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Integration of biocontrol agents and chemical for the management of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* complex on French bean

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Abstract
Combine effect of root knot nematode, *Meloidogyne incognita* and soil borne plant pathogen like *Sclerotinia sclerotirum* with synergistic effect can cause severe crop of many diseases. Indiscriminate uses of chemical have different ill effect. Present study was conducted to study the effect of biocontrol agents and sublethal doe of chemicals for the management of disease complex caused by root knot nematode, *Meloidogyne incognita* and soil borne plant pathogen like *S. sclerotiorum.* Seed treatments with *T. harzianum* @ 5ml/ kg + seed treatment with carbendazim 0.05% w/w + soil application with carbofuran @ 0.5 kg a.i/ha was found to be most effective in increasing plant growth parameters of French bean. The treatment with carbofuran @ 1 kg a.i/ha was found to be best in suppressing the number of galls, eggmasses and final nematode population in soil. Minimum was recorded in the seed treatment with *T. harzianum*@ 5ml/kg + seed treatment with carbendazim @ 0.05% w/w + soil application with carbofuran @ 0.5 kg a.i/ha.

Keywords: *Trichoderma harzianum*, carbendazim, carbofuran, *Meloidogyne incognita*, *Sclerotinia sclerotiorum*, efficacy, French bean

Introduction
French bean (*Phaseolus vulgaris*, Family: Leguminaceae) is an important pulse crop grown in India.In an area of 60,000 ha with a total production of 12,50,000 tonnes and productivity of 20.8 t/ha. In Assam it occupies an area of 2,250 ha with a total production of 20,593 tonnes and productivity of 9.15 t/ha [1]. The annual production of the crop in India is much lower than that of other advance countries. Among the different factors root-knot nematode, *Meloidogyne incognita* is considered to be one of the important limiting factor for lower production. Pathogenicity of *M. incognita* on French bean has already been established[2], in which they reported that the nematode causes characteristic root galls or root-knot by which their infestation can be recognized. Earlier reported that the nematode may interfere with bacterial establishment and nodulation in roots and thus hamper the crop indirectly [3]. It was identified root-knot nematode, *M. incognita* alongwith *Pratylenchus brachyurus* as the causative agents of decline in French bean cultivation in Northern Parana, Brazil [4] and recorded 43.48% yield loss in French bean due to *M. incognita* [5].
French bean is also susceptible to a wide range of disease causing pathogens among which *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most common that causes serious and unpredictable yield losses as high as 100% [6, 7]. It affect all the stages of the crop i.e, seedling stage, vegetative stage, poding stage and harvesting stages. The organism also causes infection after harvesting of the crop i.e. in storage, during transportation etc.The disease has been found to be most destructive in French bean as reported by several workers[8,9].The fungus is pathogenic to more than 500 species of higher plants [10]. White mold epidemics of beans are produced by sclerotia of *S. sclerotiorum* [11].
Soil is a complex substrate and multiple associations of different organisms occur. The root-knot nematode, *M. incognita*, *R. solani* and *S. sclerotiorum* are soil borne pathogens infecting a wide range of vegetable crops. Plants are rarely subjected to association of one potential pathogen. Combine attack of *M. incognita* and *R. solani* results in greater damage to the crops compared to the damage caused by either pathogen alone. The association forms complexes where nematode predisposes and makes the host more susceptible to fungal attack on okra and tomato [12], on brinjal [13].

Among the various biocontrol agents, *Trichoderma* spp. plays an important role in various diseases management. Reduction of nematode due *T. harzianum* may be attributed to the direct parasitism of eggs and larvae through the increases in chitinase and protease activities, which could be indicator of eggs infection capabilities.

But the work on effect of biocontrol agents in combination with compatible chemicals on *M. incognita* and *S. sclerotiorum* is rare. So, the present study was conducted with an aim to find out the effective combination of biocontrol agents and chemicals to curb the problem of nematode (*M. incognita*) and soil borne pathogen (*S. sclerotirum*) at the same time.

Materials and Methods

Pot Experiment

Experimental site

The experiment was conducted in pot condition in the net-house at Department of Nematology, AAU, Jorhat, Assam during the *rabi* season.

Pot preparation

Pot (25 cm) was prepared by mixing soil: sand: compost at 2:1:1 ratio. Pots were filled with 5 kg soil mixtures and low to settle for 1 week. Mass culture of *S. sclerotiorum* was prepared in wheat bran media and applied @ 0.2 w/w in each pots one week prior to seed sowing and pots were also inoculated with 1000 freshly hatched second stage juvenile of *M. incognita*.

Treatment Combination

The French bean seeds treated with *T. harzianum* based liquid bioformulation (Org-Trichojal) @ 5ml/litre of water, fungicides (carbendazim @ 0.3%) and nematicides (carbofuran 3G) were applied as per the treatment combinations as,

T_1 : Control (Nematodes + Fungus),

T_2 : Seed treatment with *T. harzianum* @ 5 ml/kg,

T_3 : Seed treatment with carbendazim @ 0.3% w/w,

T_4 : Soil application with carbofuran @ 1kg a.i/ha,

T_5 : Seed Treatment with *T. harzianum* @ 5 ml/kg + Seed treatment with carbendazim@ 0.05% w/w,

T_6 : Seed treatment with *T. harzianum* @ 5 ml/kg + Soil application with carbofuran @ 0.5 kg a.i/ha,

T_7 :Seed Treatment with *T. harzianum* @ 5 ml/kg + Seed treatment with carbendazim @ 0.05% w/w + Soil application with carbofuran @ 0.5 kg a.i/ha,

T_8 : Seed treatment with carbendazim @ 0.05% w/w + Soil application with carbofuran @ 0.5 kg a.i/ha.

Carbofuran 3G was applied before 5 days of sowing. All the treatments were replicated for five times and laid in completely randomized block design.

Regular watering was done till the harvesting of the crop. Insect pests when appeared were killed manually. Propping was done by using a small bamboo stick to prevent the crop from lodging.

Observation recorded

For recording the observations on different parameters, 5 plants of each treatment were uprooted carefully at 60 days after inoculation of nematode. The entire root system was taken out from the pot and kept in a plastic bucket half filled with water for half an hour and then washed carefully with tap water. Observations were made on shoot length, fresh and dry weight of shoot and root, number of galls, eggmasses per root system, final nematode population in soil and disease incidence (%). For recording the dry weights, the shoots and roots were separately cut into small pieces and kept in an oven running constantly at 60 °C. The materials were weighted at every 24 hours interval till a constant weight was obtained.

For recording the final nematode population at first the entire amount of soil from each pot was mixed homogenously and drawn 250 ml of it and processed by following Cobb's modified sieving technique as mentioned earlier.

The disease incidence% was calculated by using the following formulae.

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of infected plant units}}{\text{Total number (healthy and infected) of units assessed}} \times 100$$

Results and Discussion

The present study revealed that the maximum increase in plant growth parameters viz., shoot length, fresh weight of shoot, dry weight of shoot, fresh weight of root and dry weight of shoot were recorded in the seed treatment with *T. harzianum* @ 5ml/kg (w/v) + seed treatment with carbendazim @ 0.05% (w/w) + soil application with carbofuran @ 0.5kg a.i/ha (Plate 1 and 2). The seed treatment with *T. harzianum* @ 5ml/kg + soil application with carbofuran @ 0.5 kg a.i/ha and seed treatment with *T. harzianum* @ 5ml/kg + seed treatment with carbendazim @ 0.05% (w/w) were statistically at par with each other. Further, it was observed that the soil application with carbofuran @ 1 kg a.i/ha and seed treatment with carbendazim @ 0.3% (w/w) were also statistically at par (Table 1) in enhancement of plant growth parameters of French bean. These results of the present study are in agreement with the findings of earlier worker who observed that application of *T. harzianum* and *T. viride* were effective in increasing the plant growth parameters of tomato and reducing the population of *M. incognita* [14]. It was reported that carbofuran + *T. harzianum* was highly effective in increasing number of fresh weight of seedlings of brinjal [15] and also found that maximum length of shoot and root length was obtained in soil application of *T. harzianum* colonized compost @ 20 q/ha[16] and the treatment with *T. harzianum* @ 1.25 kg/ha + carbofuran @ 1 kg a.i/ha were found to be best in increasing the plant growth characters and yield of French bean [17].

Data presented in Table 1 showed that, all the treatments were effective in increasing the plant growth of French bean over control (Plate 3). The increase plant growth observed in the study may be due to the suppression of plant diseases caused by the pathogen. Two mechanisms involved in *Trichoderma* antagonism against root-knot nematode (i) *Trichoderma* produced metabolites with antinematode activity that immobilized J_2 thus reduced root penetration and (ii) direct parasitism by *Trichoderma*. *Trichoderma* is highly rhizosphere competent i.e. able to colonize on roots as it develops, thus promote plant growth. It may also exert several mechanisms such as tolerance to stress through enhanced root and plant development, induced resistance, inactivation of pathogen's enzymes in improving plant growth and suppressing plant pathogens. All the treatments except carbendazim @ 0.3% (w/w), brought about significant decrease in number of galls and eggmasses in the root of French bean. The maximum reduction in galls and eggmasses were recorded in the treatment with carbofuran @ 1 kg a.i/ha

(Table 2). The treatment with carbendazim @ 0.3% (w/w) was statistically *at par* with control. The seed treatment with *T. harzianum* @ 5 ml/kg + seed treatment with carbendazim @ 0.05% (w/w) + soil application with carbofuran @ 0.5kg a.i/ha and the seed treatment with *T. harzianum* @ 5ml/kg + soil application with carbofuran @ 0.5 kg a.i/ha were statistically at par with each other. These results are in agreement with the previous findings of who reported that carbofuran was effective in suppressing *M. incognita* on *Hyoscyamus muticus*. It was reported that carbofuran was highly effective against nematodes and reduced the severity of root-knot [13].

The maximum reduction in nematode population was recorded in the treatment with carbofuran @ 1kg a.i/ha (Table 2) and the treatment, carbendazim @ 0.3% w/w are statistically *at par* with control. Seed treatment with *T. harzianum* @ 5ml/kg + seed treatment with carbendazim @ 0.05% w/w + soil application with carbofuran @ 0.5 kg a.i/ha and the seed treatment with *T. harzianum* @ 5ml/kg +soil application with carbofuran@ 0.5 kg a.i/ha were statistically at par for decreasing the nematode population and disease incidence. These results are in agreement with the findings that carbofuran was most effective among the treatments in reducing *M. incognita* population densities in soil. Similarly, it was also recorded reduction in population of *M. incognita* with carbofuran @ 1.5 kg a.i/ha in pigeon pea [19]. The reduction of disease incidence as observed in the present study might be due to the effect of the treatment on nematode population as in one hand carbofuran impairs nematode neuromuscular activity by inhibiting the function of the enzyme acetyl cholinesterase resulting in reduced movement and ability of invasion and multiplication[19, 20] and the nematodes may also be killed 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 [12] and on the nematicidal and antifungal activity of *T. harzianum* is an well-known fact as reported by many worker. The minimum reduction in disease incidence per cent (20.00%) was found in the treatment with *T. harzianum* @ 5ml/kg + seed treatment with carbendazim @ 0.05% w/w + soil application with carbofuran @ 0.5 kg a.i/ha (Table 2). These results are in agreement with the findings of Bora (2009) who found that maximum reduction in disease incidence was recorded when the soil was treated with *T. harzianum* colonized compost @ 20 qt/ha infested with *S. sclerotiorum* controlled white mold of French bean.

In the present investigation the seed treatment with *T. harzianum* @ 5ml/kg + carbendazim @ 0.05% (w/w) + seed treatment with carbendazim @ 0.05% (w/w) + soil application with carbofuran @ 0.5 kg a.i/ha was found to be best in increasing the shoot length, fresh and dry weight of shoot and root and in reducing the disease incidence per cent in French bean. The treatment with carbofuran @ 1 kg a.i/ha was found effective in suppressing number of galls, eggmasses and nematode population in soil.

Table 1: Effect of *Trichoderma harzianum,* carbendazim and carbofuran on the plant growth parameters of French bean (Mean of 5 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)
T_1 : Control (Nematode + Fungus)	13.88	7.69	0.75	1.02	0.31
T_2 : Seed Treatment with *Trichoderma harzianum* @ 5ml/kg	28.50	20.12	2.46	2.46	1.08
T_3 : Seed treatment with carbendazim @ 0.3 % w/w	22.22	14.71	1.53	1.71	0.72
T_4 : Soil application with carbofuran @ 1kg a.i/ha	22.46	14.83	1.59	1.64	0.69
T_5 : Seed treatment with *Trichoderma harzianum* @ 5ml/kg + seed treatment with carbendazim @ 0.05 % w/w	29.04	21.48	2.99	2.68	1.23
T_6 : Seed treatment *Trichoderma harzianum* @ 5ml/kg + soil application with carbofuran @ 0.5 kg a.i/ha	29.28	21.51	3.08	2.63	1.19
T_7 : Seed treatment with *Trichoderma harzianum* @ 5ml/kg + seed treatment with carbendazim @ 0.05 % w/w + soil application with carbofuran @ 0.5 kg a.i/ha	30.32	22.76	3.69	2.81	1.41
T_8 : Seed treatment with carbendazim @ 0.05 % w/w + soil application of carbofuran @ 0.5 kg a.i/ha	27.50	19.49	2.10	1.89	0.85
S Ed (±)	0.24	0.07	0.05	0.06	0.03
$CD_{0.05}$	0.49	0.15	0.11	0.12	0.05

Means followed by the same letter in the superscript(s) are not significantly different

Table 2: Effect of *Trichoderma harzianum,* carbendazim and carbofuran on host infection, nematode multiplication and disease incidence on French bean (Mean of 5 replications)

Treatments	No. of galls	No. of eggmasses	Final nematode population/ 250 cc soil	Disease incidence (%)
T_1 : Control (Nematode + Fungus)	91.20 (9.58)	45.20 (6.76)	384.80 (19.96)	100.00 (5.59)
T_2 : Seed treatment with *Trichoderma harzianum* @ 5ml/kg	53.00 (7.31)	30.80 (5.59)	163.40 (12.80)	60.00 (50.77)
T_3 : Seed treatment with carbendazim @ 0.3% w/w	86.00 (9.35)	43.60 (6.64)	383.00 (19.55)	60.00 (50.77)
T_4 : Soil application with carbofuran @ 1 kg a.i/ha	26.00 (5.15)	12.40 (3.58)	111.60 (10.59)	80.00 (63.43)
T_5 : Seed treatment with *Trichoderma harzianum* @ 5 ml/kg + seed treatment with carbendazim @ 0.05% w/w	47.50 (6.95)	27.80 (5.32)	149.60 (12.25)	20.00 (26.57)
T_6 : Seed treatment with *T.harzianum* @ 5 ml/kg + soil application with carbofuran @ 0.5 kg a.i/ha	38.80 (6.26)	21.40 (4.68)	131.40 (11.48)	40.00 (39.23)
T_7 : Seed treatment with *T. harzianum* @ 5ml/kg + seed treatment with	34.00	19.60	128.60	20.00

carbendazim @ 0.05% w/w + soil application with carbofuran @ 0.5kg a.i/ha	(6.07)	(4.48) ^{e}	(11.36)	(26.57)
T_8 Seed treatment with carbendazim @ 0.05% (w/w) + soil application with carbofuran @ 0.5kg a.i/ha	43.40 (6.62)	25.40 (5.08)	140.00 (11.85)	40.00 (39.23)
S.Ed (±)	0.14	0.11	0.07	0.64
$CD_{0.05}$	0.28	0.22	0.14	1.30

(-) = Increase; (+) = Decrease; INP = Initial Nematode Population
Initial nematode population 260/250 ml of soil
Values of number of galls, eggmasses and final nematode population within parentheses are √ (x + 0.5) transformed data
Values of disease incidence percentage are angular transformed values
Means followed by the same letter in the superscript(s) are not significantly different.

Plate 1: General view of the pot Experiment

Plate 2: Effect of different treatments on plant growth of French bean

Plate 3: Effect of different treatments on root growth of French bean

Conclusion
The best treatment found in the present study i.e. seed treatment with *T. harzianum* @ 5ml/kg + carbendazim @ 0.05% (w/w) + seed treatment with carbendazim @ 0.05% (w/w) + soil application of carbofuran @ 0.5 kg a.i/ha can be recommended for management of complex disease of *S. sclerotiorum* and *M. incognita* on French bean in field condition.

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