	135.3	50.94	29.9	46.12	2.3	46.51	3.4	21.42
Untreated	275.8	% increased over treated 103.84	55.5	% increased over treated 85.61	4.3	% increased over treated 86.95	2.8	% loss in yield over treatment 15.78
Cal. 't' Value	96.9	-	19.66	-	6.9	-	6.1	-
Table 't' Value	2.26	-	2.26	-	2.26	-	2.26	-

(Mean of ten replication)*

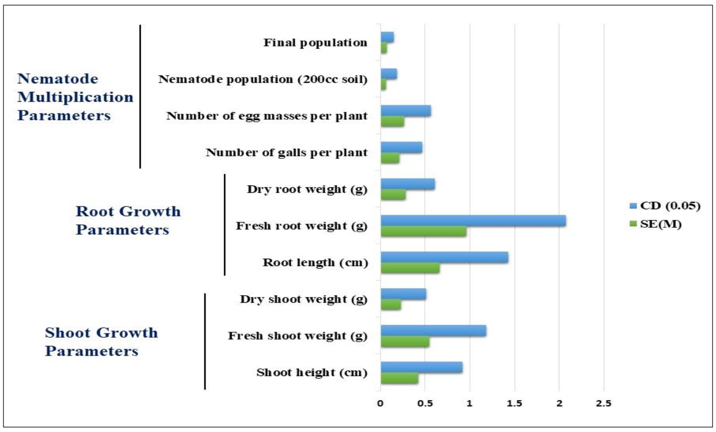

Fig 4: Comparison of shoot, root and nematode multiplication parameters after different inoculums of *Meloidogyne incognita* in cowpea (CD: Confidence distribution; SE (M): Standard error of the mean).

from 1451.50 to 36695.00 by an increase of inoculum level from 10 to 10,000 Juveniles (Table 2). However, no significant difference was recorded between the total nematode populations of pots with an initial inoculum level of 1000 Juveniles and 10,000 Juveniles. The mean final population of root knot nematode in untreated plots increased because of presence of suitable host and it decreased in treated plots because of efficient killing effect of the nematicide applied (Pradhan *et al.,* 2020).

The reproduction rate of M. *incognita* was inversely related to the inoculum levels and was maximum at a lower level (10 J2 /pot) and minimum in the highest nematode (10000 J2 /pot) inoculum. Maximum multiplication (145.15) was recorded in treatment with 10 J2 per pot as against the minimum (3.66) in 10,000 J2/pot (Table 2). The multiplication rate was 28.11 in 1000 J2 per pot. The high rate of multiplication at low levels of inoculum, on the other hand, could be due to positive factors like abundance of food, lack of competition and the inability of the host to support these levels of the population (Das and Swain, 2013). The rate of nematode multiplication showed a declining trend with the increase in the initial inoculum level (Fig 4).

Yield loss assessment

Results of yield loss assessment (Fig 3) in cowpea due to root knot nematode depicted in Table 4 showed that yield in treated plot 3.4 kg was significantly higher than untreated plot 2.8 kg, thereby leading to 15.78% avoidable loss in cowpea yield due to *M.incognita.* Root knot index at harvest in treated plot 2.3 was lower than untreated plot 4.3 (Table 4). Significant differences were also noticed between the treated plot and untreated plot with respect to root knot nematode population in 5 g of root and 200cc of soil, Plant growth character like plant height, Fresh and dry weights of shoot and roots. Root knot nematode population of 275.80 per 200cc soil and 55.5 in 5 g root was recorded from untreated plot as against 135.3 and 29.9, respectively from treated plot. (Table 4). Mean plant height of 116.45 cm along with fresh shoot weight 34.58 g and fresh root weight of 15.31 g were observed from the treated plot whereas the untreated plots had produced the plants of 75.30 height having 27.24 g weight of shoot and 10.77 g fresh weight of roots (Table 3). Jaiswal *et al.*, 2011 and Vinod *et al.,* 2011 were of the opinion that the increase in the nematode population and subsequent reduction in yield of cowpea are directly influenced by initial density of nematodes in the soil. This view holds true with the present finding where in plant growth was proportionately affected with the increase in the initial inoculum levels of the nematode. These results are also agree with those reported by Abbasi and Hisamuddin, 2014 on *Vigna radiate*.

It was noted that nematode population (soil as well as root) and root knot index suppressed in plots that are treated with nematicide. This was due to the efficacy of carbofuran in controlling root knot nematode and its utility as an effective nematicide. The nematicide effect of carbofuran is reported by (Adegbite, 2011) on cowpea. The increased final population of nematode because of the presence of the suitable host and decreased population in the treated plot because of efficient killing effect of the applied chemical were reported by Ali, 2009. Nematodes not only suppress the plant growth but also interfere in the nodulation, nitrogen fixation and adversely affect the yield (Rehman *et al.,* 2012).

CONCLUSION

A significant reduction was observed at and above 1000J_2/ Kg of soil compared to other inoculums where a maximum decrease in phenotypical changes and yield loss of plants was observed. So it is advised to farmers they can cultivate cowpea up to some extent, where there is less pathogenicity of *Meloidogyne incognita* in their cultivated field or they can go for cultural methods of integrated nematode management. But in nematode prone areas infected to *Meloidogyne incognita* farmers are advised to apply carbofuran (Furadan 3G) @ 3 kg ai/ha before planting cowpea seeds followed by light watering for the better enhancement and yield of the crop cowpea.

Conflict of interest: None.

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