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Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp ciceri on chickpea

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Abstract
In the study on the interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp *ciceri* on chickpea, the result indicated that dual inoculation treatments significantly decreased plant growth parameters over the treatment with *M. incognita* @1000 J2/kg soil and *F. oxysporum* @ 2% (w/w). The treatment with *M. incognita* @1000 J2/kg soil + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation was statistically superior in decreasing the plant growth parameters of chickpea. However, number of galls, egg masses, and final nematode population was found maximum in single inoculation treatment than dual inoculation treatments. The highest number of galls, egg masses, and final nematode population were observed in the treatment with *M. incognita* @1000 J2/kg soil. The Maximum disease incidence was recorded in the treatment with *M. incognita* @1000 J2/kg soil and *F. oxysporum* @ 2% (w/w) after 15 days of inoculation.

Keywords: *Meloidogyne incognita*, *Fusarium oxysporum*, dual inoculation, superior, growth parameters, inoculation

Introduction
Chickpea (*Cicer arientinum*), commonly known as Bengal gram, is one of the most important pulse crop grown in India. It belongs to the Family leguminosae. It is one of the previous pulse recognized and grown both in Asia and Europe being its place of origin lies in South Western Asia. It is rich source of proteins (21.1%), carbohydrates (61.5%) and fats (4.5%). The area under chickpea in India is 105.61million hacters with an annual production of 112.29 million tonnes and productivity of 1063 kg/ha (Anon., 2017-18) [2]. In Assam, the crop is grown in rabi season during the month of October and November and grown in an area of 2193 thousand hacters with a production of 1544 thousand tonnes and productivity of 704 kg/ha (Anon., 2017) [2]. Chickpea is grown mainly in 27 districts of Assam among which Dhubri, Dhemaji, Sonitpur, Jorhat and Lakhimpur are the major districts. Phytonematodes are important group of pathogens which cause considerable damage to pulse crops (Sasser, 1989) [22] but the major damage is caused by *Meloidogyne* spp., which are known to inhabit inside the roots (Ali *et al.*, 2003) [1]. M. incognita is actually the most abundant in causing injure to chickpea plants (Sharma and Sharma, 1988) [25]. Yield losses due to *Fusarium* wilt of chickpea recorded up to 72.16% (Kumar and Bourai, 2012) [11]. Crop losses due to *Meloidogyne incognita* have been estimated to be 24.09% loss in yield (Borah, 2017) [4]. In India Upadhya and Dwivedi (1987) [27] reported 4% loss due to M. incognita and wilt of chickpea caused by *Fusarium oxysporum* f.sp *ciceri* in chickpea. The disease intensity caused by the nematodes often gets aggravated in presence of other microorganisms like fungi, bacteria, virus, mycoplasma etc. The interaction with wilt pathogens leads to increase in severity of the disease, breakdown of resistance to wilt pathogens and predispose plants to fungi. Simultaneous or sequential infection caused by root-knot nematodes and *Fusarium* spp. may increase the severity of wilt and rate of death of plants. The nematode advances the arrival of wilt from 31 to 16 days and rise the incidence of disease from 25-50% (Ramnath and Dwivedi, 1981).

Materials and Methods
This experiment was carried out in the net house of Department of Nematology, Assam Agricultural University, and Jorhat during rabi season 2017 to study the interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp *ciceri* on chickpea, and was laid out in Completely Randomized Design (CRD). Required quantities of earthen pots were collected, Filled with 1kg of autoclaved soil and labeled according to the allotted treatments and replications.

Sources of root-knot nematode

Eggmasses of *M. incognita* was collected from infested roots of tomato plant already maintained as pure culture in the culture house of the Department of Nematology, AAU, and Jorhat and kept for hatching. After 24 hours, the freshly hatched second stage juvenile of *M. incognita* were inoculated in tomato plants grown in earthen pots filled with sterilized soil. These inoculated tomato plant were maintained in pots and used as source of inoculum for subsequent use. The culture was checked periodically for its purity. Sterilized 1kg pot mixed with finely dried cowdung and sand in the ratio of 2:1:1 and was inoculated with freshly hatched second stage juveniles of *M. incognita* @ 1000 J2 /pot. Chickpea Seeds were sown in the pots. Moisture content of the pot was maintained by watering regularly and observation were recorded on plant growth parameters, number of galls, number of egg masses, soil and disease incidence (%).

Sources of *Fusarium oxysporum* f.sp Cicero

The pure culture of the fungus (*Fusarium oxysporum* f.sp *ciceri*) was collected from Department of Plant Pathology, AAU, and Jorhat. Pure culture of fungus *Fusarium oxysporum* f.sp *ciceri* was maintained throughout the period of experimentation on PDA media by sub-culturing on fresh media and stored at 4 °C. Pathogenicity of F. *oxysporum* was proved in pot condition by soil inoculation the mass cultured inoculum (mass culture of *F. oxysporum* was done in 4% maize meal sand medium) in pot condition.

Preparation of Mass culture of *Fusarium oxysporum*. fsp *Cicero*

Maize Meal Sand Medium (MMSM) was used for preparation of mass inoculum in laboratory. For preparation of mass inoculum 4.5 g crushed maize grain was added to 150 g of clean sand. Those were mixed thoroughly by pouring 20 ml of distilled water. The medium was filled in 250 ml volume conical flasks up to ¾th volume and were plugged tightly with nonabsorbent cotton and autoclaved at 15 lb pressure per square inch for 20 min for two consecutive days. The flasks were inoculated with 5 mm mycelial discs of the fungus under study. After 30 days the medium flasks were covered with white mycelium. This mass culture was used for inoculation of the pathogen in the pots. For soil inoculation, 15 days old culture of *F. oxysporum* f.sp *ciceri* grown in MMSM were used. The rate of inoculation of the fungus was 1% (W/W).

Results and Discussions

In the present investigation, the maximum plant growth parameters *viz*., shoot length, fresh and dry weight of shoot and root were recorded in control while the minimum was recorded in the treatment *M. incognita* @1000 J2 + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation. All the treatments differed significantly over control. (Table 1). The treatment *Meloidogyne incognita* alone @ 1000 J2/kg and *Fusarium oxysporum* @ 2% (w/w) + *Meloidogyne incognita* @ 1000 J2/kg soil after 15 days of inoculation were statistically at par in case of shoot length as well as fresh root weight. In case of fresh weight of shoot the treatments *M. incognita* @1000 J2 + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation and *F. oxysporum* @ 2% (w/w) + *M. incognita* @ 1000 J2/kg of soil with simultaneous inoculation were found statistically at par. Further, it was also observed that the treatment with *Fusarium oxysporum* @ 2% (w/w) + *M. incognita* @1000 J2/kg soil after 15 days of inoculation and the treatment with *F. oxysporum* @ 2% (w/w) + *M. incognita* @ 1000 J2/kg of soil with simultaneous inoculation were statistically at par. No statistical differences were also observed in the treatment with *Meloidogyne incognita* alone @ 1000 J2/kg of soil and the treatment with *Fusarium oxysporum* @ 2% (w/w) + *M. incognita* 1000 J2/kg soil after 15 days of inoculation in dry weight of shoot. The treatments with *Meloidogyne incognita* alone @ 1000 J2/kg of soil and the treatment with *Fusarium oxysporum* @ 2% (w/w) was statistically at par in case of dry root weight. The result of the present investigation were in agreement with the findings of Kumar *et al.* (2017) [9] who reported that significant decline in plant height was recorded in the treatment where the nematode was inoculated first and fungus seven days after followed by the inoculation by fungus first and nematode seven days after on black gram. Similar results were also observed by Vijayashanthi *et al*. (2020) [28] who reported that growth of plant was affected in all treatments where the nematode and fungus inoculated to the plant both individually and in combination on cucumber. Meena *et al*. (2021) [15] reported that plant growth was adversely affected when *M. inocgnita* inoculated prior to *F. oxysporum* in tomato. When the inoculation of fungus was done seven days prior to nematode inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously (Sharma and McDonald., 1990). They also reported that the presence of *Meloidogyne incognita* exasperated the disease situation *by F. oxysporium* f.sp. *ciceri* on chickpea. In a sequential etiology, one pathogen of the disease complex infects host before the invasion by the other pathogen and brings about definite histophysiological and biochemical alterations within the host, rendering it more suitable substratum for establishment and growth (Anwar and Khan, 2002) [3] McDonald. Presence of nematodes not only predisposed the host but also reduced the period of incubation for disease expression (Fazal *et al*., 1994, Malhotra *et al*., 2011) [5, 13]. Even though, each pathogen was able to reduce the plant growth, the combined infection of nematode and fungus resulted in synergistic effect. Haseeb *et al.* (2007) [7] and Ravishankar and Singh (2008) [20] observed that there was significant reduction in all the plant growth parameters when inoculation of *R. solani* was 15 days prior to M. *incognita* in Vigna mungo compared to *M. incognita* 15 days prior to *R. solani*. The possible reason for comparatively higher damage in plant inoculation of nematode 15 days before the inoculation of fungus as compared to fungus before the application of nematode may be due to the prior invasion of nematode into roots thereby making the host more suitable for fungal penetration providing a metabolic rich substrate or nematode might modify the rhizosphere thereby favouring the fungal growth (Owens and Specht, 1966) [17]. The maximum number of galls (93) and eggmasses (57.7) were recorded in the treatment with *Meloidogyne incognita* alone @ 1000 J2/kg of soil while the minimum number of galls (26.7) and egg mass (28.8) were recorded in the treatment with *F. oxysporum* @ 2% (w/w) + *M. incognita* 1000 J2/kg soil after 15 days of inoculation. All the treatments differed significantly over control. (Table 2). The treatments with *Fusarium oxysporum* @ 2% (w/w) alone were statistically at par with control. Further, it was observed that the treatment with *F. oxysporum* @ 2% (w/w) + *M. incognita* 1000

J2/kg soil after 15 days of inoculation and the treatment with *F. oxysporum* @ 2% (w/w) + *M. incognita* 1000 J2/kg of soil with simultaneous inoculation were statistically at par in case of eggmass. The maximum nematode population (192.4) was recorded in the treatment with *Meloidogyne incognita* alone @ 1000 J2/kg of soil while the minimum nematode population (138.7) was recorded in the treatment *F. oxysporum* @ 2% (w/w) + *M. incognita* 1000 J2/kg soil after 15 days of inoculation. The treatments with *Meloidogyne incognita* alone @ 1000 J2/kg of soil and *M. incognita* @1000 J2 + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation were statistically at par. All the treatments differed significantly over control. These results were in agreement with the findings of Patel *et al*. (2000) [18] who observed that the maximum nematode population was recorded in nematode alone treatment in chickpea. Gogoi *et al*. (2011) [6] also that reported maximum galls, egg masses and final nematode population were recorded in the treatment inoculated with nematode only in french bean. Similar results were also recorded by Kumar and Haseeb (2009) [12] who reported highest reproduction rate in tomato plants inoculated with nematode alone followed by nematode prior to F, N-F simultaneously and F prior to N respectively. The minimum number of galls, egg mass, nematode population were recorded in the treatment F. oxysporum @ 2% (w/w) + M. incognita 1000 juvenile/kg soil after 15 days of inoculation. This findings were in agreement with Roy and Mukhopadhyay (2004) [21] who observed reduced galling and population density in presence of F. oxysporum in brinjal. Haseeb *et al*. (2007) [7] reported that nematode reproduction and roots galling decreased with pre-inoculation of fungus in *Pisum sativum.* The reduction of nematode population is due to adverse effects of metabolites of fungus on the juveniles of *M. incognita* in terms of their development and reproduction. Decrease in the reproductive rate of nematode and galling in the presence of fungus due to the fact that fungus makes roots less vulnerable for nematode attack or the fungus secretion produced adverse effects on the juveniles (James, 1966). Infection of fungi in the plants reduced nematode population which might be due to the formation of mycelial mat over the roots which create unfavourable condition for the nematodes to enter into the roots and ultimately cause sex reversal in nematodes. I.e. female nematodes were converted into males during the unfavourable environmental condition and the male nematodes thus formed 56 leave the root without feeding which creates reduction in the final nematode population in the roots. Report of Nagesh *et al*. (2006) [16] supported the finding where they observed reduced gall index of *M. incognita* in tomato in the combined inoculation of nematodes and the fungal pathogen. The maximum disease incidence (43%) was recorded in the treatment with *M. incognita* @1000 J2 + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation while minimum disease incidence (26%) was recorded in the treatment with *F. oxysporum* @ 2% (w/w) (Table 2). The results were in agreement with Kumar *et al*. (2017) [9] who reported that there was a considerable increase in percent disease incidence when *M. incognita* inoculation preceded the fungal inoculation in black gram. Patil *et al*. (2018) [19] observed that inoculation of nematode M. incognita inoculated 7 days prior to the fungus *Fusarium oxysporum* f. sp. *cucumerinum* shows high level of disease incidence followed by concominant inoculation of nematode and fungus than the control in case of cucumber. Sreegayathri *et al*. (2018) [26] reported that maximum incidence of wilt was recorded in the treatment where *Meloidogyne incognita* was inoculated 15 days prior to the inoculation of *Fusarium solani* on bitter gourd. The nematode cause injury on root surface, weakening the root tissue thereby making the host plant more prone to fungal attack (Senthamarai *et al.*, 2008) [23]. It was proved in the study that the nematode acts as a pre- disposer for the spread of secondary fungal pathogens. Thus the nematode acts as a primary invader for causing disease complex (Meena *et al.*, 2015) [14]

Table 1: Effect of *Meloidodyne incognita* and *Fusarium oxysporum* alone and in combination on plant growth parameters of chickpea (Mean of 10 replications)

Treatments	Shoot length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T1: *M. incognita* alone @ 1000 J2/kg soil	17.53c	4.76c	1.37bc	3.08b	0.60b
T2 : *F.oxysporum* @ 2% (w/w)	19.82b	5.01b	1.47b	3.26b	0.64b
T3 : *M. incognita* @ 1000 J2 + *F. oxysporum* 2%(w/w) after 15 days of inoculation	11.16e	2.65e	0.37e	0.89e	0.10e
T4 : *F. oxysporum* @ 2%(w/w)+ *M. incognita* alone @ 1000 J2/kg soil	17.02c	3.55d	1.03cd	2.46c	0.41c
T5 : *M. incognita* @ 1000 J2 + *F.oxysporum* 2%(w/w) simultaneous inoculation	16.15d	2.79e	0.724de	1.40d	0.28d
T6 : Check (UC)	27.75a	6.13a	2.74a	3.90a	0.784a
S.Ed.±	0.266	4.44	0.204	0.157	0.025
C.D (0.05)	0.533	8.89	0.409	0.315	0.41

Means followed by the same letter in the superscript(s) are at par

Table 2: Effect of *Meloidodyne incognita* and *Fusarium oxysporum* alone and in combination on host infection, nematode multiplication and disease incidence on chickpea (Mean of 10 replications)

Treatments	No. of galls	No. of eggmass	Final nematode population/250cc soil	Disease incidence (%)
T1: *M. incognita* alone @ 1000 J2/kg soil	93	57.7	192.4	0.00
	(9.66)a	(7.62)a	(13.88)a	(0.707)c
T2 : *F. oxysporum* @ 2% (w/w)	0.00	0.00	0.00	26.00
	(0.707)e	(0.707)e	(0.707)d	(5.10)b
T3 : *M. incognita* @ 1000 J2 + *F. oxysporum* 2% (w/w) after 15 days of	74.8	40.1	189.8	43.00

inoculation	(8.67)b	(6.36)b	(13.79)a	(5.56)a
T4 : *F. oxysporum* @ 2%(w/w)+*M. incognita* alone @ 1000 J2/kg soil	26.7	28.8	138.7	31.00
	(5.20)d	(5.40)c	(11.79)c	(5.55)b
T5 : *M. incognita* @ 1000 J2 + *F. oxysporum* 2% (w/w) simultaneous inoculation	68.1	30.7	178.6	38.00
	(8.28)c	(5.58)d	(13.38)b	(6.15)a
T6 : Check (UC)	0.00	0.00	0.00	0.00
	(0.707)e	(0.707)e	(0.707)d	(0.707)c
S.Ed.±	0.83	0.101	0.061	0.281
C.D (0.05)	0.168	0.205	0.122	0.563

Values within parentheses are → (x + 0.5) transformed data. Means followed by the same letter in the superscript(s) are at par

Fig 1: Pure culture of *Fusarium oxysporum*f.sp *ciceri* on PDA plate

Fig 2: Mass culture of *Fusarium oxysporum* f.sp *ciceri* on Maize and Sand Medium

Fig 3: General view of the pot experiment

Fig 4: Effect of different treatments on root growth of chickpea

Conclusion

In the investigation, it was observed that all treatments significantly reduced plant growth parameters over uninoculated control. The dual inoculation treatments brought significant reduction in plant growth parameters *viz.*, shoot length, fresh and dry weight of shoot and root over single inoculation treatment. Maximum reduction in shoot length, fresh and dry weight of shoot and root was recorded in *M. incognita* 1000 J2/kg + *F. oxysporum* @ 2% (w/w) after 15 days of inoculation. Maximum increase in number of galls, eggmasses, final nematode population and disease incidence was observed in the treatment with M. incognita @ 1000 J2/kg of soil. Minimum number of galls, egg mass, nematode population were recorded in the treatment *F. oxysporum* @ 2% (w/w) + *M. incognita* 1000 J2/kg soil after 15 days of inoculation.

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