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Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* on Black gram (*Vigna mungo* L)

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Abstract
The interaction between root knot nematode *Meloidogyne incognita* and the fungus *Fusarium oxysporum* was studied on Black gram cultivar TU 98-14.The results indicated that plant growth was adversely affected in all the cases where the plant was inoculated with *M. inocgnita* and *F. oxysporum* when compared with uninoculated control. The data revealed that significantly reduced (13.40) plant height was recorded in the treatment where nematode inoculated first and fungus seven days after followed by the inoculation by fungus first and nematode seven days after. Simultaneous inoculation by both the organisms recorded 16.06 cm plant height which was at par with nematode inoculation only (15.36) cm). Fungus inoculated plants attained 16.56 cm plant height against 18.62 cm plant height in control. Minimum (11.52 cm) length of root was noticed in the treatment were nematode preceded fungus followed by the treatment where fungus inoculated first and nematode seven days after (12.98 cm). Reduced root length was also noted in nematode (14.28 cm) and fungal inoculation (13.06 cm) which were at par with each other. Significant increase in root length was also recorded in simultaneous inoculation by both the organisms. Uninoculated control recorded maximum (19.02 cm) root length.

Keywords: *Fusarium oxysporum, Meloidogyne incognita*, Black gram, Interaction

1. Introduction
In Indian agriculture pulses, play an important role in maintaining soil fertility and supplying protein to the large vegetarian population of the country. Nearly 11,000 species of legumes are known and many are of importance as industrial, medicinal of food plants. Black gram (*Vigna mungo* L), one of the pulses, is mostly produced in Asian countries as their tropical climate and soil type suits its cultivation. India occupies 31 lakh hectares of land with 14 lakh tones production and 451.61 kg/ha productivity. Madhya Pradesh alone contributes 4.72 lakh hectares of land with 1.66 tones production and 351.69 kg/ha productivity (Anon. 2012). Urdbean contains about 26 percent proteins. In addition, being an important source of human food and animal feed**,** it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Several fungi have been reported in black gram *viz*. *Aspergillus flavus, A. niger, Curvularia lunata, Colletotrichum* sp*. Fusarium solani, F. oxysporum, and Macrophomina phaseolina.* Some of these are seed borne in nature and seed transmissible (Raut and Ahire, 1988, Rahman *et al.*1999) [20, 19] causing considerable losses in yield. *Meloidogyne incognita is* considered to be one of the most severe pests of blackgram. The nematodes adversely affect nodulation Nitrogen fixation and yield (Hussaini and Seshadri, 1975) [9]. *Meloidogyne* infection, which primarily impairs water and nutrient uptake, and upward translocation by the root system (Karssen and Moens, 2006) [12]. Disease complexes involving nematodes and fungal pathogens may cause significantly more crop losses than individually (Hussey and McGuire, 1987) [10]. The interaction between the root infecting fungus and the nematode results in the reduction of seed emergence and increase in both galling and nematode fecundity (Kassab & Ali 1996) [13]. Simultaneously the disease development caused by soil-borne fungal pathogens is also stimulated (Shawadfy & Mousa, 1997) [24]. *Meloidogyne incognita* has evolved a specialized adoptive mechanism with the vascular wilt fungus *F. oxysporum* to cause a disease complex etiology in a variety of crop plants (Powell, 1971; Mai and Abawi, 1987) [11, 16]. Such a disease complex, involving both the organisms has also been reported in black gram inflicting an appreciable loss in yield to the tune of 49 percent (Mahapatra and Swain, 1999) [15].

2. Material and methods

Seed of Blackgram (var. TU 98-14) ware used for the present studies. A mixture of soil and SSM was specifically prepared as per the method described earlier in the ration of 100 kg soil and 1kg Sorghum. This soil mixture was subsequently autoclaved at 1.05 kg/cm^2 for two hours before use. Ten cm earthen pots holding 500 cm^3 of soil were used in the present studies. The treatments consisted of control without fungus and nematode, nematode alone (N), Fungus alone (F), simultaneous inoculation of nematode and fungus (NF), nematode at the time of sowing and fungus one week after (N1F7) fungus at the time of sowing and nematode after one week (F1N7). A constant level of 1000 second stage juveniles was inoculated per pot as per treatment. The technique for extraction and disinfection of nematodes was the same as described in earlier. The nematodes were pipette around the pregerminated seeds growing in sterile moist chambers prepared out of Petri dishes. The radical length at the time of sowing ranged between 0.3 to 0.5 mm. Pregerminated seeds were dibbled 2cm deep. Due precautions were observed to avoid contamination from one pot to another. Wherever, the nematodes were to be inoculated, one week after, three glass rods were fixed two cm deep in a circle of two cm in diameter. At the time of inoculation, the glass rods were removed and the nematode suspension was evenly distributed in the holes and these were then plugged with sterile soil. For inoculating the soil with fungus, *Fusarium oxysporum*, and 50g content of each flask containing SSM inoculated with the fungus was mixed in each pot for fungus, fungus + nematode one week after, and simultaneous inoculation of fungus and nematode.

The other pots which were to be inoculated by nematode, nematode + fungus, fungus one week after, and control were mixed with un inoculated SSM. When the fungus was to be inoculated on week after nematode inoculation, the technique suggested by Grewal and Pall (1974) was adopted with slight modification of placing three glass rods equidistant in a circle of two cm diameter and plugging the holes with sterile soil after introducing the actively growing *Fusarium oxysporum* plug derived from the culture.A total of 30 pots were thus randomized over the glass house bench following CRD and watered daily with an equal quantity of sterilized distilled water if and when required. The experiment was concluded after 45 days after inoculation. The glass house temperature during the course of experiment was ranged from 27 to 34^{0}C. The observation on plant height, fresh and dry shoot and root weights, root length, number of galls, nematode population in soil and root and number of galls were recorded. The entire root system along with the soil was taped out of the pot and washed in a container with a gentle stream of water. For obtaining fresh weight, the method described earlier was followed. The roots were then kept in an oven maintained at 60 ± 1^{0}C for 72 hr. to record oven dry weights.

3. Results

The experiment on interaction between *M. incognita* and *F. oxysporum* was conducted under pot conditions and the data is presented in the Table (1). The data revealed that significantly reduced (13.40) plant height was recorded in the treatment where nematode inoculated first and fungus seven days after followed by the inoculation by fungus first and nematode seven days after. Simultaneous inoculation by both the organisms recorded 16.06 cm plant height which was at par with nematode inoculation only (15.36) cm). Fungus inoculated plants attained 16.56 cm plant height against 18.62 cm plant height in control.

Minimum (11.52 cm) length of root was noticed in the treatment were nematode preceded fungus followed by the treatment where fungus inoculated first and nematode seven days after (12.98 cm). Reduced root length was also noted in nematode (14.28 cm) and fungal inoculation (13.06 cm) which were at par with each other. Significant increase in root length was also recorded in simultaneous inoculation by both the organisms. Uninoculated control recorded maximum (19.02 cm) root length. The fresh shoot weight significantly declined (0.60g) in the treatment where nematode preceded fungus seven days before followed by fungus inoculated first and nematode seven days after (0.61g) simultaneous inoculations of nematode and fungus (0.64g) and nematode alone (0.63g). Fungus alone exhibited 0.68g root weight against maximum (0.79g) in control.

Similarly minimum (0.58g) root weight was recorded in the same treatment were nematodes inoculated first and fungus after words followed by fungus inoculated first and nematode seven days after (0.61g) followed by nematode alone (0.63g) and simultaneous inoculations by both the organisms (0.64g). Fungus alone exhibited 0.68g fresh root weight against maximum (0.79g) in control. On dry weight basis minimum (0.29g) shoot weight was recorded in nematode inoculation followed by fungal inoculations and fungus inocufirst and nematode seven days after (0.32g) simultaneous inoculation by both the organism and nematode inoculation remained at par. Dry weight of root also exhibited the same trend.

Table 1: Influence of *Meloidogyne incognita* and *Fusarium oxysporum* on plant growth parameters.

S.no	Treatment	Plant height (cm)	Root length (cm)	Fresh weight(g)		Dry weight(g)		No of galls/plant	Total No. of nematode
				Shoot	Root	Shoot	Root		
1	Control	18.62	19.02	0.83	0.79	0.43	0.41	0.00 (0.70)	0.00 (0.70)
2	N	15.36	14.28	0.65	0.63	0.33	0.34	14.40 (3.79)	1849.00 (43.00)
3	F	16.56	13.06	0.68	0.68	0.34	0.31	0.00 (0.70)	0.00, (0.70)
4	N+F	16.06	15.24	0.62	0.64	0.33	0.33	10.20 (3.19)	1549.00 (39.35)
5	N 1 + F7	13.40	11.52	0.60	0.58	0.29	0.26	14.00 (3.74)	1655.00 (40.68)
6	F1 + N7	14.10	12.98	0.63	0.61	0.32	0.27	11.40 (3.37)	1545.00 (39.30)
S.E.(M) ±		0.810	0.737	0.033	0.030	0.011	0.010	1.282	66.711
CD (P=0.05)		2.378	2.163	0.098	0.089	0.032	0.029	3.764	195.875

*Mean of four replication

** Values in parentheses are √n+1 transformation

Maximum (14.00) number of galls was observed in nematode inoculated first followed by fungus inoculated first and nematode seven days after and nematode alone. Simultaneous inoculation by both the organisms exhibited minimum (10.20) number of gall followed by fungus inoculated first and nematode after words. Maximum numbers (1849) of

nematodes were recovered with inoculation of nematode alone followed by nematode inoculated first and fungus seven days after (1655). Fungus inoculated first and simultaneous inoculation of both the organism remained at par.

4. Discussion

Studies were carried out under pot conditions to determine the effects of cohabitation of *Meloidogyne incognita* and *Fusarium oxysporum* on disease development and growth parameters in blackgram. The results indicated that plant growth was adversely affected in all the cases where the plant was inoculated with *M. inocgnita* and *F. oxysporum* when compared with uninoculated control. Generally, the treatments receiving the nematode inoculation prior to fungus resulted in higher reduction of plant growth than the other treatments. When nematode inoculation was done seven days prior to fungal inoculation, showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression (Fazal *et al.* 1994, Malhotra *et al.* 2011) [4, 17]. In a sequential etiology, one pathogen of the disease complex infects host before the invasion by the other pathogen and brings about certain histophysiological and biochemical alterations within the host, rendering it more suitable substratum for establishment and growth (Anwar and Khan, 2002) [1].

Although, each pathogen was able to reduce the plant growth, the combined infection of nematode and fungus resulted in synergistic effect. Haseeb *et al.* (2005) [6] and Ravishankar and singh (2008) [21] observed that inoculation of *M. incognita* 15 days prior to *R. solani* significantly reduced all the plant growth parameters as compared to inoculation of *R. solani* 15 days prior to *M. incognita* in *Vigna mungo*. Similar effect on suppression of plant growth of tomato than alone treatment was also recorded by (Samuthiravalli & Sivakumar 2008) [23]. The host infestation by *M. incognita* as represented by root knot index was maximum in plants inoculated nematode seven days prior to fungus followed by nematode and fungus simultaneously, and fungus seven days prior to nematode, nematode alone inoculations showed maximum galling, number of galls and egg masses. This was Followed by plants inoculated with fungus first and nematode a week after. The reduction might be due to reduced root system, thus nematode faced competition for food. In addition to fungal disruption of nematode feeding sites, plants affected by disease complexes may be more prone to early senescence and death which in turn might prevent nematode from completing its life cycle leading to reduced reproduction. Similar type of reduced galling and population density in presence of *R. solani* was also reported by Roy and Mukhopadhya (2004) [22]. Visually highest root infection by *F. oxysporum* was observed in nematode-fungus simultaneously inoculated plants followed by nematode seven days prior to fungus; seven days prior to nematode and fungus alone inoculated plants, respectively. There was a significant increase in percent disease incidence when *M. incognita* inoculation preceded the fungal inoculation. The results corroborate with the research findings of Bhagwati *et al*. (2007) [2]. Minimum root infection in plants inoculated with *F. oxysporum* alone than the ones where nematode was present together with fungus suggested that delay in entry of fungus was due to absence of predisposing agent (*M. incognita*) or due to absence of nutrient rich cells, which were responsible for attracting the fungus to galled roots. Similar types of results were reported by Haseeb *et al*. (2007) [7] on *Pisum sativum*.

These observations on nematode-fungal interaction suggested that they were due to nematode providing a readymade means of entry into the host for the fungus. This occurred when root knot nematode caused superficial root injury and so enhances fungal access. The results of the present investigation suggested that *M. incognita* and *F. oxysporum* together caused greater damage to black gram than either of them alone. Anwar and Khan (2002) [1] observed slight to moderate galling in nematode inoculated prior to fungus in soybean. This difference might be due to the variety used during the study. The reduction in galling and nematode population could be possibly attributed to deleterious effects of metabolites of *F. oxysporum* on the juveniles of root-knot nematode. This is further supported by greater reductions when fungi and nematodes were inoculated simultaneously. These results are in conformity with the results recorded by Haseeb *et al*. (2007) [7] on *V. mungo.* Minimum nematode population was recorded when fungus was inoculated first and nematode a week after. Similar results in reduction of population of nematode in presence of *R. solani* was also reported by Goswami *et al.* (1975) [5], Kumar and Haseeb (2009) [14] and Vidyasagar (2012) [25]. This is possibly attributed to delitorious effects of metabolites of *fungus* on the juveniles of root knot nematode in terms of their development and multiplication. Decrease in the rate of nematode multiplication and galling in the presence of fungus showed antagonistic effect of the fungus on the development and reproduction of nematodes. This could be ascribed to the possible toxic effect of the fungus metabolites on nematodes (Haseeb *et al*. 2007) [7]. This was followed by simultaneous inoculations with nematode and fungus in terms of total nematode population (Root +Soil). These findings are in accord with of Anwar and Khan (2002) [1] who obsesrved lowest reproduction of *M. incognita* during the simultaneous inoculation by fungus and nematode. The increase in nematode population in the treatment where nematode inoculated first and fungus a week after might be due to the fact that nematode has colonized the feeding site first and modifies the host substrate unfavourable for fungus similar results has been recorded by Bhatt (1986). The treatments receiving the nematode inoculation prior to fungus resulted in higher reduction and plant growth than the other treatments. When nematode inoculation was done seven days prior to fungal inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression.

5. Summary and conclusion

The treatments receiving the nematode inoculation prior to fungus resulted in higher reduction and plant growth than the other treatments. When nematode inoculation was done seven days prior to fungal inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression. *M. incognita* not only predisposed the host but also shortened the incubation period for diseases expression.

6. Suggestions for further work

Systemic and comprehensive surveys of blackgram growing areas are needed to work out the economic importance of nematode fungus wilt complex involving *M. incognita* and *Fusarium oxysporum*. Agro ecological factors have to be studied to know the distribution and prevalence of this complex disease. Genetic basis of mechanism of interaction, physiologically and histological changes in diseases complex have to be workout. Characterization of translocatable metabolites in nematode fungal interactions and determination of their biochemical nature and mode of action and specificity are the areas of further research.

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