

Original Research Article

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Interaction of White Rot Fungus [*Sclerotinia sclerotiorum* (Lib.) de Bary] and Root-Knot Nematode [*Meloidogyne incognita* (Kofoid and White) Chit.] in Cabbage (*Brassica oleracea* L.)

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ABSTRACT

Keywords

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Cabbage is one of the most widely cultivated vegetables of the temperate zone. Cabbage is susceptible to many foliar and soil borne diseases, among the soil-borne fungal diseases of cabbage, white head rot disease caused by *Sclerotinia sclerotiorum* is a potential threat to cabbage production and limits the crop cultivation and it is also associated with root-knot nematode (*Meloidogyne incognita*). An experiment was conducted to study the interaction of *S.sclerotiorum* and *Mincognita* in cabbage. Sequential and concomitant inoculation of the rot-knot nematode (*M. incognita*) and fungus (*S. sclerotiorum*) was done in cabbage to observe the disease severity due to the individual effect and interactive effect of the nematode and fungus. It was observed that the white rot disease was found to be more severe (95.41 per cent) with the sequential inoculation of nematodes followed by fungus than with the fungus alone treatment. From the present study, it is well established that the root-knot nematode in cabbage predispose the plants to infect *S. sclerotiorum* pathogen.

Introduction

Cabbage (*Brassica oleracea* L. or *B. oleracea* var. *capitata* L.) is one of the most widely cultivated vegetables of the temperate zone. It is a leafy green or purple biennial plant, grown as an annual vegetable crop for its dense-leaved heads. It is closely related to other Cole crops, such as Broccoli, Cauliflower and Brussels sprouts. Cabbage is one of the most popular cruciferous vegetables grown throughout India and is one of the richest sources of energy (Vitamin C) and health benefits

(Aggie Horticulture, 2013). It is susceptible to many foliar and soil borne diseases, among them white rot disease caused by *Sclerotinia sclerotiorum* is a potential threat to cabbage production and limits the crop cultivation and it is also associated with root-knot nematode, *Meloidogyne incognita*. The nematodes usually assist and enhance the pathogenicity mechanism of the fungus towards modifications in the host plant tissues (Jordan, 1987; Bowers *et al.*, 1996). The disease intensity of many soil-borne fungal pathogens

is aggravated by association with root-knot nematode especially *Meloidogyne* spp. (Golden and Van Gundy, 1975). Loganathan *et al.*, (2010) reported the positive correlations between head rot disease intensity and population of root-knot nematode, *M. incognita* in cabbage. Interactive effect of disease intensity increases by population of root-knot nematode *M. incognita* and also reduces plant growth and seed yield of *Plantago ovate* (Anisha and Akhtar, 2012). Management of this disease is becoming very difficult due to its soil borne nature and complex with nematode.

Materials and Methods

Source of root-knot nematode

Cabbage roots infested with *M. incognita* were collected from the farmer's fields and the roots were examined for the presence of the nematode. A monoculture of *M. incognita* was raised in sterile soil on tomato plant under glasshouse conditions. The culture was checked periodically for its purity. Sterilized pot mixture (500 g) was taken in 15 cm diameter pots. The soil was inoculated with freshly hatched second stage juveniles of *M. incognita* @ one juveniles/gm and on which seeds of cabbage were sown. Optimum watering was done every day and observation was made on 25th day after inoculation. The gall development was assessed using gall index scale developed by Headle *et al.*, (1989). Observations on number of egg mass per gram root and gall index using 1-5 scale (Gall index: 1= no galls; 2= 1-25% galls; 3= 26-50% galls; 4= 51-75% galls; 5= 76-100% galls per root system) were recorded.

Source of *Sclerotinia sclerotiorum*

Cabbage heads showing characteristic head rot symptoms were collected and the pathogen was isolated by tissue segment

method (Yella Goud, 2011) on potato dextrose agar medium. Pure culture of the isolate was obtained by single hyphal tip method (Yella Goud, 2011). The morphological characters of the pathogen were observed under microscope and the pathogen was maintained on PDA slants at 4°C.

The pathogenicity was proved by head detached technique on cabbage head under laboratory conditions, by making a hole using cork borer and pathogen was inoculated in to the hole and closed with wet absorbent cotton.

After 10 days of inoculation, the cabbage head was observed for rotting symptoms and sclerotial bodies (Siddique and Yasmin, 1998).

For soil inoculation, *S. sclerotiorum* was multiplied in sand-maize medium (Riker and Riker, 1936). The substrate containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 800 ml of water kg⁻¹ and packed in polypropylene bags.

The bags were autoclaved at 1.4 kg cm⁻² for two hr for two consecutive days and each bag was inoculated with two 9 mm culture discs of the fungus. These bags were incubated at room temperature (28 ± 2°C) for 15 days and the pathogenicity of the fungus was confirmed by Koch's postulates. Earthen pots of 30-cm-dia were filled with five kg of pot mixture (red soil : sand : FYM at 1:1:1 w/w/w), sterilized at 1.4 kg cm⁻² for two h for two successive days and inoculated by mixing 10 g of inoculum of the fungus multiplied on sand maize medium.

Cabbage seedlings were planted in pots and proper controls were also maintained. The pots were maintained in glasshouse by uniform and judicious watering. The plants were observed for development of disease symptoms.

Interaction of *Sclerotinia* and *Meloidogyne* in cabbage

A pot culture experiment was conducted with six treatments each replicated four times in a completely randomized block design to study the interaction of root knot nematode and head rot pathogen in cabbage. Sterilized pot mixture was used to fill the 3/4 dia mud pots. In each pot 10% w/w of fungus and 1500 juveniles of nematode were inoculated in each pot.

The treatments are as follows, T₁ - Fungus alone @ 10% w/w, T₂ - Root knot nematode alone @ 1500 juveniles, T₃ – Fungus was inoculated first and root knot nematode was inoculated after 7 days, T₄ - Root knot nematode was inoculated first and fungus was inoculated after 7 days, T₅ – Simultaneous inoculation of both fungus and root knot nematode, T₆ – Control (Uninoculated). The inoculation of fungal pathogen and nematode was done as described above. The nematode root knot index and population of nematode were recorded on 45 DAI in concerned treatments. Infection on root and stem was recorded at 45 DAI and the head rot disease incidence was recorded by Per cent infection. The plant growth parameters *viz.*, germination percentage, plant height and plant biomass were also recorded in each treatment.

Result and Discussion

Identification of *Meloidogyne* species associated with Cabbage

Meloidogyne species collected from cabbage were identified by the presence of root galls (Picture a), and female egg (b,c) in root portion and determining their morphological characters. Mature females are pear-shaped, globose, 0.4-1.3 mm long, usually embedded in root tissue which is often swollen or galled, soft body, pearl-white in color, stylet is shorter, 10-24 µm under stereo microscope.

Identification of *S. sclerotiorum* associated with cabbage

The species was identified based on the morphological characters identification. Observation under microscope revealed hyaline, septate, branched and multinucleate hyphae (Picture e). Mycelium appeared as white to tan in culture (Picture f). No asexual conidia were observed. The sclerotia were black in colour, large in size and irregular in shape.

Effect of *Sclerotinia sclerotiorum* and *Meloidogyne incognita* in cabbage on head rot and gall index in cabbage

Sequential inoculation of nematodes 7 days prior to fungus inoculation significantly increased the severity of white head rot incidence to 95.4 per cent followed by the concomitant inoculation of nematode and fungus, which recorded a white rot incidence of 84.31 per cent, indicating that nematodes predispose plants to infection by fungus and aggravate the disease incidence, which ultimately leads to the death of plants. The nematode gall indices were found to be 4 and 3 in the above treatments, respectively. Highest gall index of 5.0 was observed when the nematode alone was inoculated. When the fungus alone was inoculated 75.4 per cent head rot incidence was observed. However, when fungus was inoculated 7 days prior to inoculation of nematode recorded a head rot incidence of 73.96 per cent and the nematode gall index was found to be 2. When the number of egg masses were considered, a highest egg masses of 27.49/gram of root tissue was observed when the nematode was inoculated 7 days prior to fungus inoculation. However, a egg masses of 14.27/gram of root tissue was observed when the fungus was inoculated 7 days prior to nematode inoculation. A maximum egg masses of 24.52/gram root tissue was observed, when the nematode alone inoculated (Table 1).

Effects of *Sclerotinia sclerotiorum* and *Meloidogyne incognita* on head rot disease severity and plant growth parameters of cabbage

In general all the plant growth parameters were decreased significantly in all the treatments when compared to uninoculated control. Sequential inoculation of nematodes 7 days prior to fungus inoculation significantly decreased the germination percentage with 53%. The plant growth

parameters viz., plant height and plant biomass were also recorded with 6.4cm and 1.91g respectively followed by the concomitant inoculation of nematode and fungus which recorded a germination percentage of 55.2%, and a plant height and plant biomass of 6.8cm and 1.97g respectively. The reduction in plant growth parameters indicating that nematodes predispose plants to infect fungus and aggravate the disease incidence which ultimately leads to plants death (Table 2; Fig. 1).

Table.1 Effect of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on head rot and Gall in dextrin cabbage

Treatment(s)	Root gall index (0-5 scale)	No. of eggs mass/g of root	Per cent white rot disease incidence
<i>S. sclerotiorum</i> alone @ 10% w/w	0 (4.05)	0	75.4 (60.29)
<i>M. incognita</i> alone @ 1500 juveniles/pot	5 (12.92)	24.52	7.5 (15.89)
Inoculation of <i>S. sclerotiorum</i> @ 10% w/w 7days prior to inoculation of <i>M. incognita</i> @ 1500 juveniles/pot	2 (8.12)	14.27	73.96 (59.31)
Inoculation of <i>M. incognita</i> @ 1500 juveniles/pot 7days prior to inoculation of <i>S. sclerotiorum</i> @ 10% w/w juveniles	4 (11.48)	27.49	95.41 (78.41)
Concomitant inoculation of <i>S. sclerotiorum</i> @ 10% w/w and <i>M. incognita</i> @ 1500 juveniles/pot	3 (9.97)	21.94	84.31 (66.7)
Uninoculated control	0 (4.05)	0	0 (0.70)
CD (P=0.05)	0.19	0.56	4.48

Mean of Four replications

Values in parantheses are arc sine transformed values

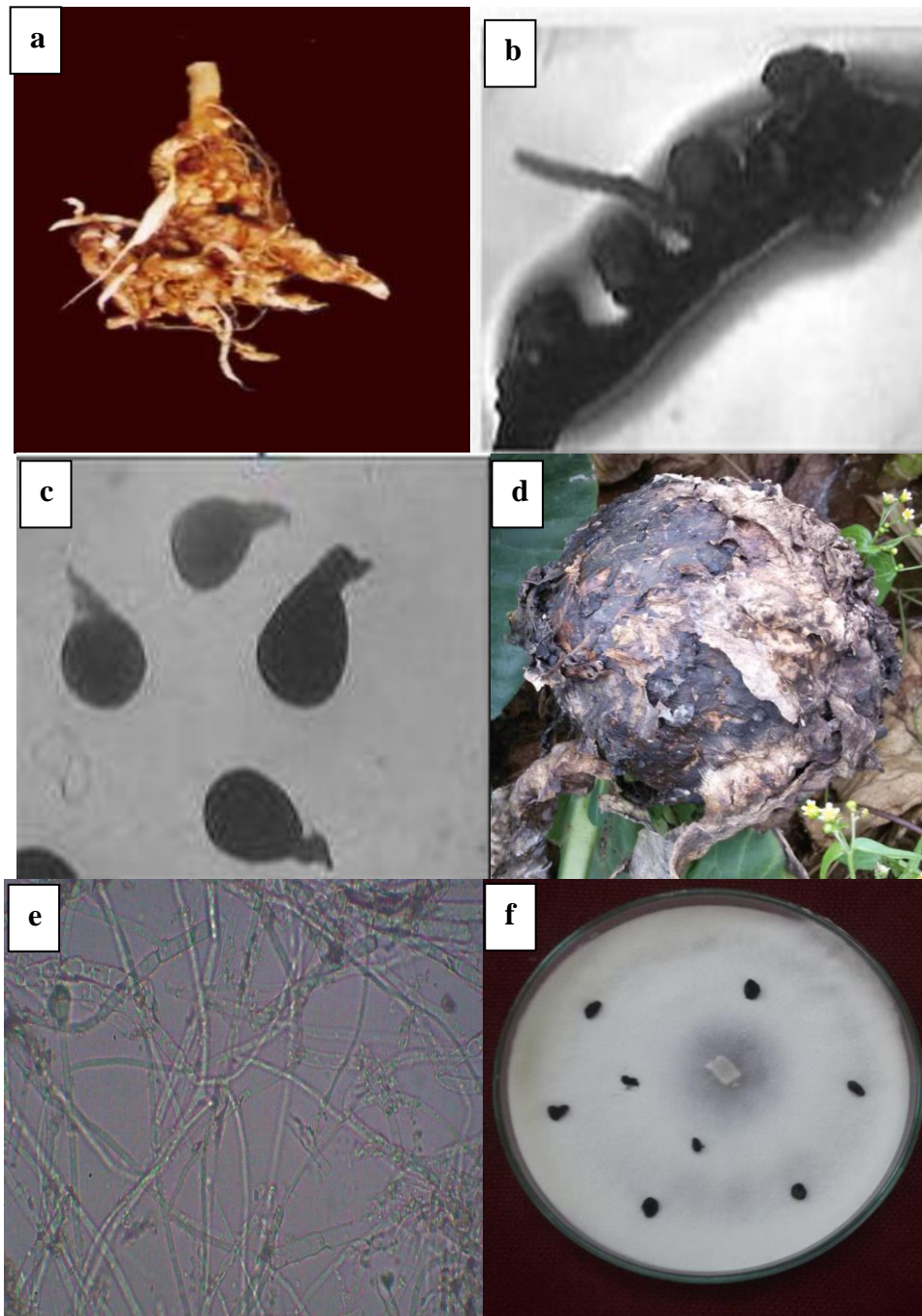
Table.2 Effect of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on germination and Plant growth parameter of cabbage

Treatment(s)	Germination (%)	Plant height (cm)	Plant biomass (grams)
<i>S. sclerotiorum</i> alone @ 10% w/w	62% (51.94)	7.4	2.23
<i>M. incognita</i> alone @ 1500 juveniles/pot	63.8% (53.0)	7.0	2.06
Inoculation of <i>S. sclerotiorum</i> @ 10% w/w 7days prior inoculation of <i>M. Incognita</i> @ 1500 juveniles	59.4% (50.42)	7.1	1.91
Inoculation of <i>M. incognita</i> @ 1500 juveniles/pot 7days prior to inoculation of <i>S. sclerotiorum</i> @ 10% w/w	53% (46.71)	6.4	1.75
Concomitant inoculation of <i>S. sclerotiorum</i> @ 10% w/w and <i>M. incognita</i> @ 1500 juveniles/pot	55% (47.87)	6.8	1.97
Uninoculated control	72% (58.05)	9.9	3.60
CD (P=0.05)	1.62	0.44	0.21

Mean of four replications

Values in parentheses are arc sine transformed values

Fig.1 a. Root-knot symptom in cabbage b. Egg masses of *M. incognita* on roots of cabbage c. Female nematode d. Symptom of head rot on cabbage e. mycelial characters of *S. sclerotiorum* f. Culture of *S. sclerotiorum* with sclerotia



The diseased cabbage plants were collected from conventional cabbage growing areas of Tamil Nadu. The pathogenic isolates of *S. sclerotiorum* was established by the standard isolation procedure and the isolate possessed

the morphological characters similar to *S. sclerotiorum* as described by Pedro *et al.*, (2015), that the young growing mycelium was fluffy white. The black sclerotia were developed from the mycelium growing in

strands at its tip initiates sclerotial formation and sclerotia were first white and becoming light brown to dark brown at maturity.

Nematode infected roots were collected and the juveniles were inoculated in the soil where tomato seedlings were planted to maintain the nematode population and gall formation was noticed in the inoculated plants as described by Meena *et al.*, (2015) on cabbage.

When the pathogen was inoculated on the head of cabbage and incubated at 18 to 20°C for a week, rotting symptoms was observed and sclerotia were also developed on the head. It was commensurate with the findings of Siddiqui and Yasmin (1998). By inoculation of root-knot nematodes root galls were developed on the root of inoculated plant as reported by Sasanelli *et al.*, (2013).

Many species of plant parasitic nematodes predispose the plants for infection by certain fungi or in same way enhance the severity of the diseases (Taylor, 1990; Abrantes *et al.*, 1994; Jonathan and Rajendran, 1998). Wounding of hosts by nematode by predisposition, appears to have a restricted role in promoting interactions between nematode, *M. incognita* and soil borne pathogen, *S. sclerotiorum* (Anisha and Akhtar, 2012). Nematodes act as a predisposer in the spread of secondary fungal pathogens (Meena *et al.*, 2015).

The above mechanism suggested that the nematode bring about severe host susceptibility for fungal infection. In a similar way, *M. incognita* would aggregate the head rot symptoms when the nematode was inoculated previously or simultaneously with fungal pathogen in cabbage. Loganathan *et al.*, (2010) reported earlier that there was a positive correlation between white rot disease intensity and population of root-knot nematode *M. incognita* in cabbage.

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