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Studies on the management of root-knot nematode, *Meloidogyne incognita*-wilt fungus, *Fusarium oxysporum* disease complex of green gram, *Vigna radiata* cv ML-1108

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Abstract: Studies were conducted under pot conditions to determine the comparative efficacy of carbofuran at 1 mg a.i./kg soil, bavistin at 1 mg a.i./kg soil, neem (*Azadirachta indica*) seed powder at 50 mg/kg soil, green mould (*Trichoderma harzianum*) at 50.0 ml/kg soil, rhizobacteria (*Pseudomonas fluorescens*) at 50.0 ml/kg soil against root-knot nematode, *Meloidogyne incognita*–wilt fungus, *Fusarium oxysporum* disease complex on green gram, *Vigna radiata* cv ML-1108. All the treatments significantly improved the growth of the plants as compared to untreated inoculated plants. Analysis of data showed that carbofuran and *A. indica* seed powder increased plant growth and yield significantly more in comparison to bavistin and *P. fluorescens*. Carbofuran was highly effective against nematode, bavistin against fungus, *A. indica* seed powder against both the pathogens and both the bioagents were moderately effective against both the pathogens.

Key words: *Meloidogyne incognita*, *Fusarium oxysporum*, Disease complex, Management, *Vigna radiata*
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INTRODUCTION

Green gram (*Vigna radiata*) is an important short duration pulse crop. Due to its high nutritive value, it is grown throughout the tropical countries of South and Southeast Asia, particularly in India (Vavilov, 1951). Among various pests and diseases, nematodes-fungus disease complex particularly of *Meloidogyne incognita* and *Fusarium oxysporum* poses a great problem to the cultivation of pulse crops by inflicting severe yield losses (Perveen *et al*., 1999; De *et al*., 2000; Mahapatra and Swain, 2001). The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops including green gram (Sikora and Greco, 1993). The infection starts with root penetration of second stage juveniles (J2) hatched in soil from eggs encapsulated in egg masses laid by the females on the infected roots (Barker *et al*., 1985). Wilt fungus (*Fusarium* spp.), deutromycetous fungus, causes wilting of the infected plant that leads to death. Yield loss due to this fungus had been reported on various crops (Agrios, 1983).

Management of disease complexes appears to less straightforward than one might anticipate. The most obvious solution is to use chemical methods to control one of the interacting organisms and thus prevent the disease complex from occurring. However, it is fundamental to have prior knowledge of the interaction involved, as even low densities of fungi or nematode can result in a disease complex of significant importance (Bowers *et al*., 1996). Carbofuran belonging to the carbamates group is a non-fumigant and had been reported to cure root-knot nematodes on various crops (Butool *et al*., 1998). It mainly affects nematode neuromuscular activity by inhibiting the enzyme acetyl cholinesterase (Evans, 1973; Wright, 1981). Similarly, bavistin (benzimidazole group) has already been proved to cure fungi belongs to deutromycotina, particularly *Fusarium* spp. by inhibiting

mitosis that ultimately causes the disorganization of the fine structure of fungal cells in plants (Howard and Aist, 1977).

Besides chemicals, various workers suggested other control measures in view of the need to replace highly toxic and potentially polluting chemicals used to control plant parasitic nematodes and fungi, with less dangerous chemicals or preferably with biological control agents and botanicals (Oostendrop and Sikora, 1989). Some species of the genus *Trichoderma* have been used as biocontrol agents against soilborne phytopathogenic fungal pathogens (Chet, 1987). These fungi may also promote plant growth (Inbar *et al*., 1994). Several attempts have also been made to use *Trichoderma* spp. to control plant parasitic nematodes. Windham *et al*.(1989) reported reduced egg production in the root-knot nematode, *M. arenaria* following soil treatments with *T. harzianum* (T-12) and *T. koningii* (T-8) preparations. Besides fungi, bacteria especially rhizospheric bacteria have also been used for biological control of soil borne plant pathogens. Fluorescent *Pseudomonas* spp. is among the most effective rhizospheric bacteria in reducing soil–borne diseases in disease suppressive soils (Weller, 1988). These bacteria can antagonize soil-borne pathogens through various mechanisms e.g. bacterial siderophores inhibit plant pathogens through competition for iron, antibiotics suppress competing microorganisms, and chitinases and glucanases lyse microbial cells (Bakker *et al*., 1991). Along with chemical and biomanagement practices for the management of nematodes and fungi, bioactive products from neem (*A. indica*) have been proved economic and ecofriendly approach due to the presence of an array of complex compounds, triterpenes, or more specifically limonoids (Kraus, 1995; Musabyimana and Saxena, 1999).

Considerable work has been carried out on the pathogenic potential and management of *M. incognita*-*F. oxysporum* disease complex on different pulse crops, but information on pathogenic potential of *F. oxysporum* and that to with *M. incognita* on *V. radiata* have not so far been reported and that is why *F. oxysporum* has not been considered as a major pathogen of *V. radiata*, but during survey of Aligarh district of Uttar Pradesh in India we observed severe symptoms of *F. oxysporum* on *V. radiata* (Haseeb *et al*., 2005). Therefore, the present study was carried out to determine the comparative efficacy of chemicals, *A. indica* seed powder and microbial antagonists against *M. incognita*-*F. oxysporum* disease complex on *V. radiata* cv ML-1108.

MATERIALS AND METHODS

Preparation of inoculum of *M. incognita* and *F. oxysporum*

A single egg mass of the *M. incognita* picked by hand with fine forceps from infected eggplant root was surface sterilized in 1:500 (*V*/*V*) aqueous solution of "chlorax" (sodium hypochlorite) for 5 min. It was then transferred to a small coarse sieve lined with tissue paper, placed beforehand in a petriplate containing sufficient amount of water. The petriplates were incubated at room temperature (27±5) °C for 5 d (den Ouden, 1958). Seedlings of eggplant raised in autoclaved soil, were inoculated with the progeny of the single egg mass in order to get regular supply of the inoculum for the experiments.

Pure culture of *F. oxysporum* isolated from the infested plants from the field was maintained on PDA (Potato Dextrose Agar) in petriplates at (27±5) °C in order to mass-produce pure culture of the fungus to be transferred to flasks containing sorghum seeds soaked overnight in 5% (*m*/*V*) sucrose solution. The flasks were incubated in a BOD (Biological Oxygen Demand) incubator at a temperature of (27±1) °C for 10 d. During incubation, the flasks were shaken three times in a day, to ensure proper growth of the fungal mycelium on the sorghum seeds. Spore suspension was made from the colonized sorghum seeds so that final CFU (colony forming units) of *F. oxysporum* was maintained at 10^8 CFU/ml.

Preparation of inoculum of *T. harzianum* and *P. fluorescens* and procurement of *A. indica* seed powder

Pure culture of *T. harzianum* isolated from the experimental fields of Aligarh Muslim University, was maintained on PDA in petriplates at (27±5) °C. Healthy sorghum seeds were soaked in 5% (*m*/*V*) sucrose solution for 16 h and then strained and placed into 500 ml conical flasks to give 200 cm^3 of sorghum seeds/flask. Flasks with sorghum seeds were plugged with cotton and sterilized by autoclaving for 30 min at

1 kg/cm² pressure. The conical flasks containing sterilized sorghum seeds were inoculated with 1 cm-diameter PDA discs punched from the periphery of actively growing 5 d-old culture of *T. harzianum* and then placed in an incubator at (27±1) °C and the fungi were allowed to grow with periodic shaking of the flasks, so that the surface of all sorghum seeds was colonized and CFU reached above to 10^8 CFU/g culture.

Pure culture of *P. fluorescens* isolated from the same experimental fields. The culture tubes, each containing 10 ml King's 'B' (Broth) were autoclaved for 30 min at 1 kg/cm² pressure. After the culture tubes were cooled, each tube was inoculated with a single colony of *P. fluorescens* strain Pf-1 from pure bacterial culture maintained on King's 'B' agar. The culture tubes were then placed in a BOD incubator for 48 h at (30±1) °C for the multiplication of *P. fluorescens*. For mass production, one-liter conical flasks containing 500 ml King's 'B' (Broth) were autoclaved at the same pressure and time as mentioned above. After the flasks were cooled, each flask was inoculated with 1.0 ml of *P. fluorescens* cultured broth. The flasks were kept at (30±1) °C in the BOD incubator for 120 h and were shaken two times a day. Inoculum culture was mixed with talc at ratio of 1:4, and then the amount of talc was adjusted so that the final CFU of *P. fluorescens* was maintained at 2×10^8 CFU/ml.

Whole seeds or dehulled seeds of *A. indica* were first dried in sunlight for 24 h and then in oven at 70 °C for 1 h, were manually pounded in a large steel mortar with pestle to produce a fine neem seed powder. Powdered form was used to allow easy decomposition of *A. indica* seeds in soil.

Experimental procedure

Experiment was conducted in polyhouse ((26.7±3) °C, 73.5%±11% Relative Humidity and 0.918 kPa) in 21 earthen pots (18 cm top diameter) filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5) and compost (4:1). One seed was sown initially and the treatments (carbofuran at 1 mg a.i./kg soil, bavistin at 1 mg a.i./kg soil, *A. indica* seed powder at 50 mg/kg soil, *T. harzianum* at 50 ml/kg soil and *P. fluorescens* at 50 ml/kg soil) were applied at the time of sowing of seeds. Six pots were untreated. Each treatment was replicated 3 times in a completely randomized block design and watered daily so that each pot got about 500 ml water. After 4 d i.e. at two-leaf stage of seedlings all the pots except the 3 untreated pots were inoculated with 2000 freshly hatched second stage juveniles of *M. incognita* and 2 ml spore suspension of *F. oxysporum* into 1 cm holes around the base of the plant which were then filled with soil. Uninoculated pots and nematode+fungus inoculated pots served as controls.

Recording of data

Sixty days after inoculation the plants were uprooted and root gall indices were determined on a scale 0~4, where 0=no infection or root galling, 1=slight infection (1%~25%), 2=moderate infection (26%~50%), 3=severe infection (51%~75%) and 4=very severe infection (76%~100%) (Taylor and Sasser, 1978). In order to determine the extent of *F. oxysporum* infection washed roots of inoculated plants were cut into 1.0 cm pieces, then treated with 10% KOH solution and finally kept at 90 °C for 1 h. These root segments were washed again with distilled water, then acidified and stained with trypan blue (0.5% (*V*/*V*) in lactophenol) as described by Philips and Hayman (1970). Five stained pieces of each taproot were mounted on slides in lactophenol and presence of mycelium of the fungus was estimated. The root infection was calculated by measuring the infected portion in relation to total length of root pieces (Biermann and Lindermann, 1981).

Fresh and dry root and shoot weights, seed and husk weight were obtained. Final nematode population in the entire soil volume was extracted by Cobb's sieving and decanting technique along with Baermann funnel and in roots by macerating 5 g root tissues in a Warring blender (Southey, 1986), and counted as per the procedure suggested by Doncaster (1962). Data were analyzed by analysis of variance (Cochran and Cox, 1957) and significant differences among treatments were tested by the least significant difference test (LSD) at probability levels of 5% ($LSD_{0.05}$) and 1% ($LSD_{0.01}$).

RESULTS

Regular observations showed that no treatment

affected seed germination except *A. indica* seed powder at 50 mg/kg soil, where the germination of seeds was delayed by 4 d as compared to other treatments, this was also observed earlier by Chakrabarti *et al*.(2001).

Data (Table 1) shows that the effect of various treatments on all the growth and yield parameters viz. shoot length, fresh and dry weights of shoot and root, seed and husk weights were mostly significant ($P \leq 0.05$) as compared to untreated inoculated plants. However, no significant differences were found in shoot height of plants treated with bavistin (24.1 cm) and *P. fluorescens* (22.6 cm). Maximum increase in all the growth and yield parameters was found in carbofuran treated plants followed by *A. indica* seed powder, *T. harzianum*, bavistin and *P. fluorescens* treated plants as compared to untreated inoculated plants.

The suppressive effect of the treatments on nematode population both in roots and soil was highly significant as compared to untreated inoculated plants, but no differences were observed in soil populations of nematode of plants grown in carbofuran (500) and *A. indica* seed powder (500) treated pots. The reproduction rate of *M. incognita* was significantly suppressed by all the treatments as compared to untreated inoculated plants. Similarly all the treatments were found to be highly effective in their ability to reduce root-knot index (RKI) when compared with untreated plants.

Data presented in Table 2 shows that the greatest suppression of nematode reproduction i.e. reproduction factor (Rf) and root-knot index was achieved by application of carbofuran (*Rf*=0.46, *RKI*=0.25) followed by *A. indica* seed powder (*Rf*=0.99, *RKI*=0.50), *T. harzianum* (*Rf*=1.27, *RKI*=1.50), *P. fluorescens* (*Rf*=1.38, *RKI*=1.75) and bavistin (*Rf*=1.50, *RKI*=2.25) respectively as compared to controls (*Rf*=1.74, *RKI*=3.00). Bavistin was highly effective in suppression of taproot colonization by fungus (8% root colonized) followed by neem seed powder (30%), *T. harzianum* (35%), *P. fluorescens* (65%) and carbofuran (75%) respectively.

DISCUSSION

V. radiata is highly susceptible to *M. incognita-F. oxysporum* disease complex as indicated by severity in root-knot development, nematode population densities, root colonization by fungus and plant growth suppression in the inoculated controls. Our results indicate that carbofuran is most effective among the treatments in improving plant growth and reducing *M. incognita* population densities in soil. Carbofuran impairs nematode neuromuscular activity by inhibiting the function of the enzyme acetyl cholinesterase resulting in reduced movement and ability of invasion and multiplication (Evans, 1973; Wright, 1981). The nematodes may also be killed

Table 1 Effect of carbofuran, bavistin, *Azadirachta indica* seed powder, *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Meloidogyne incognita* and *Fusarium oxysporum* disease complex on *Vigna radiata* cv ML-1108

Treatments	Shoot height (cm)	Fresh weight (g)		Dry weight (g)		Seed weight (g)	Husk weight (g)
		Root	Shoot	Root	Shoot		
Control	30.8	7.5	27.5	2.50	8.21	8.50	3.33
Nematode+fungus	22.1 (28.3)	4.8 (36.2)	18.6 (32.3)	1.62 (35.3)	5.63 (31.4)	5.34 (37.1)	2.12 (36.2)
Nematode +fungus+carbofuran	29.2 (5.2)	7.0 (6.8)	25.9 (5.8)	2.36 (5.4)	7.85 (4.3)	7.88 (7.2)	3.13 (5.9)
Nematode+fungus +neem seed powder	27.8 (9.7)	6.7 (10.2)	24.8 (9.8)	2.26 (9.7)	7.50 (8.7)	7.62 (10.4)	3.02 (9.4)
Nematode+fungus +*T. harzianum*	26.3 (14.6)	6.4 (15.1)	22.7 (17.3)	2.14 (14.5)	6.85 (16.5)	7.12 (16.2)	2.85 (14.3)
Nematode +fungus+bavistin	24.1 (21.8)	6.0 (20.1)	22.1 (19.7)	2.06 (21.4)	6.54 (20.4)	6.77 (20.4)	2.66 (20.1)
Nematode+fungus +*P. fluorescens*	23.8 (22.6)	5.4 (22.8)	21.9 (20.5)	1.91 (23.5)	6.46 (21.3)	6.66 (21.7)	2.65 (20.5)
$LSD_{0.05}$	2.5	0.6	2.0	0.22	0.61	0.70	0.26
$LSD_{0.01}$	3.6	0.9	2.8	0.30	0.85	0.98	0.37

Each value is an average of three replicates; Values in parenthesis represent percent reduction over uninoculated control

Table 2 Effect of carbofuran, bavistin, *Azadirachta indica* seed powder, *Trichoderma harzianum* and *Pseudomonas fluorescens* on root-knot development, reproduction of *Meloidogyne incognita* and infection of *Fusarium oxysporum* on *Vigna radiata* cv ML-1108

Treatments	Final nematode population (Pf)			Reproduction factor *Rf=Pf/Pi*	Root-knot index (*RKI*)	Disease index (%)
	Total root	1 kg soil	Total			
Control	–	–	–	–	–	–
Nematode+fungus	1488	2000	3488	1.74	3.00	95
Nematode+fungus+carbofuran	420	500	920	0.46	0.25	75
Nematode+fungus+neem seed powder	1474	500	1974	0.99	0.50	30
Nematode+fungus+*T. harzianum*	1536	1000	2536	1.27	1.50	35
Nematode+fungus+bavistin	1508	1500	3008	1.50	2.25	8
Nematode+fungus+*P. fluorescens*	1512	1250	2762	1.38	1.75	65
$LSD_{0.05}$	144.1	121.9	258.4	0.11	0.14	6.0
$LSD_{0.01}$	204.9	173.3	367.3	0.15	0.20	8.5

Each value is an average of three replicates; *Pi*: Initial population

while feeding on root tissues by the systemic action of these nematicides when they are absorbed by the plant roots and translocated in the plant system (van Berkum and Hoestra, 1979). Butool *et al.*(1998) found similar effectiveness of carbofuran in suppressing *M. incognita* on *Hyoscyamus muticus*.

Bavistin was found most effective in controlling root colonization by fungus. It inhibits the nuclear division of fungi by inactivating the spindle, which is composed of microtubules. Various scientists have also been reported, bavistin as an important control measure against *F. oxysporum* (Etebarian, 1992; Prasad *et al*., 2000; Haseeb and Shukla, 2002). To maintain a low inoculum load by continuous application of systemic fungicide alone is not practical for the control of wilt disease. To cope with this, *A. indica* seed powder and other bioagents may be applied. It is clear from the results that besides chemicals *A. indica* seed powder was sufficiently effective against both the pathogens, this may be due to presence of active principles and toxic chemicals in *A. indica* seed powder (Singh and Sitaramaiah, 1970).

Results showed significant suppression of both *M. incognita* and *F. oxysporum* by *T. harzianum*. The possible mechanism involved in *Trichoderma* antagonism had been studied intensively in terms of antibiotic and enzyme production as hyphal interactions (Dennis and Webster, 1971; Elad *et al*., 1982). Production of chitinases may have direct significance in the parasitism of *Trichoderma* on *F. oxysporum* as these enzymes function by breaking down the polysaccharides, chitin and β-glucan that are responsible for the rigidity of fungal cell walls thereby destroying cell wall integrity (Howell, 2003). Significant suppression of nematode multiplication by *P. fluorescens* was due to its capability of altering root exudates, which could alter nematode behavior and suppress nematode population in root system (Oostendrop and Sikora, 1989). Initial investigations on antagonistic rhizobacteria against root-knot nematodes, include work by Kloepper *et al*.(1992). *P. fluorescens* was found not only effective against *M. incognita* but also against wilt causing fungi. This finding accords with the results of Vidhyasekaran and Muthamilan (1995).

Our results show that the *Meloidogyne incognita-Fusarium oxysporum* disease complex can cause severe yield losses in *V. radiata* as in other crops. Although chemicals viz. carbofuran and bavistin showed a significant effect in increase of growth parameters and in suppression of the disease complex, these can be replaced to some extent by *A. indica* seed powder and microbial antagonists viz. *T. harzianum* and *P. fluorescens* to avoid the hazards of chemicals.

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