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ORIGINAL ARTICLE

# Interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on green beans

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Synergism

**Abstract** The interaction between *Meloidogyne incognita* (race 2) and *Rhizoctonia solani* (AG 4) in a root rot disease complex of green beans (*Phaseolus vulgaris*) was examined in a greenhouse pot experiment. Three week-old seedlings (cv. Contender) were inoculated with the nematode and/or the fungus in different combinations and sequences. Two months after last nematode inoculation, the test was terminated and data were recorded. The synchronized inoculation by both pathogens (N + F) increased the index of *Rhizoctonia* root rot and the number of root galls; and suppressed plant growth, compared to controls. However, the severity of root rot and suppression of plant growth were greater and more evident when inoculation by the nematode preceded the fungus (N → F) by two weeks. Nematode reproduction (eggs/g root) was adversely affected by the presence of the fungus except by the synchronized inoculation. When inoculation by nematode preceded the fungus, plant growth was severely suppressed and roots were highly damaged and rotted leading to a decrease of root galls and eggs.

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## 1. Introduction

Green beans (*Phaseolus vulgaris* L.) is a very important summer vegetable crop in Saudi Arabia, and grown in the open fields and greenhouses mainly for its green pods. The crop is frequently attacked by *Meloidogyne javanica* (Treub), Chitwood and *Meloidogyne incognita* (Kofoid & White) Chitwood (Al-Hazmi, 1985; Al-Hazmi et al., 1995). Green beans are also very susceptible to *Rhizoctonia solani* Kuhn (Hall, 1991). In a field survey of fungal pathogens associated with green beans in the central region of Saudi Arabia, 17 species of pathogenic fungi were recorded (Al-Osaimi, 2005). Among these fungi, *R. solani* was found to be the second most common species and the most severe on green beans. The fungus *R. solani* was found frequently associated with the root-knot nematodes. Interaction between these two pathogens in our field soils might play a very damaging role in our green bean fields.

Since the first recorded case of nematode–fungus interaction in 1892 (Atkinson, 1892), interest in such interactions and their damages to many economic crops has been attracting many scientists. Interactions between the root-knot nematodes (*Meloidogyne* species) and the root rot fungus *R. solani* have been studied and documented in several host crops including

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green beans. Several reviews on the subject have been published (Powell, 1971; Back et al., 2002; Shahzad and Ghaffar, 1992; Mai and Abawi, 1987; Evans and Haydock, 1993).

Several reports indicated that *Rhizoctonia*-root rot was more severe in the presence of root-knot nematodes, including the root rot disease complex caused by *R. solani* and *M. incognita* on green beans (France and Abawi, 1994; Mokbel et al., 2007; Abuzar, 2013; Ali and Venugopal, 1992; Batten and Powell, 1971; Chahal and Chhabra, 1984; Shahzad and Ghaffar, 1995; Sharma and Gill, 1979; Anwar and Khan, 2002; Reddy et al., 1979; Bhagwati et al., 2007). Most of these reports indicate a synergistic interaction between these two important pathogens. *Rhizoctonia*-root rot generally affects seedlings, but fungus can also infect mature plants and induce root rot leading to plant wilt and finally death of infected plants.

The objective of this present study was to evaluate the interaction of *M. incognita* (race-2) and a local isolate of *R. solani* in a root rot disease complex of green beans (cv. Contender) under the greenhouse conditions.

## 2. Materials and methods

This greenhouse experiment consisted of individual, concomitant and sequential inoculation of *M. incognita* (race-2) and *R. solani* (AG4). The test included six treatments, namely: (1) *M. incognita* alone (N); (2) *R. solani* alone (F); (3) both pathogens simultaneously (N + F); (4) nematode first and then fungus two weeks later (N → F); (5) fungus first and then nematode two weeks later (F → N); and (6) control (non-inoculated seedlings) (Table 1).

The nematode inoculum consisted of eggs which were extracted in 0.05% sodium hypochlorite (Hussy and Barker, 1973) from a pure greenhouse culture of *M. incognita* (race-2) on tomato plants. The egg suspension was immediately washed several times with sterilized distilled water, and then, adjusted to contain 1200 eggs/ml of the suspension. The nematode inoculum used was 12,000 eggs/seedling.

A pure culture, on Potato Dextrose Agar (PDA), of *R. solani* originally isolated from green bean plants from local fields, was obtained from the Mycology Unit (Dr. Saleh El-Hussaini), laboratory of fungal plant diseases, Department of Plant Protection, King Saud University, Riyadh, Saudi Arabia. The fungus was, then, maintained on PDA in petri plates (at 25–27 °C) for a week. For inoculum preparation, 250 ml conical flasks, each containing about 10 g of barley grains soaked overnight in sterilized distilled water, were used. The media in flasks were autoclaved for 30 min in two consecutive days. After the flasks were cooled, each one was inoculated with a small block (5 mm diam.) taken from the periphery of the 7-day-old cultures on PDA. The flasks were, then, incubated at 27 ± 2 °C for two weeks. During incubation, the flasks were shaken twice a day to ensure the proper growth of fungal mycelium on the barley seeds. The fungal-colonized barley seeds were used as inoculum at the rate of 15 g/seedling.

The green bean (*Phaseolus vulgaris* L.) cultivar used in this study was "Contender" which is known to be susceptible to both *M. incognita* (race-2) and the fungus *R. solani*. Uniform 3-week-old seedlings were transplanted singly into sterilized plastic pots (14 cm diam.) containing a steam-sterilized mixture (1500 g soil) of equal parts of sand, soil, and peat moss. The seedlings were fertilized with Hogland's solution and left in the greenhouse for two weeks before treatments.

At inoculations with nematode and/or fungus, each seedling was inoculated with 12,000 eggs and/or 15 g of the fungus inoculum on barley grains depending on the designated treatment (Table 2). Non-inoculated seedlings served as control. The nematode egg inoculum, suspended in 10 ml of water, was equally distributed through three small holes made in the soil around the seedling stem and deep enough to contact the roots. Inoculation with the fungus was made by distributing and mixing the fungal inoculum thoroughly with the soil surface of the designated pots. Each treatment was replicated five times, and treatments were arranged on a bench in the greenhouse (25–27 °C) in a completely randomized design. All seedlings were irrigated and fertilized with Hogland's solution as needed.

Sixty days after inoculation, plants were uprooted, washed under tap water and growth parameters were recorded. Nematode infection was determined by the number of root galls and host growth, whereas the nematode reproduction was determined by the number of eggs on roots. Gall index (Taylor and Sasser, 1978) and reproduction factor (RF) of the nematode (Oostenbrink, 1966) were calculated. Root rot of each root system was determined according to four categories of root system necrosis: 0 = none; 1 = less than 25%; 2 = 26–50%; 3 = 51–75%; 4 = 76 = 100% (Aoyagi et al., 1998). Disease severity of root rot was also calculated according to the formula by Aoyagi et al. (1998).

**Table 1** Effects of *Meloidogyne incognita* (N) and *Rhizoctonia solani* (F) singly and combined on the severity of root rot of green beans.

| Treatment* | % root area infected | Root rot dis. index (0–4)** | % Severity of root rot*** |
|---|---|---|---|
| F | 51 bc | 2.2 c | 52 bc |
| N + F | 58 b | 2.8 b | 58 b |
| N → F | 84 a | 3.6 a | 82 a |
| F → N | 42 c | 2.0 c | 42 c |

Values are means of five replicates. Means, in each column, followed by the same letter (s) are not significantly, different at $P \leqslant 0.05$.

[*] Sequences: N + F = simultaneous inoculation, N → F = nematode applied 2 weeks before fungus, F → N = fungus applied 2 weeks before nematode.

[**] On a scale of 0–4, where 0 = healthy; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%, and 4 = 76–100% root area is infected (Aoyagi et al., 1998).

[***] % disease severity $= \sum \frac{\text{Disease index} \times \text{No. plants in each category of the index}}{\text{Higher value of the index} \times \text{No. of all inoculated plants}} \times 100$ (Aoyagi et al., 1998).

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