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Study on pathogenic interaction of root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chit.] and white rot causing fungus, *Sclerotinia sclerotiorum* [*Sclerotinia sclerotiorum* (Lib.) de Bary] in 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. Therefore, a study on interaction of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on French bean was conducted and the results indicated that the dual inoculation of nematode and fungal pathogen significantly decreased plant growth parameters over the single inoculation of *S. sclerotiorum* @ 0.2% W/W and *M. incognita* @ 1000 J_2/kg of soil. The treatment with *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2 % W/W simultaneous inoculation was statistically superior in decreasing the plant growth parameters of French bean. However, the number of galls, egg masses and final nematode population in soil were found maximum in single inoculation treatment than dual inoculation treatments. The highest number of galls, eggmasses and nematode population were observed in the treatment with *M. incognita* @ 1000 J_2/kg of soil. The maximum disease incidence was recorded in the treatment with *M. incognita* @ 1000 J_2/kg soil + *S. sclerotiorum* @ 0.2% w/w after 15 days inoculation.

Keywords: French bean, *Interaction*, *Meloidogyne incognita*, *Sclerotinia sclerotiorum*

Introduction

Meloidogyne incognita, the root- knot nematode causing species is considered as the most widespread and the serious plant-parasitic nematode causing diseases to a wide range of legume crops including French beans in the tropics and subtropical regions. Due to the infection the infected French beans plants shows symptom of stunting followed by wilting and death of the plants. Aboveground symptoms of severe root knot infestation include patches of chlorotic, stunted, necrotic, or wilted plants. Infested plants that are also under moisture or temperature stress may wilt earlier than other plants. Nematodes develop and reproduce on the roots of bean plants. Pathogenicity of *M. incognita* on French bean has already been established [1], 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 [2]. 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 [3] and recorded 43.48% yield loss in French bean due to *M. incognita* [4].

Sclerotinia sclerotiorum a soil borne plant pathogen causes white rot in French bean is a destructive disease and is a potential threat to the cultivation of the crop with a maximum yield loss of 100% [5, 6]. The pathogen can cause infection in all above-ground parts of French bean plants from seedling to harvesting stage. This disease infects flowering crops, especially when the weather is cool (temperatures ranging from 5-30^0 C, with an optimum of 20-25 ^{0}C) and moist and the crop canopy is dense. Besides, French bean, the fungus is pathogenic to more than 500 species of higher plants [7]. White mold epidemics of beans are produced by sclerotia of *S. sclerotiorum* [8].

The disease caused by *S. sclerotiorum* is also associated with root-knot nematode, M. incognita. The nematode, usually assist and enhance the pathogenicity mechanism of the fungus towards modification of the host plant tissues. Worker also reported that the disease intensity of soil borne plant pathogen is aggravated by the association with the root-knot nematode especially, *Meloidogyne* spp. It means that there is a positive between both the pathogen and due to which Management of such complex this disease is becoming very difficult. The present study was conducted to study the interaction of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* in French bean as the literature on this aspect is rare.

Materials and Methods

Source of root-knot nematode

M. incognita infested French bean roots showing typical symptom were collected from framer's field and were examined for presence of nematode. Monoculture of M. incognita was raised in sterile soil on tomato plant under glasshouse conditions of Department of Nematology, Assam Agricultural University, Jorhat, Assam, India. The culture was checked periodically for its purity. Sterilized 1000 g of pot mixture was taken in 25 cm diameter pots and was inoculated with freshly hatched second stage juveniles of *M. incognita* @ 1000 juvenile /pot. French bean

seeds were sown in the pots. Moisture content of the pot was maintained by watering regularly and observation were recorded on, number of galls, number of egg masses, final nematode population/250 cc soil and disease incidence (%).

Source of *Sclerotinia sclerotiorum*

French bean plants with typical symptom of white riot infection were collected and the pathogen was isolated as per standard method under the guidance of pathologist, Dr. P. Dutta and purified by standard single hyphal tip culture method on potato dextrose agar (PDA) media. Cultural and microscopical characteristics of the pathogen were recorded and pure culture was maintained on PDA slants by periodic subculture and storing at 4^0 C.

Pathogenicity of *S. sclerotiorum* was proved in pot condition by soil inoculation the mass cultured inoculum (mass culture of *S. sclerotiorum* was done in 4% maize meal sand medium) in pot condition.

Preparation of inoculum of *S. sclerotiorum*

Inoculum of *S. sclerotiorum* was prepared in wheat bran media in polypropylene bags. The media was sterilized in autoclave consecutively for two days. Bags containing sterilized media was inoculated with two mycelial disc (7 mm dia.) of 7 days old culture of *S. sclerotiorum*. These bags were incubated in BOD incubator at 18 ± 2^0C for 15 days and the pathogenicity of the fungus was conformed by Koch's postulates. Earthen pots (25 diam) were filled with 5000 g of pot mixture (soil: sand : compost at 2 : 1 : 1 W/W/W), sterilized with 4% formalin solution. After 7 days of sterilization, 60 mg of inoculam of *S. sclerotiorum* prepared in 4% maize meal sand medium.

French bean seeds were sown in pathogen inoculated pots with required control pots. The pots were maintained in glasshouse by uniform and judicious watering. Observation on development of disease symptom were recorded at frequent intervals.

Interaction of *Meloidogyne incognita* and *Sclerotinia sclerotiorum*

Pot experiment

To study the interaction of *M. incognita* and *S. sclerotiorum* a pot experiment was conducted with six treatment combinations, viz., T_1 : *S. sclerotiorum* @ 0.2% w/w,. T_2 : *Meloidogyne incognita* @ 1000 J_2/kg of soil, T_3 : *Meloidogyne incognita* @ 1000 J_2/kg of soil + *S. sclerotiorum* @ 0.2% w/w after 15 days of inoculation, T_4 : *S. sclerotiorum* @ 0.2% w/w + *M. incognita* @ 1000 J_2/kg of soil after 15 days of inoculation, T_5 : *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2% w/w simultaneous inoculation and T_6 : Un-inoculated control. All the treatments were replicated for ten times in a completely randomized block design. Mass culture of *S. sclerotiorum* was inoculated @ 0.2% w/w and mixed with the soil two days before sowing and ten days old plants were inoculated with 1000 freshly hatched second stage juvenile of *M. incognita* at root zone at 1 cm depth. 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 on number of galls, number of egg masses, final nematode population/250 cc soil, disease incidence (%) and plant growth parameter were recorded during the period of study. For recording the observations on different parameters, 10 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. 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.

Results and Discussion

Identification of *Meloidogyne* species associated with French bean

Meloidogyne species collected from French bean were identified by the presence of galls and eggs in roots of French bean. The morphological characters were also determined. Mature females are pear-shaped, 0.43-1.45 mm long, globose, usually embedded in root tissue which is often swollen or galled, soft body, pearl-white in color, stylet was found shorter, 9.5-22 µm under stereo microscope.

Identification of *S. sclerotiorum* associated with French bean

The fungal pathogen causing white rot was identified based on the cultural and morphological characters. On PDA medium frosty white mycelial growth was observed that covered the 90 mm PDA plates within 4-5 days. Microscopic observation showed mycelium

As hyaline, septate, branched and multinucleate hyphae. The fungus produced black colored, irregular shaped sclerotia with 2-5 mm in size.

Study on interaction of *M. incognita* and *S. sclerotiorum*

In the present study lowest plant growth parameters viz., shoot length, fresh weight of shoot, dry weight of shoot, fresh weight of root and dry weight of root were recorded in T5: *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2 % W/W simultaneous inoculation (Plate 1 and 2). This was followed by T3: *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2 % (W/W) after 15 days of inoculation. The recorded plant growth parameter for T1 and T2 are statistically at par with each other (Table 1). These results of the present study are in agreement with the findings of earlier worker [10] who reported that inoculation of M. incognita causes reduction in plant height, shoot and root fresh weight, root length of French bean [9]. Similarly, it was also reported [10] that there was a significant reduction in shoot weight (fresh and dry) of the plant when inoculated with 10 or above second stage larvae of *M. incognita* per pot of French bean and also found that *M. incognita* and *R. solani* together, significantly reduced the shoot length, fresh and dry weight of shoot and root of French bean [11]. It was also recorded that simultaneous inoculation of *M. incognita* and *Colletotrichum lagenarium* significantly reduced plant growth parameters on ivy gourd [12]. White mould symptoms on French bean plants inoculated with *S. sclerotiorum* resulted in rotting of collar region with white mycelial growth on the infected plant parts followed by chlorosis of leaves, wilting and dyeing of the infected plants. These symptoms were found to be typically similar to those described

by Steadman (1983) [13]. The possible explanation for comparatively greater damage in plants inoculated with nematode and fungus simultaneously or where nematode inoculation preceded 15 days to fungus as compared to fungus preceded to nematode may be due to the prior invasion of nematode into the roots thereby making the host more suitable for fungal infection providing a metabolic rich substrate and/or nematode might also modify the rhizosphere thereby favouring the fungal growth [14]. On the other hand, fungus inoculation followed by nematode caused less reduction in plant growth. The fungus made the roots less favourable for nematode attack or the fungus secretion produced adverse effects on nematodes [15].

The maximum number of galls (122.70) and egg masses (62.90) were recorded in T2: *M. incognita* @ 1000 J_2/kg of soil, which was significantly higher than the other treatments and dual inoculation treatments (Table 2). These findings are in agreement with that of earlier worker who found that maximum number of galls and egg masses were recorded in the plants inoculated with 1000 juveniles of *M. incognita* on French bean [16]. Similar, observations was also recorded that host infection (galls and egg masses in root) was significantly higher in the treatment with nematode alone and lower in dual inoculation treatments in French bean [17].

The maximum final nematode population (494.60/250 cc soil) in soil was recorded in treatment with *M. incognita* @ 1000 J_2/kg of soil. The nematode population in the treatment with *M. incognita* @ 1000 J_2/kg of soil was significantly higher than the dual inoculation treatments. These results are in agreement with findings of earlier worker reported that the final nematode population in soil increased gradually with increasing inoculums level of 10 to 1000 juveniles in French bean [18]. Similar, observations was also recorded by worker that maximum nematode population in soil in the treatment with nematode alone and lower in dual inoculation treatments in French bean [19]. The number of galls and find nematode population of nematode were reduced in presence of fungus. This may be due to the impairment of nutrient supply to the developing eggs and laying adult nematodes through giant cells [20].

The maximum disease incidence (80.00 %) was recorded the treatment T3: *M. incognita* @100J_2 /kg of soil+ *S. sclerotiorum* @ 0.2 per cent W/W after 15 days of inoculation. This was followed by T5: *M. incognita* @1000 J_2 /kg of soil+ *S. sclerotiorum* @ 0.2 per cent W/W simultaneous inoculation and T4: *S. sclerotiorum* @ 0.2 per cent W/W a+ *M. incognita* @1000 J_2 /kg of soil after 15 days of inoculation with disease incidence of 70.00%. and 60.00% respectively. These results are in agreement with findings of workers that nematode cause injury on root surface, weakening the root tissue by causing rotting or lesions, thereby making the host plant more prone to fungal attack [21].

Table 1: Effect of interaction of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* alone and in combinations on the plant growth parameters of French bean (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)
T_1: *Sclerotinia sclerotiorum* @ 0.2% w/w	18.93^b	12.39^b	1.91^b	1.72^b	0.88^b
T_2: *Meloidogyne incognita* @ 1000 J_2/kg of soil	18.48^b	12.27^b	1.89^b	1.69^b	0.85^c
T_3: *M. incognita* @ 1000 J_2/kg of soil + *S. sclerotiorum* @ 0.2% w/w after 15 days of inoculation	15.86^d	10.12^d	0.95^d	1.12^d	0.37^e
T_4: *S. sclerotiorum* @ 0.2% w/w + *M. incognita* @ 1000 J_2/kg of soil 15 days after of inoculation	17.63^c	11.11^c	1.45^c	1.38^c	0.55^d
T_5: *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2% w/w simultaneous inoculation	12.97^e	8.52^e	0.79^e	0.87^e	0.16^f
T_6: Uninoculated control	27.73^a	17.86^a	2.74^a	2.12^a	1.10^a
S.Ed. (±)	0.34	0.08	0.02	0.12	0.08
$CD_{0.05}$	0.68	0.17	0.06	0.24	0.15

Means followed by the same letter in the superscript(s) are not significantly different Check (UC) = Check (Uninoculated Control)

Table 2: Effect of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* alone and in combination on host infection, nematode multiplication and disease incidence on French bean (Mean of 10 replications)

Treatments	No. of galls	No. of egg Masses	Final nematode Population/250 cc Soil	Disease Incidence (%)
T_1: *Sclerotinia sclerotiorum* @ 0.2 % w/w	0.00 (0.71)^e	0.00 (0.71)^e	0.00 (0.71)^e	50.00 (45.00)^d
T_2: *Meloidogyne incognita* @ 1000 J_2/kg of soil	122.70 (11.09)^a	62.90 (7.93)^a	494.60 (22.24)^a	0.00 (0.91)^e
T_3: *Meloidogyne incognita* @ 1000 J_2/kg of soil + *Sclerotinia sclerotiorum* @ 0.2 % w/w after 15 days of inoculation	72.80 (8.56)^b	46.00 (6.81)^b	400.31 (20.01)^b	80.00 (63.43)^a
T_4: *Sclerotinia sclerotiorum* @ 0.2 % w/w + *Meloidogyne incognita* @ 1000 J_2/kg of soil after 15 days of inoculation	42.10 (6.51)^d	24.90 (5.02)^d	251.40 (15.87)^c	60.00 (50.77)^c
T_5: *M. incognita* @ 1000 J_2 and *S. sclerotiorum* @ 0.2% simultaneous inoculation	64.00 (8.03)^c	32.50 (5.74)^c	341.90 (18.50)^d	70.00 (56.79)^b
T_6: Uninoculated control	0.00 (0.71)^e	0.00 (0.71)^e	0.00 (0.71)^e	0.00 (0.91)^e
S.Ed.(±)	0.09	0.12	0.11	0.16
$CD_{0.05}$	0.19	0.24	0.22	0.32

Values within parentheses are $\sqrt{(x + 0.5)}$ transformed data. Means followed by the same letter in the superscript(s) are not significantly different. Check (UC) = Check (Uninoculated Control).

Plate 1: General view of pot experiment

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

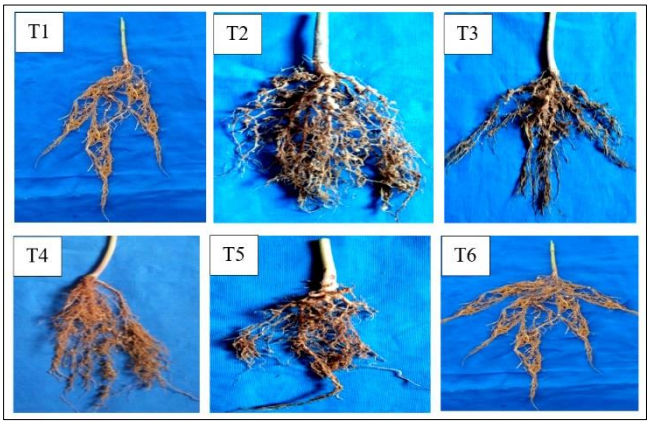

Plate 3: Effect of different treatments on root growth of French bean

Conclusion

In the present study, the maximum reduction in plant growth parameters *viz.*, shoot length, fresh and dry weight of shoot and root were due to the treatment with *M. incognita* @ 1000 J_2/kg of soil and *S. sclerotiorum* @ 0.2% W/W simultaneous inoculation, while the maximum number of galls, egg masses, and nematode population in soil were observed in the treatment with *M. incognita* @ 1000 J_2/kg of soil and also maximum disease incidence was observed in the treatment with *M. incognita* + *S. sclerotiorum* 0.2% W/W after 15 days of inoculation

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