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Estimation of Yield Loss of Cowpea, [*Vigna unguiculata* (L.) Walp.] with Reaction Response of Few Genotypes against Root Knot Nematode, *Meloidogyne incognita* (Kofoid and White), Chitwood

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ABSTRACT

Background: Root knot nematode, *Meloidogyne incognita* is one of the main biotic constraints in reducing the quantity and quality of the cowpea crop in most of the growing areas of the world. The nematode produces galls on roots and disrupt the physiology of the plant by reducing crop yield and product quality and are therefore of great economic importance.

Methods: Studies were conducted to estimate the yield loss of cowpea in field condition along with evaluation of few genotypes under net house condition against root knot nematode based on gall index, reaction response and number of egg masses/ 5 g of root at Directorate of Research, BCKV, West Bengal. Estimation of cowpea yield loss was conducted with the parameter of mean yield, percentage avoidable and increase in yield, Root Knot Index (RKI in 1-5 scale) and final nematode population reduction.

Result: Under treated condition, cowpea yield was significantly increased ranging from 10.93 to 14.58% with 37.19 to 38.17% reduction of nematode population. The range of avoidable yield loss was 9.85 to 12.72%. In response reaction, 28 genotypes were found as moderately resistant. Local cowpea cultivar, Kashikanchan may be avoided at nematode infection area due to its high susceptibility and the moderately resistant genotypes may be suggested for the use in future breeding studies as a parental material to develop the root knot nematode resistant cultivars.

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

INTRODUCTION

Globally, cowpea [*Vigna unguiculata* (L.) Walp] is a very valuable leguminous crop due to its nutritive value. It is an important food legume and essential component of cropping systems in the drier region of the tropics and subtropics (Singh, Ehlers, Sharma and Freirefilho, 2003) and it is an important livelihood of millions of people (Quin, 1997). The grain contains between 20 and 25 per cent proteins (Kay, 1979). Cowpea is a valuable and dependable commodity that produces income for many smallholder farmers and traders in sub-saharan Africa (Langyintuo, Lowenberg-DeBoer and Faye, 2003). It is a deep rooted crop which does well in sandy soil and is more tolerant to drought than soybean (Dadson, Hashem, Javaid, Joshi and Allen, 2003). It forms a major component of the tropical farming system because of its ability to improve soil fertility through nitrogen fixation (Abayomi, Ajibade, Sammuel and Saadudeen, 2008). It is an important fodder which is also used for erosion control. In addition to being well adapted to the drier regions of the world, it can produce yield where other crops fail (Hall *et al*., 2002). Cowpea can fix about 240 kg ha^{-1} atmospheric nitrogen and makes about 60-70 kg ha^{-1} nitrogen available for succeeding crops grown in rotation (Aikins and Afuakwa, 2008). Plant parasitic nematode is a serious pest and constitutes a major production constraint to cowpea in most growing areas of the world (Sikora and Greco, 1990; Sikora, Greco and Silva, 2005). Symptoms of nematode damage on cowpea include stunted growth, yellowing, presence of galls, excessive root branching and reduced functioning of root systems. Poor germination and death of seedlings may be observed in case of heavy infestations (Mishra, 1992). Several nematode species are known to cause losses to cowpea throughout the world. Caveness and Ogunfowora (1985) listed 55 species of plant parasitic nematode associated with cowpea production. The root knot nematode *Meloidogyne incognita* and *Meloidogyne javanica*, are documented to cause major losses, with *M. incognita* indicated to be the most detrimental species to cowpea

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(Sarmah and Sinha, 1995). However, cowpea is very susceptible to root knot nematode and suffers massive infections which have caused serious economic constraints in the production of the crop (Yakub, Izuogu , 2013). Several researchers have screened cowpea genotypes for resistance to *Meloidogyne* spp. In India, Subramaniyan *et al.* (1997) identified three of 37 lines as resistant to *M. incognita.* In Cuba, eight of nine cultivars were rated as resistant (Rodriguez *et al.*, 1996) and in Venezuela, one of eight varieties was resistant to *Meloidogyne* spp. (Renato *et al*., 1995). Recently several breeding lines of cowpea have been developed in Nigeria, but little is known about their reaction to *Meloidogyne* species under field conditions. In developing nations, control of nematodes may be uneconomical and producers may not be trained in methods of application. Use of resistant varieties reduces labor cost and is an environmentally sound management strategy for reducing production cost (Roberts *et al*., 1996). Present investigation was undertaken for estimation of cowpea yield loss in field condition along with assessment of the reactions of few cowpea genotypes under net house condition against root-knot nematodes.

MATERIALS AND METHODS

A. Yield loss estimation

The experiment was conducted on nematode sick plots at Central Research Farm, Gayeshpur, Nadia, West Bengal of Bidhan Chandra Krishi Viswavidyalaya during the *kharif,* 2015 and 2016 to evaluate the yield losses due to root-knot nematode, *M. incognita* on cowpea. Paired plot technique given by (Leclerg, 1971)was employed to conduct the experiment, *viz.*, (T1) treated and (T2) untreated and each treatment was replicated ten times in plot size measuring 3.5m × 3 m each. After land preparation initial soil nematodes population was determined based on counting number of nematodes (2nd stage juveniles) by Cobb's sieving and decanting method from multiple samples and presented as number of INP/ 200 cm^3 soil. The requisite buffer area between the plots maintained was 1m X 1m. Recommended fertilizer doses and other agronomic practices were followed for management of the crops. Seeds were sown at a spacing of 40 cm X 30 cm plant to plant and row to row, respectively. Ten plots were treated with Carbofuran 3G granules @ 3 kg a.i./ha prior to seed sowing and the rest ten plots were kept as untreated. Plants from each plot were uprooted and washed carefully under the tap water to remove adhering soil particles at harvest. Number of galls on roots per plant was recorded and gall indices 1 to 5 scales were worked out. Yield (kg/plots) was calculated from each plots and then converted to quintal/ha. For final nematode population soil sample comprising of several subsamples were collected from different sites of each plot and mixed thoroughly and 200 cc of soil was drawn as a composite sample and processed by Cobb's sieving and decanting method followed by modified Baermann's technique.

Statistical analysis

Per cent increase in yield and avoidable loss was calculated as per the formula given in the following example.

Method of computation of t-value

Yields from a field experiment conducted to compare treated and untreated plots and some of the computations necessary for a test of significance between the two treatments.

Paired plots (No.)	Yield/plot (kg) Treated (x_1)	Yield/plot (kg) Untreated (x_2)	Difference (x_1-x_2)	Deviations from the mean of the difference (d)	Square of the deviations from the mean (d^2)
1	14.6	6.2	8.4	- 0.1667	0.0278
2	12.6	3.3	9.3	0.7333	0.5377
3	15.0	6.8	8.2	- 0.3667	0.1345
4	15.6	6.6	9.0	0.4333	0.1877
5	12.7	4.2	8.5	- 0.0667	0.0044
6	12.0	4.0	8.0	- 0.5667	0.3211
Sum	82.5	31.1	51.4		1.2132
Mean	13.75	5.18	8.5667		

From first set of paired plots in Table, the values in the last three columns are determined as follows:

$$\text{Difference, } x_1\text{-}x_2 = 14.6 – 6.2 = 8.4$$

Deviation from the mean of the difference (d)

$$d = 8.4 - 8.5667 = 0.1667$$

Square of the deviation from the mean difference (d^2)

$$d^2 = (- 0.1667)^2 = 0.0278$$

Similar procedure is followed to obtain the values for the remaining paired plot data. In this example:

n = number of paired plots =6

(n-1) = degree of freedom *i.e.* 6-1 = 5

Mean difference = 51.4/6 = 8.5667

S = Standard deviation

$$= \frac{\sqrt{\text{sum of } d^2}}{\text{n-1}} = \frac{\sqrt{1.2132}}{5} = \sqrt{0.2426} = 0.4925$$

$$\text{Standard error (Sd)} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

Sd = standard error of mean difference

i.e.

$$S = \frac{0.4925}{\sqrt{n}} = \frac{0.4925}{\sqrt{6}} = 0.201$$

Substituting in Equation (1), we obtain:

$$T = \frac{X_1\text{-}X_2}{sd} = \frac{8.57}{0.201} = 42.64 = t$$

This calculated value of 't' needs to be compared with tabulated value to ascertain whether the observed value is statistically significant at 0.05 and 0.01 probability levels.

From the two mean tables in Table, one can compute the percentage reduction in actual yield per cent and avoidable losses in yield.

$$100 \times \frac{13.75-5.18}{13.75} = 62.3\%$$

Per cent avoidable losses =

$$\frac{\text{Yield in treated plots-Yield in untreated plots}}{\text{Yield in treated plots}} \times 100$$

Per cent increase in yield =

$$\frac{\text{Yield in untreated plots-Yield in untreated plots}}{\text{Yield in untreated plots}} \times 100$$

B. Screening of genotypes

The present study was carried out in 2016 in the net house of AICRP Nematodes at Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The experiment was laid out in completely randomized design and the treatments were replicated three times. The required amount of soil was sterilized by 10% formaldehyde solution to destroy the pre-plant population of plant parasitic nematode. It was done by thorough mixing of formaldehyde solution with the required amount of soil.The treated soil was then covered by polythene sheet. The cover was removed after 48 hours. The soil was pulverized thereafter to facilitate the volatilization of gas from the soil. The soil was ready to use after 15 days from the removal of polythene cover. The earthen pots were then filled with sterilized soil @ 500 gm soil/ pot.The seeds were sown in a pot and each genotype was sown in three pots having 3 plants per pot. After 10 days of sowing thinning was done leaving only one seedling per pot. Collection of inoculum (*Meloidogyne incognita* race 2) as egg mass was done from brinjal grown in the root knot nematode sick plot. The collected egg masses were then allowed to hatch in a petridish containing tap water. Second stage juveniles (J_2) were collected from Petridish and kept in beaker allowing the juveniles to settle down at the bottom. Then excess water was drained out without disturbing the juveniles. To facilitate counting of inoculum, 2ml of the sample was taken in a counting disc and was observed under stereoscopic binocular. Four to five samples were counted to find out the average number of inoculum (J_2) per ml of suspension. Inoculation @ 500 juveniles per pot was done at 15 days after sowing of the cowpea germplasm in the net house. The required amount of inoculum as per treatment was taken in micropipette and poured in the root zone after making few small holes with the help of a glass rod. This was done to facilitate proper entry of inoculum in the root zone.The cowpea plants were carefully uprooted after 45 days of inoculation to avoid the damaging of roots and other plant parts. The observations on number of galls per plant, number of egg mass/5g root were recorded and gall indexing was done as per the following chart (Table 1).

RESULTS AND DISCUSSION

A. Assessment of avoidable yield losses

The study on assessment of yield losses due to *Meloidogyne incognita* on cowpea revealed that the application of carbofuran 3G @ 3 kg a.i. ha-1 significantly increased the yield of the crop by 10.93 % with significant reduction in *M. incognita* population by 38.17 per cent (Table 3) during 2015. It was found that an infestation of root-knot nematodes on the crop inflicted an avoidable yield loss of 9.85 per cent (Table 2). In 2016, the performance of the crop with reference to all growth attributes under study was significantly better

Table 1: Rating chart for evaluation of host response.

Observations	Gall Index	Host Reaction
No galls and egg masses	0 - 1.0	Highly Resistant
1-10 galls/egg masses	1.1 - 2.0	Resistant
11-30 galls/egg masses	2.1 - 3.0	Moderately Resistant
31-100 galls/egg masses	3.1 - 4.0	Susceptible
101 and above galls/egg masses	4.1-5.0	Highly Susceptible

Source: Used in AICRP on Nematodes.

Table 2: Estimation of yield loss due to *Meloidogyne incognita* in cowpea during 2015.

Crop	Mean yield in treated plot (q/ha)	Mean yield in untreated plot (q/ha)	% avoidable yield loss	% increase in yield
Cowpea	70.34	63.41	9.85	10.93
Paired t-test value (P=0.05)	2.84			
Tabulated t	2.20			

Table 3: Performance of plant growth attributes along with nematode population in cowpea during 2015.

Crop	Germination /M		Nodules/plant		RKI(1-5 scale)		Final nematode population ($200cm^3$ soil + root) *M. incognita*	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cowpea	5.50	5.56	4.13	3.73	1.98	2.63	204.54	330.83
Paired t-test value (P=0.05)	0.48 (NS)		0.66 (NS)		(-) 2.59		(-) 3.91	
Tabulated t	2.20		2.20		2.20		2.20	
Nematode	% reduction of *M. incognita* population over untreated control							
M. incognita	38.17							

INP (Initial nematode population)= 230 J_2/200 cm^3 soil, M= Meter, RKI= Root Knot Index.

in carbofuran 3G @ 3kg a.i. ha^{-1} compared to untreated check. Application of carbofuran 3G @ 3 kg a.i. ha^{-1} significantly increased the yield of cowpea by 14.58 per cent with significant reduction in *M. incognita* population by 37.19 per cent (Table 5). It was found that an infestation of root knot nematodes on the crop inflicted an avoidable yield loss of 12.72 per cent. The reduction in number of gall index in treated plots was 41.91 per cent in the year 2015 and 30.95 per cent in 2016. The average initial population of larvae of root knot nematodes was 230 and 215per 200 cm^3 of soil for two years (2015 and 2016) respectively. In untreated plot, the nematode population at the time of final crop harvest was increased upto 43.47 and 49.77 per cent for two years, respectively (Table 2 and 4).

The statistical analysis of the data revealed that performance of cowpea local variety with Carbofuran 3G at 3 kg a.i./ha was significantly better for two years as compared to the untreated control. The decreasing yield data recorded for the untreated cowpea local variety was probably a result of the stunting action of root-knot nematode (*M. incognita*). The treated plants started profuse vegetative growth than the untreated. Early vegetative growth is very important because it affects the yield at the time of harvesting the plants. The yield of the crop was found to be higher with the application of nematicide-Carbofuran at 3kg a.i/ha. A significant reduction in the yield of the crop in untreated plots was mainly attributed to direct damage of the root system by the feeding activities of root knot nematode (*M. incognita*). The ovicidal effect of carbofuran is more effective in preventing penetration of nematodes into the root. This may suggest that carbofuran acts directly on the nematodes in the soil thereby affecting hatching of eggs and the movement of larvae into the root. This is in agreement with the work of Di-Sanzo (1973), Kinloch (1974, 1982), Adegbite and Agbaje (2007) and Adegbite and Adesiyan (2001). The two seasons data from yield losses on cowpea cv local indicated that the root knot nematode is responsible for an average yield loss to the extent of 11.30 per cent and adoption of suitable management practices against the nematode can enhance average yield of the crop upto12.75 per cent. There is very little information available regarding avoidable yield loss assessment by root knot nematode in cowpea. Toler *et al.,* (1963) also reported 5-10 per cent yield loss in cowpea due to *M. incognita*. Ali (2009) also assessed that unavoidable yield loss up to 25.6 per cent in chick pea and 15 per cent each in pea and lentil was incurred by RKN. Bridge (1972) reported 40 per cent yield loss on cowpea due to *Meloidogyne incognita.* Avoidable yield losses due to infestation of *Meloidogyne incognita* in field pea cv. Bonnevilla was upto 18.32 per cent in untreated plots as compared to treated plots (Haider *et al.,*2009).The present findings are also compared with some other vegetable crops. The results obtained under study are in conformity with those of Darekar and Mhase, 1988 who reported 36.72 per cent yield losses in bitter gourd *(M. charantia*) CV. Coimbatore White long due to *M. incognita* race 3 and Krishnaveni and Subramanian, 2002 and Khanna and Kumar (2003) also recorded 69.2 per cent and 22.9 to 42.8 per cent losses in yield of cucumber and bitter gourd respectively due to *M. incognita*. Similar findings were also reported by Khan *et al*. (2014) on bottle gourd, snake gourd, bitter gourd, cucumber and pumpkin due to the infestation of root-knot nematodes. Lack of awareness among the farmers increases the losses caused by these tiny creatures if proper management strategies are not adopted on the crops. Therefore, necessary steps should be taken in the field of awareness as well as management of these nematodes to minimize economical losses to the farmer.

Table 4: Estimation of yield loss due to *Meloidogyne incognita* in cowpea during 2016.

Crop	Mean yield in treated plot(q/ha)	Mean yield in untreated plot (q/ha)	% avoidable yield loss	% increase in yield
Cowpea	55.00	48.00	12.72	14.58
Paired t-test value (P=0.05)	3.35			
Tabulated t	2.20			

Table 5: Performance of plant growth attributes along with nematode population in cowpea during 2016.

Crop	Germination % at 15DAS		Plant stand at 15 DAS		Plant stand at harvest		RKI (1-5 scale)		Final nematode population (200cm³ soil + root) *M. incognita*	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Cowpea	97.00	97.00	130	132	86	93	2.52	3.30	202.25	322.02
Paired t-test value (P=0.05)	(-) 0.13 (NS)		(-) 0.92 (NS)		(-) 1.07 (NS)		(-) 4.53		(-) 2.26	
Tabulated t	2.20		2.20		2.20		2.20		2.20	
Nematode	% reduction of *M. incognita* population over untreated control									
M. incognita	37.19									

INP (Initial nematode population)= 215 J_2/200 cm^3 soil, M= Meter, RKI= Root Knot Index.

Table 6: Screening of cowpea genotypes against *Meloidogyne incognita* race 2.

Germplasm	Gall Index	Reaction	No. of egg-masses /5 g of root
COPB var. 1 (2014)	4.0	S	6
COPB var. 2 (2014)	3.3	S	8
COPB var. 3 (2014)	2.5	MR	4
COPB var. 4 (2014)	2.2	MR	6
COPB var. 5 (2014)	2.7	MR	3
COPB var. 6 (2014)	4.5	HS	7
COPB var. 5 (2015)	4.2	HS	10
COPB var. 6 (2015)	3.0	MR	5
KashiKanchan CP-4	4.5	HS	15
BCCP-5	4.5	HS	9
IC 2196	2.5	MR	4
IC 9883	4.5	HS	10
IC 19797	3.0	MR	6
IC 20451	3.0	MR	10
IC39835	3.0	MR	9
IC 39869	5.0	HS	5
IC 39890	5.0	HS	8
IC 39916	5.0	HS	6
IC 39945	4.5	HS	11
IC 39947	2.5	MR	6
IC 52094	2.5	MR	5
IC 52110	2.5	MR	5
IC 199701	2.5	MR	6
IC 201095	5.0	HS	7
IC 202705	2.5	MR	3
IC 202711	2.5	MR	2
IC 202717	2.5	MR	9
IC 202718	3.0	MR	6
IC 202720	2.5	MR	6
IC 202730	3.0	MR	5
IC 202762	3.0	MR	5
IC 202775	2.5	MR	1
IC 202821	4.5	HS	10
IC 202825	2.5	MR	7
IC 202827	3.1	S	5
IC 202891	3.5	S	7
IC 202926	2.5	MR	6
IC 214757	3.0	MR	2
IC 219607	4.5	HS	9
IC 249133	4.5	HS	10
IC 249137	5.0	HS	9
IC 249584	3.5	S	11
IC 300039	4.0	S	5
EC – 2	4.1	HS	6
EC 100092	5.0	HS	5
EC 232352	5.0	HS	3
EC 328650	3.5	S	3
EC 341722	2.5	MR	2
EC 367702	4.2	HS	3
EC 367710	5.0	HS	5
EC 367704	5.0	HS	9
EC 390258	2.5	MR	2
EC 390263	2.5	MR	1
EC 390264	2.5	MR	5
EC 390277	4.5	HS	3
EC 390280	2.5	MR	7
Check (Kasikanchan)	5.0	HS	6

Table 6: Continue...

NB: S-Susceptible, HS- Highly susceptible and MR- moderately resistant.

B. Screening of germplasms

The experiment on the study of screening of germplasm in cowpea consists of 57 genotypes including the var. Kashikanchan as the check.

The results presented in Table 6 revealed that the different genotypes showed different levels of gall index and reaction of host plant against *M. incognita*. Out of 57 genotype, 22 showed high level of susceptibility, 7 were susceptible in reaction and 28 were found as moderately resistance to root-knot nematode, *M.incognita*. All the germplasm screened for resistance against root-knot nematode, *M. incognita* favoured nematode development in terms of gall index or root system and population densities in root. Among the tested germplasms no germplasm was highly resistant or resistant against *M. incognita*. The rest of the germplasm showed considerable susceptibility towards *M. incognita*. All the germplasm showed great variation in their response to *M. incognita* from moderately resistant to susceptible with 2.2-5.0 root knot index. Lowest root-knot index (2.2) was observed in COPB var. 4 (2014) and highest (5.0) were found in 10 genotypes including check variety KashiKanchan.

CONCLUSION

It is concluded from the experiment on avoidable yield losses of cowpea under West Bengal field conditions that the avoidable losses ranged between 9.85 -12.72 per cent and application of Carbofuran 3G at 3 kg a.i./ha increased the yield of the crop by 10.93 to 14.58 per cent. Therefore, management of root knot nematode (*M. incognita*) is necessary to get maximum yield of the crop. The moderately resistant genotypes (28 Numbers) are being suggested to use in breeding studies as a potential material to develop the root knot nematode resistant cultivars.

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