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Reaction of Chickpea Germplasm Lines against Root-knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and its Management using Bioagents under Field Condition

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ABSTRACT

Background: Chickpea (*Cicer arietinum*) is one of the most important food legumes grown worldwide. Its cultivation in India is hampered considerably due to regular occurrence of root knot nematode and reported to reduce the chickpea yield by 9-40%.

Methods: The present study was conducted to evaluate some chickpea germplasm for resistance against root knot nematode, *Meloidogyne incognita*. The field experiment was also conducted for the management of root-knot nematode infesting chickpea with seven treatments including an untreated control. Mainly the experiment was conducted to check the efficacy of some biopesticides.

Result: The screening result revealed that none of the germplasm was found highly resistant against the root knot nematode, however three germplasms exhibited moderately resistant reaction having root gall index between 2.1 to 3.0. All the treatments were significantly superior over the untreated control in reducing the root-knot nematode population, gall index and increasing yield of chickpea at termination. However, soil application of *Pseudomonas fluorescens* @ 20 g/m^2 + Neem cake @ 100 g/m^2 was found to be the most effective in reducing root-knot nematode population (47.88 %) and increasing the yield of chickpea (50.20%) at termination. This was followed by soil application of *Perpureocillium lilacinum* @ 20 g/m^2 + Neem Cake @ 100 g/m^2. The reduction in root knot nematode population and increase of yield over control were 37.46% and 36.40% respectively.

Key words: Biopesticides, Chickpea, Legumes, Management, *Meloidogyne incognita*, Root knot ematode, Screening.

INTRODUCTION

Pulse crops possess an important position in India as they contain nearly three times as much protein as in the cereals. Basically, these are the main source of dietary protein (Jeswani and Vanchaik,1986; Chand and Srivastava,1982) for a large vegetarian population in our country. On an average, pulses contain 22 to 24 per cent protein as against 8 to10 per cent in cereals. In India, chickpea was grown on 9.01 million hectares area with total production of 7.58 million tones and an average productivity of 841 kg/ha. The enormous disparity between the actual and expected yield of chickpea is due to biotic stresses, caused by insects, bacteria, fungi, nematodes and viruses and abiotic stresses, such as drought, nutrient deficiencies, salinity and chilling (Roorkiwal *et al*., 2016). Among these phyto nematodes cause considerable damage to pulse crops. There are 97 listed nematode species associated with chickpea on global basis, out of which 64 have been reported from India, but the major damage is caused by three endoparasitic nematodes *viz*., *Meloidogyne* spp., *Heterodera* spp. And *Rotylenchulus reniformis*, which are known to inhabit inside the roots (Ali *et al*., 2003). Three species of the root-knot nematode, *M. incognita*, *M. javanica* and *M. arenaria* are associated with chickpea. Of these species, *M. incognita* is apparently the most predominant which is closely followed by *M. javanica* (Sharma and Sharma, 1988). To control nematode attacks biological control is one possible safe alternative to pesticides for disease management and is likely to be free from toxic residual effects. *Pseudomonas fluorescens* and *Trichoderma spp*. are among the most commonly used biocontrol agents (BCAs) against plant nematodes (Sikora, 1992; Khan *et al*., 2009).

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MATERIALS AND METHODS

Evaluation of germplasm

Thirty chickpea germplasm lines received from the Project coordinator AICRP on nematodes, ICAR New Delhi, were screened in net house condition at the Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, during November 2019 - January, 2020 to investigate response of the germplasm against root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood race 2.

For the experiment pure culture of the nematode was maintained on brinjal roots in the net house. Then nematode eggs were extracted by using modified method (Hussey and Barker, 1973). Juveniles were also extracted from infested brinjal roots, using modified Baermann tray method. Counting was done three times to obtain the mean number of juveniles. On the other hand, a potting medium comprising of soil, sand and vermicompost in the ratio of 3:1:1 was sterilized by 10% formaldehyde solution to make the media free from nematodes. The potting media was ready to use after three weeks of sterilization. The earthen pots (6 “) were filled with this sterilized soil @ 1000 cc pot. Then sowing of chickpea seeds was done. Three seeds were sown in each pot and only one plant per pot was allowed to grow after one week of germination. The inoculation of previously extracted nematode juveniles was done at 3-4 leaves stage (15 days after sowing) @ one J2 per cc of soil *i.e.*, 1000 J2 pot. For the inoculation three to four holes to a depth of 3-5 cm were made with the help of glass rod near the rhizosphere. The second stage juveniles (J2) of *Meloidogyne incognita* @ 1000 J2 per pot were released with the help of 10 ml pipette. Holes were subsequently covered with soil and pots were watered after inoculation. The chickpea plants were uprooted after 15 days of inoculation carefully to avoid the damage of roots and other plant parts. After uprooting the roots were gently washed in tap water and made cut at the junction of the shoot and root. Observation on length (cm) of shoot and root, weight (g) of shoot and root were recorded thereafter. Roots were brought to the laboratory for further studies. Counting of galls and egg masses were carried in the laboratory under stereoscopic binocular microscope. After counting roots as well as shoots were kept in paper packets for drying in dry air oven at 45°C for 4-5 days and then taken the dry weight. The degree of resistance was indicated by the root knot index and it was done as per Heald *et al.(1989).* The critical difference (CD) at 5% level of significance was worked out from the data recorded during experiment and the data was analyzed in CRD.

Management of nematode

The experiment was carried out from Dec 2019 to March 2020 in an established sick plot infested with *Meloidogyne incognita*, at central research farm of B.C. K.V. Goyeshpur, Nadia, West Bengal. The farm is located at 23°N latitude and 89°E longitudes at an elevation of 9.75 meter from the mean sea level. The land is topographically referred as a medium land.

Details of experiment

The experiment was designed in randomized block design using seven treatments *i.e.* T1 = Pseudomonas fluorescens @ 20 g m^{-2} (CFU 2 × 10^6), T2 = Purpureocillium lilacinum 20 g m^{-2} (CFU 2 × 10^6), T3 = neem cake @100 g m^{-2},T4 = P. fluorescens 20 g m^{-2} + neem cake @100 g m^{-2} , T5 = P. lilacinum @ 20 g m^{-2} + Neem cake @100g m^{-2}, T6 = Carbofuran 3G @ 10 g m^{-2} (Standard check), T7 = Untreated control with three replications. The variety Anuradha was selected for experimental purpose. The initial nematode population (J2) of root knot nematode (212/200cc of soil) was recorded before land preparation. All the treatments were applied at the time of sowing as soil application. In case of bioagent *i.e.*, *Pseudomonas fluorescens and Perpureocillium lilacinum* application, they were mixed with sufficient quantity of FYM and kept under shade for one week before application. At final harvest, plants from each plot were carefully uprooted to observe root galling (in 1-5 scale). The final soil nematode population (J2) per plot was estimated by extracting nematode following Cobb’s decanting and sieving followed by modified Baermann’s technique. The chickpea yield from the plots was recorded and expressed in kilograms per plot that was later converted to q/ha. Incremental cost benefit ratio of various treatments was also worked out by considering the recent prices of bioagents and other treatments. The critical difference (CD) at 5% level of significance was worked out from the data recorded during experiment and compared according to Duncan’s multiple range test at 5% level of probability; the data was analyzed in CRD.

Results and Discussion

Evaluation of germplasm

The experimental result revealed that the chickpea accession Phule G 08108 was recorded for the greatest plant height, 38 cm and the lowest height, 11 cm was obtained in chickpea accession RLBGK 1. It was also found that in reference to plant height 7 accessions were having no significant difference with Phule G 08108 and all accessions had significant difference with RLBGK 1. Height of rest of the plants was significantly different from the tallest and the smallest ones. Chickpea accession Phule G 08108 was recorded for the greatest fresh shoot weight, 10 g and the lowest weight,1.4 g was obtained in chickpea accession RSG 963. It was also found that in reference to fresh shoot weight 2 accessions were having no significant difference with Phule G 08108 and 4 accessions had no significant difference with RSG 963. Fresh shoot weight of rest of the plants was significantly different from the heaviest and the lightest ones. It was observed that the chickpea accession Phule G 08108 was recorded for the greatest dry shoot weight, 1.3 g and the lowest weight, 0.05 g was obtained in chickpea accession RSG 963. It was also found that in reference to dry shoot weight, 2 accessions were at par with Phule G 08108 while 17 accessions had no significant difference with RSG 963. Dry shoot weight of rest of the plants was significantly different from the heaviest and the lightest ones.

With regard to root length, the chickpea accession, IPKC 13-163 exhibited longest root length, 22 cm whereas the smallest root length 12 cm was recorded in the accession JG 2019-155-118. It was also recorded that another 6 accessions and 17 accessions were statistically indifferent with the accessions IPKC 13-163 and JG 2019-155-118 respectively. Root length of rest 5 accessions was statistically different from both the longest and smallest root. Chickpea accession H 12 -63 was recorded for the greatest

fresh root weight, 9.3 g and the lowest weight, 3.3 g was obtained in chickpea accession BGM 1021. It was also found that in reference to fresh root weight, 4 accessions were having no significant difference with H 12-63 and 8 accessions had no significant difference with BGM 1021. Fresh root weight of rest of the plants was significantly different from the heaviest and the lightest ones. Performance trend of germplasm with regard to dry root weight of the plants was same as was noted in case of the fresh root weight. The greatest dry root weight 2.1 g, the smallest dry root weight 0.9 g, were recorded for H 12 -63 and BGM 1021 respectively.3 accessions were at par with H 12-63 while 10 were statistically indifferent with the accession BGM 1021.

In reference to root-knot index, 3 were moderately resistant and 6 germplasms were susceptible and 21 highly susceptible. However, interestingly two germplasm exhibited no statistically significant difference with the sm allest value of root-knot index (Table 1). Presence of nematode resistant genes makes the plant root less attractive for attacking nematodes. Resistance and susceptibility to plant parasitic nematodes reflect the effect of the plant on the nematode's ability to reproduce (Sharma *et al.,* 2006). Resistant and moderately resistant germplasm reduce nematode reproduction thereby directly affect the residual nematode population density under field conditions (Cook and Evans, 1987). Breeding programs for resistance to plant parasitic nematodes would be best served by selecting resistant

Table 1: Evaluation of different chickpea germplasm against root knot nematode.

	Shoot parameters			Root parameters			Root	
Germplasm	Shoot length	Fresh shoot weight	Dry shoot weight	Root length	Fresh root weight	Dry root weight	knot index	Reaction
BDNG 2017-1	30	3.5	0.5	12.3	5.66	1.5	4.8	HS
BG 4011	26.66	3.2	0.1	15	8.9	1.4	4.8	HS
NBeG 690	25	3.3	0.1	15.6	4.5	1.4	5	HS
H 12 -63	36.33	6.8	0.7	18.6	9.3	2.1	4.8	HS
BG 4005	29	4.2	0.5	17.6	7.4	1.5	4.6	HS
BGM 1021	29.66	3.9	0.4	15	3.3	0.9	3.4	S
BG20212	29	4.7	0.3	15	3.9	1	3.6	S
Phule G 1216-12	33.33	7.1	0.8	19	4.7	1.3	2.8	MR
PG239	34.66	5.3	0.4	15	8.3	1.6	4.8	HS
NBeG 810	30.6	6.6	0.5	19	7.7	1.7	4.4	HS
PG 219	28.3	4.3	0.2	13	4.3	1.5	4.4	HS
BG 20211	26.66	4	0.2	13.66	3.7	1	3.4	S
RG 2016-84	36.3	5	0.5	16.6	5.8	1.2	3.8	S
GNG 2418	27.6	4.4	0.7	14.6	6.1	1.5	4.8	HS
AKG 1506	25	3	0.1	17.33	5.8	1	5	HS
IPKC 13-163	34	4.7	0.3	22	5.8	1.4	2.6	MR
JG 2019-155-118	24	2.6	0.4	12	3.9	1.5	4.2	HS
Phule G 08108	38	10	1.3	21.66	7.7	1.2	3.4	S
HC 5	36	4.9	0.9	14.33	5	1.1	3.6	S
RSG 963	19.33	1.4	0.05	13.6	4.7	1.4	4.4	HS
Plant Gram 5	21.66	1.9	0.3	13	5.4	1.5	4.6	HS
GLK 14306	30.6	3	0.1	12.6	8.3	1.4	4.8	HS
RLBGK 1	11	4.5	0.2	13.66	7	1.3	4.4	HS
NBeG 698	18.66	4	0.1	16	6	1	4.4	HS
Phule G 1216-6	29	4.2	0.4	19.33	6.6	1.7	5	HS
Phule G 1215-1	22.66	1.9	0.5	13.66	5.2	1.6	4.6	HS
RLBGK 2	19	2.7	0.3	12.33	4.8	1.3	4.6	HS
Phule G 0517	24	3	0.4	16	5.3	1.4	4.2	HS
PG 233	32	9.7	1	20.66	8.4	1.8	5	HS
RSGD 965	35.6	9.8	1.1	13.33	5.8	1.6	2.8	MR
CD (5%)	5.57	1.53	0.39	3.74	1.54	0.46	0.69	-
CV	12.11	20.46	55.32	14.54	15.83	42.24	13.08	-

R= Resistant, MR = Moderately resistant, S = Susceptible and HS = Highly susceptible.

genotypes based on root-knot index in preliminary evaluations, followed by selection based on nematode reproduction in advanced evaluations (Hussey and Janssen, 2004). Thus, the use of resistant germplasm can be a vital component for the management of root knot nematode population in chickpea.

The correlation of root knot index with both root and shoot attributes conforms that the growth of root length, shoot length, fresh shoot weight and dry shoot weight is negatively correlated to the root knot index (r = -0.195, -0.374, -0.377, -0.361 respectively) while fresh root weight and dry root weight, were positively correlated with root knot index (r = 0.405, 0.403 respectively) (Table 2).

According to El-Sherif *et al*. (2007), root-knot nematode increases root weight for the most susceptible cultivar compared to resistant cultivar. This is because root-knot functions as metabolic sinks similar to a developing fruit as nutrients produced in the leaves are re-distributed rapidly to the roots and into the bodies of the nematodes.

Management of nematode

Effect of treatments on root-knot index and soil population of root-knot nematodes

Among the different treatments, the treatment (T4) *Pseudomonas fluorescens* + Neem cake @ (20 g/m^2 + 100 g/ m^2) was found significantly superior in reducing gall index and root- knot nematode population over the control. The treatment (T4) resulted the lowest soil population of root-knot nematodes *i.e.* 185/200 cc of soil and root gall index being 1.4. Reduction in soil nematode population over control for T4 was 47.88%. T5 *Perpureocillium lilacinum* + Neem Cake @ (20 g/m^2 + 100 g/m^2) was found as second promising treatment where gall index was 1.73 and soil population of the nematode was 222 J2/ 200cc of soil. Reduction to the satisfactory level after treatment 4 of soil population of the nematode for this treatment was 37.46%.

Effect of treatments on yield of chickpea

Yield in all the treatments were found superior over untreated control. The highest yield (3.59 q/ha) of chickpea was recorded for the treatment (T4) *Pseudomonas fluorescens* + Neem cake @ (20 g/m^2 + 100 g/m^2) followed by 3.26 q/ha for treatment (T5) *Perpureocillium lilacinum* + Neem Cake @ (20 g/m^2 + 100 g/m^2).

It was clearly revealed from the data (Table 3-4) that the treatments T1 to T6 in general performed better than the treatment T7 *i.e.,* untreated control. It also indicated that adoption of any of the above- mentioned treatments among T1 to T6 could be effective control measure against root-knot nematode. However, significant differences were observed among the treatments T1-T6 with regard to

Table 2: Correlation between root knot index and other parameters.

Parameters	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Dry root weight	Dry shoot weight
Root knot index	-0.195	-0.374	0.405	-0.377	0.403	-0.361

Table 3: Effect of treatments on gall indices and population of root-knot nematode in chickpea.

Treatments	Gall index	Population of J2/ 200cc of soil	Decrease in population % over control
T1: *Pseudomonas fluorescens* (CFU 2 × 10^6) @ 20 g/m^2	2.33	245	30.98
T2: *Perpureocillium lilacinum* (CFU 2 × 10^6) @ 20 g/m^2	2.46	263	25.91
T3: Neem cake @ 100 g/ m^2	2.53	252	29.01
T4: *Ps. fluorescens* + Neem cake @ (20 g/m^2+100 g/m^2)	1.40	185	47.88
T5: *P. lilacinum* + Neem cake @ (20 g/m^2+100 g/m^2)	1.73	222	37.46
T6: Carbofuran 3G @ 10 g/m^2	3.2	295	16.90
T7: Untreated control	3.93	355	
S.Em±	0.06	0.30	
c.d p=0.05	0.19	0.95	

INP: 212/200cc of soil.

Table 4: Effect of treatments on yield attributes of chickpea.

Treatments	Yield (gm/plot)	Yield (q/ha)	% Increase in yield over control
T1: *Pseudomonas fluorescens* (CFU 2 × 10^6) @ 20 g/m^2	220	2.93	22.59
T2: *Perpureocillium lilacinum* (CFU 2 × 10^6) @ 20 g/m^2	213	2.83	18.41
T3: Neem cake @ 100 g/ m^2	215	2.86	19.66
T4: *Ps. fluorescens* + Neem cake @ (20 g/m^2+100 g/m^2)	270	3.59	50.20
T5: *P. lilacinum* + Neem cake @ (20 g/m^2+100 g/m^2)	245	3.26	36.40
T6: Carbofuran 3G @ 10 g/m^2	210	2.79	16.73
T7: Untreated control	180	2.39	-
S.Em±		5.38	
c.d p=0.05		16.77	

parameters like yield, gall index and reduction of root-knot nematode population. The performance of the treatment T4 *i.e.* application of *Pseudomonas fluorescens* + Neem cake @ (20 g/m^2 + 100 g/m^2), was always better than T1, T2, T3 T5 and T6 with regard to the growth and yield parameters, root gall index and reduction of soil population of *M.incognita* in the experimental field. Results were in agreement with the findings of Seenivasan, 2018 who reported that the biocontrol agents *i.e. Pseudomonas fluorescens*, *Purpureo cillium lilacinum* and *Trichoderma viride* were capable of reducing root knot nematode (*Meloidogyne hapla)* juvenile (J2) population in soil, infection of female population in roots and egg numbers per gram of root at various levels in carrot. According to Qureshi *et al*. (2012) culture filtrate of several fungi including *P. lilacinum*, showed significant nematicidal activity by killing the 2nd stage juveniles of *Meloidogyne javanica*. The results are in conformity with Khan *et al*. (2018) who reported that biocontrol agent *P. fuorescens* showed killing activity against root-knot nematode, *M. incognita* and enhanced the growth and physiological parameters of Chickpea cv. 'Avarodhi' under glasshouse conditions. It may be due to the presence of various phytochemicals released from biocontrol agent which showed toxic effect on survivality of root-knot nematode. Siddiqui *et al*., 2009 reported that *Pseudomonas* spp. were better in improving plant growth and reducing galls and nematode multiplication and suggested that *Pseudomonas fuorescens* may successfully be used for the biocontrol of *Meloidogyne incognita*.

Conclusion

It may be concluded from the experiment that the use of resistant germplasm can be a vital component for the management of root knot nematode population in chickpea. Soil application of bioagents like *Pseudomonas fluorescens* or *Perpureocillium lilacinum* @ 20 g/m^2 along with Neem cake @ 100 g/m^2 may be applied for management of root knot nematodes (*M. incognita*) population in chickpea as they have some nematicidal property in addition to ability of enhancing plant growth parameters.

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Conflict of interest

The authors of the manuscript are ensured that the research was conducted ethically and in compliance with relevent guidelines and regulations. The consent from all research participants was received maintaining confidentiality and avoiding any form of coercion or deception. This includes obtaining erhical approval for the study and disclosing any financial or personal relationships that may have influenced the research.

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