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INFLUENCE OF SOIL TEXTURE AND MOISTURE ON THE INTERACTION OF *Meloidogyne javanica* AND *Macrophomina phaseolina* ON GREEN BEANS

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KEYWORDS

charcoal root rot

disease complex

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ABSTRACT

Root-knot/charcoal root rot disease complex caused by the interaction of *Meloidogyne javanica* and *Macrophomina phaseolina* is a serious disease complex attacking bean crop either in the field or greenhouses. In two different greenhouse tests, the influence of soil texture and moisture on the severity of the root-knot/charcoal root rot disease complex on green beans, *Phaseolus vulgaris* were examined. Results of the soil texture test indicated that the disease severity (suppression of plant growth and root-knot/charcoal root rot disease index), the nematode reproduction and the fungus growth in soil increased with the increase of sand content in the soil. Results of the soil moisture test showed that the greatest plant damage occurred at the soil of moisture level of 30% of field capacity, and disease severity decreased gradually as the moisture level was increased.

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1 Introduction

Green bean, *Phaseolus vulgaris* L., is an important vegetable crop worldwide. In Saudi Arabia, the crop is grown extensively in open fields and greenhouses mainly for its green pods. In different surveys, it was reported that this crop encountered simultaneous infection by the root-knot nematode (RKN), *Meloidogyne javanica* (Treub) Chitwood, and the charcoal root rot (CRR) fungus, *Macrophomina phaseolina* (Tassi) Goid (Al-Hazmi et al., 1995b; Al-Osaimi, 2005; Al-Nadhari, 2014). Infected plants are usually stunted and show extensive root gall development and severe symptoms of charcoal root rot symptoms with considerable crop loss (Al-Hazmi, 1985). This disease complex, induced by the interaction between both pathogens, has increasingly become a serious problem in the Saudi green bean fields and greenhouses. Many reports have clearly shown that the damage caused by root-knot nematodes aid in the development of diseases caused by some soil-borne fungi (Al-Hazmi, 1985; Evans & Haydock, 1993; Back et al., 2002).

Several environmental and edaphic factors can influence the interaction between these two pathogens. Subsequently, the development and severity of the resulted disease complex (root-knot/charcoal root rot) on green beans and other crops increased (Tu & Cheng, 1971; Siddiqui & Husain, 1991; Nischwitz et al., 2002).

Soil texture and moisture are important edaphic factors that can affect the activities of many soil-borne pathogens. Several studies have shown great effects of soil texture and moisture on different species of the root-knot nematodes (*Meloidogyne* spp.) on several crops (Al-Hazmi, 1988; Jaraba et al., 2014; Shahab, 2014). Similar effects were also reported on the fungus *M. phaseolina* (Srivastava & Dhawan, 1980; Pande et al., 1989; Husain & Ghaffar, 1995; Diourte et al., 1995; Lodha, 1996; Kending et al., 2000; Jimenez, 2011; Arias et al., 2013). However, effects of soil texture and moisture on the interaction between these two pathogens on green beans are very few or lacking. The severity of the root-knot/root rot, caused by the interaction of *M. javanica* and *M. phaseolina* on kenaf seedlings, *Hibiscus cannabinus* L., decreased as the seedling age at inoculation was increased from 5 to 15 days of age (Tu & Cheng, 1971). Similar results were reported on tobacco seedlings (Antoon et al., 2009). Since there is a great possibility of simultaneous natural infection by both pathogens, there is a great need for assessing the damage of their interaction, and the edaphic factors that may influence the resulted disease complex. Therefore, the aim of this work was to examine and evaluate the influence of two main soil factors namely: soil texture and soil moisture on the interaction of *M. javanica* and *M. phaseolina* and the development and severity of the root-knot/charcoal root rot on green beans under greenhouse conditions.

2 Materials and Methods

Two different greenhouse experiments were conducted to examine the effects of soil texture (test 1) and soil moisture (test 2) on the severity of the disease complex (root-knot/root rot) caused by the interaction between *M. javanica* and *M. phaseolina* on green beans.

2.1 Nematode Inoculum

Inoculum of *M. javanica* consisted of 8.0 eggs/g soil (12,000 eggs/seedling). The egg inoculum was extracted from a pure greenhouse culture on tomato (cv. Rutgers) using 0.5 NaOCl (Hussey & Barker, 1973). The extracted eggs were immediately washed several times with running tap water, and the egg suspension was then adjusted to give 1200 eggs/ml water.

2.2 Fungus Inoculum

A pure culture of the fungus *M. phaseolina* was obtained from the fungal diseases laboratory, Department of Plant Protection, King Saud University. The fungus was originally isolated from green bean plants that were naturally infected with charcoal root rot near Riyadh, Saudi Arabia. This local isolate was then cultured on potato dextrose agar (PDA) in Petri dishes at 24-26 °C for one week.

For inoculum preparation, 500-ml conical glass flasks were each filled with 150 g of barley grains and bran (3:1), and soaked with water overnight. These flasks were autoclaved for 20 min. over two consecutive days. After cooling, each flask was inoculated with a small disc (8 mm diam.) of fungus culture which was taken from the periphery of the 7-day-old cultures. Cultural flasks were incubated at 25 ± 2 °C for two weeks (Bugbee, 1974; Khanzada et al., 2012). During incubation, the flasks were shaken twice a day to ensure a uniform distribution and proper mycelial growth. To obtain the sclerotia which were used as inoculum, the cultures were thoroughly mixed and the mixture was blended in an electric blender in batches of 100 g in 250 ml sterilized distilled water. Each patch was blended five times for 30 sec. each. The contents were then passed through several layers of muslin cloth to obtain the sclerotia (Khanzada et al., 2012). The sclerotia were counted, and the suspension was adjusted to the required concentration. The fungal inoculum consisted of 8 sclerotia/g soil (12,000 sclerotia/seedlings).

2.3 Host Plant

Seeds of the green bean cv. "Contender", which is known to be susceptible to the local isolates of *M. javanica* and *M. phaseolina* (Al-Hazmi et al., 1995b; Al-Osaimi, 2005), were sown in a clean

plastic tray filled with autoclaved loamy sand soil. One week after seed germination, the seedlings were transferred, individually, into small clean plastic pots.

2.4 Effect of Soil Texture

This test was conducted to determine the effect of different soil textures on the severity of the interaction of *M. javanica* and *M. phaseolina* on green bean. In this test, the effect of five soil textures was evaluated. These soil textures included: sandy, loamy sand, sandy loam, sandy clay and clay (Table 1).

Table 1 Descriptions of soil texture treatments used in text and tables

Soil mixture	% soil particle			Soil texture
	Sand	Loam	Clay	
Soil mix 1	94.00	2.00	3.68	Sandy
Soil mix 2	83.12	6.00	10.88	Loamy sand
Soil mix 3	69.12	16.00	14.88	Sandy loam
Soil mix 4	60.32	14.00	25.68	Sandy clay loam
Soil mix 5	35.12	24.00	35.88	Clay loam

2.5 Effect of Soil Moisture

The objective of this test was to evaluate the effect of different soil moisture levels (different irrigation regimes) on the severity of the disease complex caused by the interaction of the two pathogens. Five soil moisture levels viz. 30%, 50%, 70%, 90% and 100% were used (Table 4) and these were calculated based on the percentage of soil field capacity using the pressure tablet method described by Topp & Ferre (2002). Soil moisture in each pot was checked twice every day and maintained up to its designated moisture level.

2.6 Plants Cultivation and Inoculation

At the time of seedling transplanting, nematode and/or the fungus culture were inoculated in clean plastic pots (14 cm diam.) filled with 1500 g of an autoclaved loamy sand soil. The soil of each pot was then thoroughly mixed in a plastic bag with the nematode inoculum (8 eggs/g soil) and/or the fungus (8 sclerotia/g soil). Infested soils were returned to their designated pots, according to the test treatments, and 3-wk-old seedlings of the host plants were transplanted individually into each pot. Each treatment in both experiments (soil texture and soil moisture) was replicated five times.

Pots, in each test, were arranged on a greenhouse bench (24-26°C)

in a randomized complete block design (RCBD). All seedlings, in both experiments, were fertilized every two weeks with Vauxal® (1.5-5-18-18) solution @ 10 ml Vauxal®/L water. Seedlings of the test 1 (soil texture test) were irrigated as needed, while those of test 2 (soil moisture test) were checked twice each day and always maintained up to the designated level of soil moisture.

2.7 Data collection

Sixty days after nematode and/or the fungus inoculation, plants of each treatment for both the experiments were uprooted. The disease severity, based on plant growth, number of root galls, and charcoal root rot index was determined as method described by Iqbal et al. (2010). Reproduction factor of the nematode (Oostenbrink, 1996) and percentage of the fungus re-isolation from soil (Meyer et al., 1974) were also determined.

2.8 Statistical Analysis

Data of each test were statistically analyzed (SAS, 2013), and the treatments means were separated using Fisher's protected least significant differences at $P \leq 0.05$ (FPLSD_{0.05}). Percentages data were transformed using angular transformation prior to ANOVA.

3 Results

3.1 Effect of soil texture on Disease Development

Inoculation with *M. javanica* or *M. phaseolina*, either individually or concomitantly, increased ($P \leq 0.05$) the disease severity (number of root galls and root rot) on green bean plants grown in all the tested soil textures (Table 2). However, plant growth was suppressed. The sandy soil (soil mix 1) resulted in the greatest ($P \leq 0.05$) plant damage at the concomitant inoculation. The clay loam or sand clay loam showed the least damage effect. Plant damage decreased as sand content (%) of the soil mixture was increased (Table 2). Further, reproduction of the nematode and percentages of the fungus re-isolation from soil increased with the increase of sand content (%) of soil mixture (Table 3). This increase was greatest in the sandy soil (soil mix 1) and least in the clay loam mixture (Table 3).

3.2 Effect of soil moisture on Disease development

Results of this test showed that the greatest plant damage (plant fresh weight, root galls, root rot) with both pathogens occurred at 30% moisture level (Table 4). Then, this damage gradually decreased with the increasing moisture level. Similarly, the nematode reproduction and fungal re-isolation from soil was greatest at 30% soil moisture level (Table 5). Further, results of study revealed that nematode reproduction decreased with the increasing soil moisture (Table 5).

Table 2 Effects of soil texture on the severity of the disease complex caused by *Meloidogyne javanica* and *Macrophomina phaseolina* on green beans, sixty days after inoculation

Treatment	TFW ¹		No. galls/ g root	Root-rot ² index (0-10)
	(g)	% decrease		
Control	30.62 ^a	-	-	-
<i>Meloidogyne javanica</i> (N)	25.04 ^b	18.22	19.72 ^d	-
<i>Macrophomina phaseolina</i> (F)	24.74 ^b	19.20	-	4.4 ^{bc}
N+F/ soil mix 1 (S) ³	15.14 ^e	50.55	73.93 ^a	8.2 ^a
N+F/ soil mix 2 (LS)	16.82 ^d	45.07	46.52 ^b	5.8 ^b
N+F/ soil mix 3 (SL)	17.54 ^d	42.72	43.49 ^b	5.0 ^b
N+F/ soil mix 4 (SCL)	20.68 ^c	32.46	34.91 ^c	4.4 ^{bc}
N+F/ soil mix 5 (CL)	21.74 ^c	29.00	22.30 ^d	3.8 ^c

Values are means of five replicates. Means, in each column, followed by the same letter(s) are not significantly different at $P \leq 0.05$; ¹TFW= total fresh weight of shoot and root; ²Root-rot index is based on a scale of 0-10, where 0= 0.0%, 1= 1-10%, 2= 11-20%, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7= 61-70%, 8= 71-80%, 9= 81-90%, 10= 91-100% of the root area is infected; ³(S)= sandy, (LS)= loamy sand, (SL)= sandy loam, (SCL)= sandy clay loam and (CL)= clay loam soil.

Table 3 Effects of soil texture on nematode reproduction and fungus re-isolation of green bean plants inoculated with both *Meloidogyne javanica* and *Macrophomina phaseolina*, sixty days after inoculation

Treatment	No. egg masses/root system	No. eggs/g root ($\times 1000$)	Reproductive factor (Rf) ¹	% fungus re-isolation ²
Control	-	-	-	-
<i>Meloidogyne javanica</i> (N)	232.2	4.94 ^d	4.23 ^a	-
<i>Macrophomina phaseolina</i> (F)	-	-	-	45.0 cd
N+F/ soil mix 1 (S) ³	429.4 ^a	8.27 ^a	3.97 ^b	90.0 ^a
N+F/ soil mix 2 (LS)	321.8 ^b	6.27 ^{bc}	3.56 ^c	70.0 ^b
N+F/ soil mix 3 (SL)	319.0 ^b	5.71 ^c	3.42	65.0 ^{bc}
N+F/ soil mix 4 (SCL)	301.8 ^b	4.53 ^d	3.18	35.0 ^d
N+F/ soil mix 5 (CL)	205.6 ^c	4.14 ^d	3.03	30.0 ^d

Values are means of five replicates. Means, in each column, followed by the same letter(s) are not significantly different at $P \leq 0.05$; ¹Reproductive factor (Rf)= final nematode population (Pf)/initial nematode population (Pi); ²Original data were transformed using angular transformation before analysis; ³(S)= sandy, (LS)= loamy sand, (SL)= sandy loam, (SCL)= sandy clay loam and (CL)= clay loam soil.

Table 4 Effects of soil moisture on nematode reproduction and fungus re-isolation of green bean plants inoculated with both *Meloidogyne javanica* and *Macrophomina phaseolina*, sixty days after inoculation

Treatment	TFW ¹		No. galls/ g root	Root-rot ² index (0-10)
	(g)	% decrease		
Control	30.98 ^a	-	-	-
<i>Meloidogyne javanica</i> (N)	24.94 ^b	19.50	19.37 ^e	-
<i>Macrophomina phaseolina</i> (F)	24.52 ^b	20.85	-	4.4 ^d
N+F (irrigated as needed)	16.82 ^c	45.71	46.06 ^d	6.5 ^c
N+F/ moisture at 30% ³	9.90 ^f	68.80	108.4 ^a	8.6 ^a
N+F/ moisture at 50%	12.62 ^e	59.26	75.75 ^b	7.8 ^b
N+F/ moisture at 70%	14.34 ^{de}	53.71	57.48 ^c	6.7 ^c
N+F/ moisture at 90%	15.70 ^{cd}	49.32	49.34 ^{cd}	6.9 ^c
N+F/ moisture at 100%	16.04 ^{cd}	48.22	44.98 ^d	6.6 ^c

Values are means of five replicates. Means, in each column, followed by the same letter(s) are not significantly different at $P \leq 0.05$; ¹TFW= total fresh weight of shoot and root; ²Root-rot index is based on a scale of 0-10, where 0= 0.0%, 1= 1-10%, 2= 11-20%, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7= 61-70%, 8= 71-80%, 9= 81-90%, 10= 91-100% of the root area is infected; ³Soil moisture level was based on the

Table 5 Effects of soil moisture on nematode reproduction and fungus re-isolation of green bean plants inoculated with both *Meloidogyne javanica* and *Macrophomina phaseolina*, sixty days after inoculation

Treatment	No. egg masses/root system	No. eggs/g root ($\times 1000$)	Reproductive factor (Rf) ¹	% fungus re-isolation ²
Control	-	-	-	-
<i>Meloidogyne javanica</i> (N)	236.4 ^c	4.37 ^c	4.34 ^a	-
<i>Macrophomina phaseolina</i> (F)	-	-	-	40.0 ^c
N+F (irrigated as needed)	312.8 ^{cd}	6.17 ^{cd}	3.39 ^b	65.0 ^b
N+F/ moisture at 30% ³	425.0 ^a	10.67 ^a	3.48 ^b	95.0 ^a
N+F/ moisture at 50%	382.6 ^b	8.40 ^b	3.42 ^b	80.0 ^{ab}
N+F/ moisture at 70%	332.4 ^c	6.60 ^c	3.28 ^b	65.0 ^b
N+F/ moisture at 90%	296.6 ^d	6.05 ^{cd}	3.17 ^{bc}	65.0 ^b
N+F/ moisture at 100%	288.6 ^d	4.94 ^{de}	3.62 ^c	65.0 ^b

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

¹Reproductive factor (Rf) = final nematode population (Pf)/initial nematode population (Pi); ²Original data were transformed using angular transformation before analysis; ³Soil moisture level was based on the percentage of field capacity.

4 Discussion

Results of present study declared that both soil texture and moisture had strong effects on disease severity by the synergistic interaction between *M. javanica* and *M. phaseolina* on green beans. The severity of disease symptoms and plant damage, generally, increased with the increase of sand content (%) or soil drought. This would indicate that lighter (sandier) soil texture and less soil moisture can be considered as additional major stress factors on green beans infected, simultaneously, with both pathogens. Consequently, severity of symptoms and plant damage would be much greater. Findings of this study support previous reports which show that these two edaphic factors can affect different species of *Meloidogyne* on several crops (Al-Hazmi, 1988; Al-Hazmi et al., 1995a; Jaraba et al., 2014; Shahab, 2014), and have also the same effects on *M. phaseolina* (Pande et al., 1989; Husain & Ghaffar, 1995; Jimenez, 2011; Arias et al., 2013). Similarly, Jaraba et al. (2009) concluded that cotton yield variability in the soil infested with both *M. incognita* and *Thielaviopsis basicola* is explained by sand content and soil texture.

Meloidogyne spp. are a serious problem in the Saudi green bean fields and greenhouses. Infection by this important group of plant-parasitic nematodes strongly interferes with the uptake and transport of water and nutrients from soil (Melakeberhan, 2004). Furthermore, root-knot nematodes such as *M. javanica*, provide easy and excellent penetration sites for many soil-borne fungi such as *M. phaseolina* (Tu & Cheng, 1971; Siddiqui & Husain, 1991). Movement and reproduction of root-knot nematodes are favored by sandy soils (Al-Hazmi, 1988; Al-Hazmi et al., 1995a; Robinson, 2004). Likewise many crops that are subject to severe stress caused by drought become more easily infected by the fungus *M. phaseolina* (Pande et al., 1989).

In conclusion, soils in the most of Saudi fields are sandy to loamy sand, and the climate is considered to be arid. Summer seasons are

very long with a dry and high temperature. Under such edaphic and environmental conditions, concomitant infection by *M. javanica* and *M. phaseolina* is expected to be very severe and plant damage would be high in the green beans cultivations. So, integrated control measures are strongly recommended to manage this disease complex in our green bean fields and greenhouses.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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