# ARTIGOS

# Interaction of *Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani* as root rot pathogens of *Cucumis melo*

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## ABSTRACT

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Root diseases represent one of the main reasons for yield loss in melon crops, especially root and stem rots caused by pathogens like the fungi *Fusarium solani* (Fs), *Macrophomina phaseolina* (Mp) and *Rhizoctonia solani* (Rs), frequently observed in muskmelon either alone or in combination. The objective of this study was to evaluate the effect of the interaction between the pathogens Fs, Mp and Rs on the incidence and severity of root rot and muskmelon development. Two greenhouse experiments were performed using plastic pots with substrate infested with each pathogenic agent alone or in combination. The second experiment was conducted in the same pots that were used in the first experiment. In the first experiment, the disease incidence was higher for the treatment with Fs alone. In the second experiment, the disease incidence and severity were greater for treatment Fs + Rs than for Fs alone. *Macrophomina phaseolina* was the most commonly isolated pathogen when applied to the plants in a paired mixed inoculum (Fs + Mp and Mp + Rs) in the first experiment. In the second experiment, Fs was more prevalent than the other studied pathogens. Soil infested with Fs had the lowest fresh weight of muskmelon. The pathogens Fs and Mp were more competitive than Rs.

**Keywords**: infested soil, muskmelon, root pathogens, pathogen association

## RESUMO

Porto, M.A.F.; Ambrósio, M.M.Q.; Nascimento, S.R.C.; Cruz, B.L.S.; Torres, R.M.. Interação entre *Fusarium solani, Macrophomina phaseolina* e *Rhizoctonia solani* como patógenos causadores de podridão radicular em *Cucumis melo*. ***Summa Phytopathologica***, v.45, n.4, p.355-360, 2019.

A ocorrência de doenças radiculares representa uma das principais causas de perda de rendimento na cultura do melão, com destaque para patógenos causadores das podridões de raízes e colo, como os fungos *Fusarium solani* (Fs), *Macrophomina phaseolina* (Mp) e *Rhizoctonia solani* (Rs), frequentemente observados no meloeiro de forma isolada ou associados. O objetivo deste estudo foi avaliar o efeito da interação entre os patógenos Fs, Mp e Rs sobre a incidência e severidade da podridão radicular e desenvolvimento do meloeiro. Dois experimentos foram realizados em casa-de-vegetação utilizando vasos plásticos com substrato infestado com cada agente patogênico isoladamente ou em combinação. O segundo experimento foi conduzido nos mesmos vasos do primeiro experimento. No primeiro experimento, a incidência da doença foi maior no tratamento com Fs sozinho. Já no segundo experimento, a incidência e severidade da doença foram maiores no tratamento Fs + Rs do que no Fs sozinho. *Macrophomina phaseolina* foi o patógeno isolado com maior frequência quando aplicado às plantas em um inóculo misto aos pares (Fs + Mp e Mp + Rs), no primeiro experimento. No segundo experimento, Fs foi mais prevalente do que os outros patógenos estudados. Solo infestado com Fs apresentou o menor peso fresco do meloeiro. Os patógenos Fs e Mp foram mais competitivos do que Rs.

**Palavras-chave**: solo infestado, meloeiro, patógenos radiculares, associação de patógenos

Melon (*Cucumis melo* L.) is an important member of the Cucurbitaceae for international markets. About 29 million tons were produced worldwide in 2013, and China, Turkey and Iran were the major producers (14). In the same year, Brazil ranked tenth for melon crop production worldwide, showing production of 565,900 tons and a mean yield of 25,698 tons per hectare (14). The Northeast of Brazil has excellent weather conditions for muskmelon development, such as high temperatures and sunlight, as well as modern technological practices, such as widespread adoption of automatic irrigation systems (31), which puts muskmelon in a leading position for the domestic and foreign markets.

The expansion of muskmelon production around the world, including Brazil, has led to an increase in the incidence and severity of diseases, especially those caused by soil-borne pathogens (27). The diseases caused by these pathogens are responsible for large yield losses in melon crops worldwide (1; 9; 11), in some cases, causing up to 50% loss (28). In Brazil, no reports of percentage losses exist; however, soil-borne microorganisms are known to be the main cause of diseases and economic losses (27).

Root-rot diseases can be caused by several fungi; *Fusarium solani* (Mart.), *Stagonosporopsis cucurbitacearum* (Fr.) Aveskamp, Gruyter & Verkley (=*Didymella bryoniae* (Aures.) Rehm) (29), *Lasiodiplodia theobromae* (Pat.) Grif. and Maubl, *Macrophomina phaseolina* Tassi (Goid.), *Myrothecium roridum* Tode, *Monosporascus cannonballus*

Pollack and Uecker, and *Rhizoctonia solani* Kühn are frequently found associated with muskmelon roots (6; 10; 27). The most common soil-borne pathogens of muskmelon in Brazil are *F. solani*, *M. phaseolina*, *R. solani* and *M. cannonballus*, which occur alone or in combined infections (6; 10).

Combined infestation of soil-borne microorganisms often exacerbates symptom expression in plants (17; 22) and can affect growth, development and crop production. In muskmelon, previous studies have shown that associations of soil-borne pathogens commonly occur, causing root and stem rot and collapse. For example, Aegerter et al. (1), in California, frequently found the association of *Acremonium cucurbitacearum* and *Rhizopycnis vagum* in the same lesion. In Brazil, Andrade et al. (6) reported mixed infections between *F. solani* and other soil-borne pathogens in 88.9% evaluated fields.

Control of soil-borne pathogens is difficult due to the extensive host range and specialized resistant structures produced by some fungi that can survive in soil for long periods in the absence of their hosts. Some soil-borne pathogens also survive in seeds and crop remains (26). Chemical control of these pathogens in intensive horticultural systems has been based for years on the use of methyl bromide, but restriction on the use of this fumigant has increased the risks for soil-borne pathogen outbreaks and has resulted in bigger efforts to develop chemical and non-chemical environmentally user-friendly alternative control methods (5; 10). Few reports have described association of soil-borne pathogens that cause damage in muskmelon. This piece of information will help understand the behavior of these microorganisms and will assist in management strategies. Thus, the aim of the investigation presented in this paper was to verify the effect of interactions between the pathogens *F. solani, M. phaseolina* and *R. solani* on root rot incidence and severity and on muskmelon development.

## MATERIALS AND METHODS

Two experiments were performed in a greenhouse located in Mossoró-RN (5°11'17''S, 37°20' 39''W), Brazil. A randomized complete block design (RCBD) with eight treatments [*F. solani*; *M. phaseolina*; *R. solani*; *F. solani* + *M. phaseolina*, *F. solani* + *R. solani*; *M. phaseolina* + *R. solani*; *F. solani* + *M. phaseolina* + *R. solani*; and Control (non-infested soil)] and eight replicates was used.

Soil infestation was performed only in the first experiment; the second experiment started seven days after harvest of the first experiment, with a new sowing using the same soil and pots that were used in the first experiment and following the characterization of continuous cropping to examine the behavior of pathogens from one cycle to the other. Parameters and methodologies were the same for both experiments.

The soil, characterized as Ultisol, was collected from a native area (uncultivated soil) at 0–30 cm depth; its texture composition consisted of 53% sand, 25% silt and 22% clay, while coconut fiber (Vida Verde, Mogi-Mirim, SP, Brazil) was added at a 1:2 ratio of coconut fiber:soil material. This potting mix was autoclaved twice at 120°C for 1 h on each of two consecutive days, and 3.0 L soil was placed in 3.5-L plastic pots. Each experimental unit consisted of a pot with two muskmelon plants of Iracema hybrid (Agroflora/Sakata).

**Preparation of inocula**

Isolates of *F. solani* (Me 245), *M. phaseolina* (Me 248) and *R. solani* AG-7 (Me 242) were obtained from the culture collection of the Department of Plant Sciences of "Universidade Federal Rural do Semi-Árido" (UFERSA, Brazil) and were originally collected from diseased muskmelon roots. The anastomosis group (AG) identity of *R. solani* isolate was determined via BLASTn analysis (3). The pathogenicity of all isolates was confirmed in muskmelon, before inoculum production, by the infested toothpick method (4).

*Fusarium solani*, *M. phaseolina* and *R. solani* isolates were grown for 7 days on potato dextrose agar (PDA; Himedia Laboratories Pvt. Ltd, Mumbai, India) in 9-cm Petri dishes and were maintained in a biochemical oxygen demand (BOD) incubator at approximately 28 ± 2°C in the dark. Inoculum of each fungus was prepared by separately transferring eight mycelial disks (5 mm diameter) from the growing edge of each fungus to flasks (1L) containing 500 mL arene-organic substrate (20), which consisted of three parts of cured cattle manure, one part of washed sand, 2% oat (v/w) and 20 mL distilled water for each 100 mL substrate. Flasks with the substrate were autoclaved for 1 h at 120°C on each of two consecutive days before inoculation with mycelial discs of each fungus and were incubated in BOD for 15 days at 28 ± 2°C in the dark. The flasks were shaken every two days after the growing mycelium was observed with the purpose of breaking the mycelial mass and homogenizing the infestation until use. The inoculum viability was determined before the potting mix infestation by plating on PDA + oxytetracycline (0.05 g $L^{-1}$) and observing whether it was contaminated or not.

**Soil infestation**

Inoculum of each isolate alone was spread out and mixed (about 10 cm depth) with the potting mix at a ratio of 6% v/v (potting mix/inoculum) per treatment. For treatments with two pathogens, infestation was obtained with 3% each pathogen inoculum, and for the treatment with three pathogens, infestation was obtained with 2% each pathogen inoculum. Three melon seeds were sown into the infested potting mix on the day of infestation and two plants were left after thinning. Control treatment had the same amount of sterile substrate applied to the potting mix. Irrigation was performed with a sprinkler three times a day and the amount of water was according to the plants' growth requirement (2). The greenhouse conditions of mean temperature and mean relative humidity were 34.5ºC and 38.4%, respectively, and the lighting conditions were sunny days.

Sixty days after sowing, melon plants were removed from the potting mix and taken to the laboratory, where the roots were washed under running tap water to remove residual potting mix and the disease incidence was evaluated (percentage of plants with visible disease symptoms, compared to total plants). In addition, the severity of root rot was assessed using a modified version of the scale proposed by Mao et al. (22), considering: 1 = healthy plant; 2 = 1 to 30% internal and external tissue with rot symptoms (slight disease); 3 = 31 to 60% internal and external tissue with rot symptoms (moderate disease); 4 = more than 60% internal and external tissue with rot symptoms (severe disease) and 5 = dead plant.

Isolation of pathogens present in symptomatic plants was performed by removing the fragments from the edge of a lesion in the muskmelon root or stem cortex; five pieces (approximately 0.5 $cm^2$ x 2 mm depth) were sequentially surface-treated (70% alcohol, 2% sodium hypochlorite and sterile distilled water) and placed on PDA + oxytetracycline (0.05 g $L^{-1}$). Plates were incubated in BOD for 5 days at 28 ± 2°C in the dark and were morphologically identified by optical microscopy.

After removing the fragments from roots with rot symptoms, the

plant aerial parts (everything above the soil line) were immediately weighed (fresh weight).

**Statistical analyses**

Normality was checked based on the Shapiro–Wilk test ($P \leq 0.05$). Data were analyzed according to the non-parametric Kruskal–Wallis test and the Mann–Whitney U-test at 5% probability, using the PAST (version 2.17b) program (18).

## RESULTS AND DISCUSSION

The results of both experiments are presented together for ease of explanation. The highest disease percentage among treatments occurred with the infestation of *F. solani* alone in the first experiment (Figure 1a), showing a median of 100%. This might be due to the good adaptation of this pathogen to the experimental conditions. *Fusarium solani* has a wide distribution in muskmelon fields of Northeastern Brazil and was present in all sampled melon fields (6). However, in the potted melon plants, after removal of the first set of melon plants, the incidence of *F. solani* was low, possibly due to its poor survival in the experimental substrate. As reported by Freeman et al. (15), the survival of *Fusarium oxysporum* can oscillate in soil because this fungus synthesizes a protein that is activated by environmental stress conditions in soil, especially under high temperatures, thus ensuring a varied survival of *F. oxysporum* in soil (8).

The absence of plants with root rot symptoms when the three pathogens [*F. solani* (Fs), *M. phaseolina* (Mp) and *R. solani* (Rs)] were combined in the first experiment (Figure 1a) might be due to an antagonistic action between these microorganisms (16) and/or the non-stabilization resulting from the short time pathogens were in contact with the soil before sowing, especially because all the soil used in our study was sterilized and only 2% inoculum of each pathogen was used in triple combination, which might not have been enough for the pathogens to cause disease in the first experiment. The need of a longer time for pathogens to establish in the soil was also observed by Fenille & Souza (12) while evaluating the percentage of dumping off in seedlings of common beans sown in soil infested with *Rhizoctonia solani* at different times. These authors found higher percentage of dumping off in seedlings of common beans sown after 21 days of soil infestation, when pathogens were more active in soil than after 14 days. The same results were found in our second experiment (Figure 1b), where there was an increase in the disease incidence when the three pathogens were combined, possibly due to the longer exposure time of the pathogens in the soil, which favors the survival and increase of these pathogens in this habitat. Mao et al. (22) also reported that root rot symptoms develop earlier and show greater severity in the second growing season, possibly because the exudates released by the host favored the growth and increased the pathogen population, as reported by Martyn (21). For example, ascospore germination of *Monosporascus cannonballus* is enhanced in the presence of root exudates from growing muskmelon seedlings.

Low disease severity, equal to that of control (Figure 2a), was observed when the three pathogens were combined, differently from all the other treatments in the first experiment. As previously reported for disease incidence, the high disease severity was possibly due to stabilization of the pathogens in the soil and could cause greater damage to the plant. In the second experiment, Fs + Rs showed the highest disease severity, with median of 3.75 (Figure 2b), which was statistically different from that of Fs and the control treatment, suggesting a synergistic effect among these pathogens.

Previous studies on different pathosystems have also emphasized a synergistic effect when soil-borne pathogens were combined. For example, in common beans, Tolêdo-Souza et al. (30) reported that *R.*

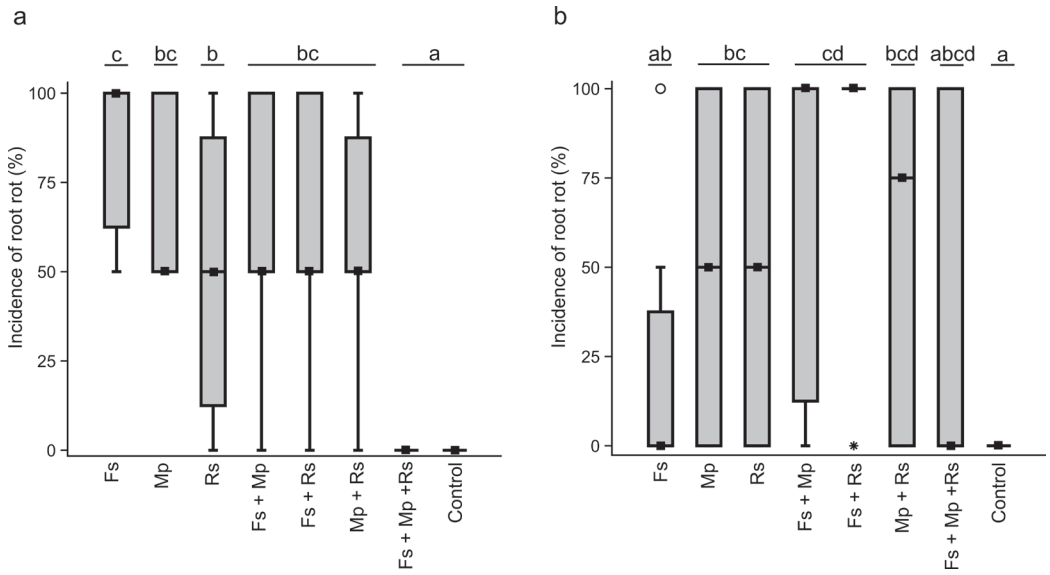


**Figure 1.** Box plot showing the incidence of root rot in melon plants in the different treatments. a = First experiment, b = Second experiment. Fs= *Fusarium solani*; Mp= *Macrophomina phaseolina*; Rs= *Rhizoctonia solani*. The dark spot in the box indicates the median value. The box is closed by upper and lower hinges; the whiskers extend to extreme values within the inner fence (the inner fence is limited by points that are 1.5 times the distance between hinges measured in either direction from the median); the stars represent values between the inner and the outer fence (the outer fence is limited by points three times the distance between the hinges measured in either direction from the median), and open circles represent outliers. Different letters indicate statistically significant differences calculated according to Kruskal–Wallis test associated with Mann–Whitney U test ($P \leq 0.05$)

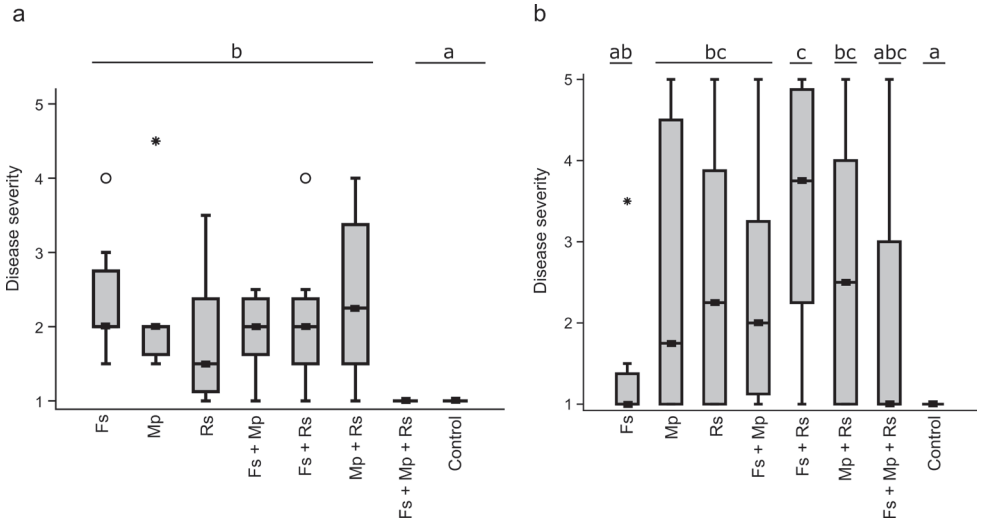


**Figure 2.** Box plot showing the severity of root rot in melon plants in the different treatments. a = First experiment, b = Second experiment. Fs= *Fusarium solani*; Mp= *Macrophomina phaseolina*; Rs= *Rhizoctonia solani*. The dark spot in the box indicates the median value. The box is closed by upper and lower hinges; the whiskers extend to extreme values within the inner fence (the inner fence is limited by points that are 1.5 times the distance between hinges measured in either direction from the median); the stars represent values between the inner and the outer fence (the outer fence is limited by points three times the distance between the hinges measured in either direction from the median), and open circles represent outliers. Different letters indicate statistically significant differences according to Kruskal–Wallis test associated with Mann–Whitney U test ($P \leq 0.05$)

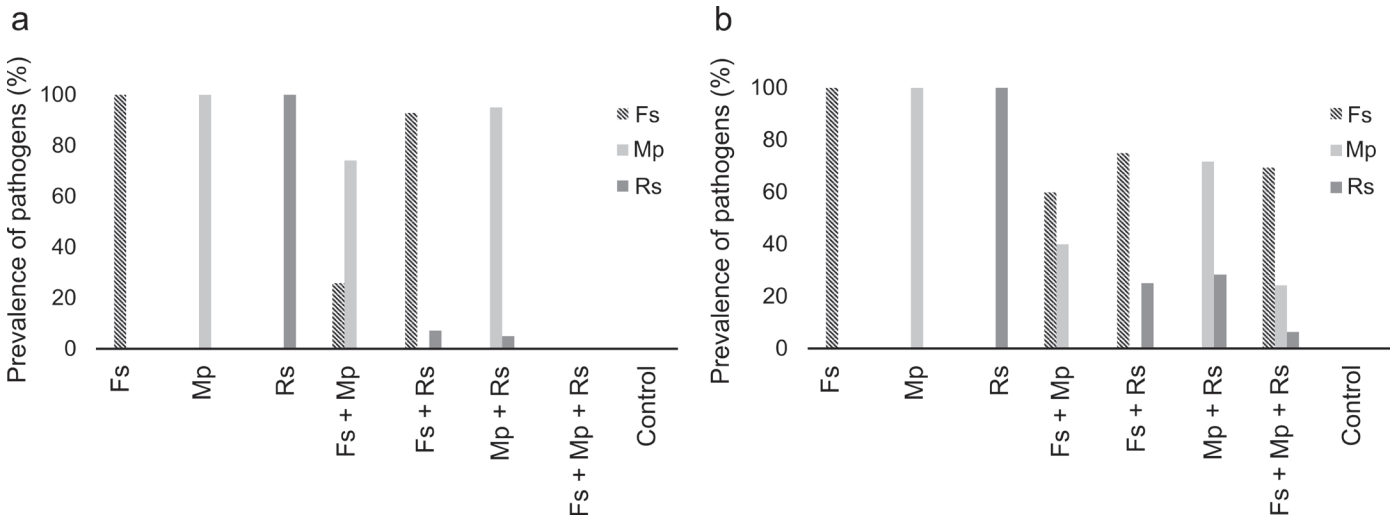


**Figure 3.** Prevalence of pathogens isolated from muskmelon with symptoms of root rot in the different treatments. a = First experiment, b = Second experiment. Fs= *Fusarium solani* Mp= *Macrophomina phaseolina*; Rs= *Rhizoctonia solani*

*solani* stimulates the expression of *F. solani* symptoms. Infestation with *R. solani* and *F. solani* f. sp. *phaseoli*, separately, caused 25 and 8% pre-emergent death, respectively. Together, these pathogens showed a synergistic effect, causing 67% pre-emergent death in common beans (25). In sugar beet, *Rhizopus stolonifer* alone did not cause disease symptoms, but root rot increased when beets were exposed to the combination of *Rhizoctonia solani* and *Rhizopus stolonifer* (17). Based on the second experiment of the present study, the combination of Fs with the other two pathogens (Fs + Rs and Fs +Mp) resulted in more disease than Fs alone (Figure 2b), suggesting a synergic effect of *Fusarium* when combined in pairs with *Rhizoctonia* or *Macrophomina*. As reported by Tolêdo-Souza et al. (30) for common beans, the severity of *F. solani* f. sp. *phaseoli* increases with the presence of *R. solani* because the initial injury caused by *R. solani* favors the capacity of *F. solani* f. sp. *phaseoli* to penetrate host roots and subsequently act as an inhibitor of *R. solani* symptoms. *Rhizoctonia solani* has an aggressive method of colonizing plants, by forming mycelial tufts that produce enzymes and toxins used for maceration and subsequent tissue penetration. *R. solani* aggressiveness was not as high as that of *F. solani* in our experiment, but *R. solani* may have stimulated *F. solani* penetration into the roots of muskmelon, as proposed by Tolêlo-Souza et al. (30) for common beans. In addition, the environmental conditions of the semi-arid region of Brazil, where the experiment was conducted, may have been more favorable for the development of *Fusarium* than for that of *Rhizoctonia*. Soils infested with each pathogen alone resulted in the presence of pathogens in all cultured tissue fragments. Considering the combination of *F. solani* and *R. solani*, *F. solani* was more frequently isolated from tissue fragments than *R. solani* in both

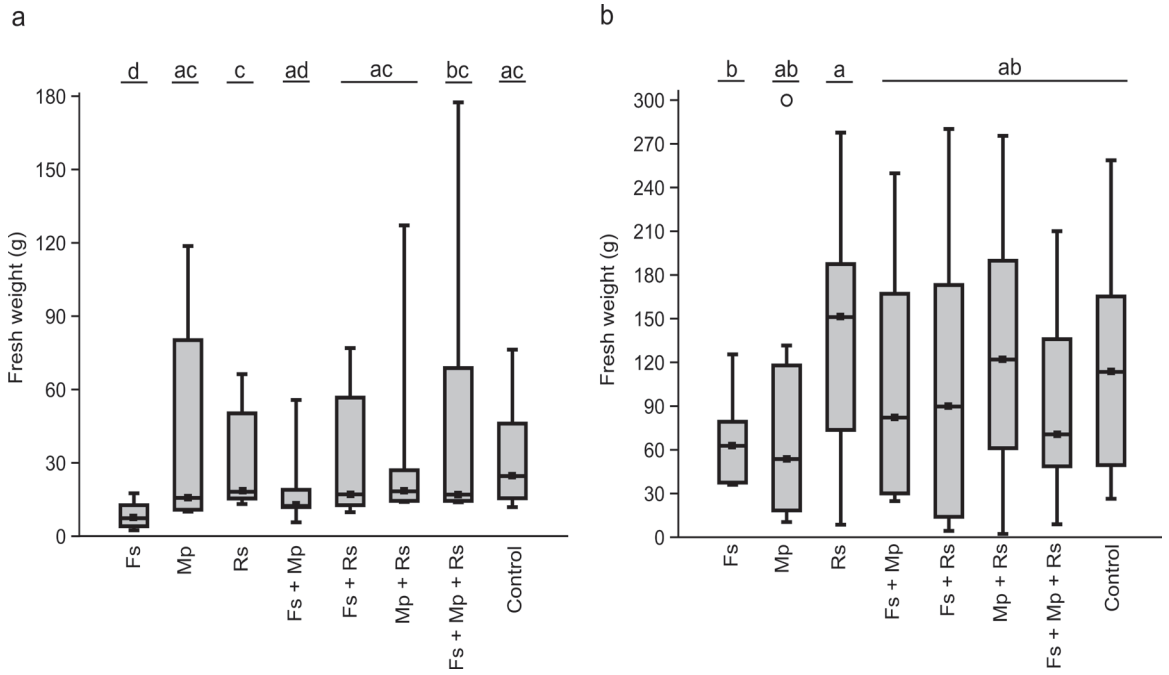


**Figure 4.** Box plot showing the fresh weight of muskmelon in the different treatments. a = First experiment, b = Second experiment. Fs= *Fusarium solani*; Mp= *Macrophomina phaseolina*; Rs= *Rhizoctonia solani*. The dark spot in the box indicates the median value. The box is closed by upper and lower hinges; the whiskers extend to extreme values within the inner fence (the inner fence is limited by points that are 1.5 times the distance between hinges measured in either direction from the median); the stars represent values between the inner and the outer fence (the outer fence is limited by points three times the distance between the hinges measured in either direction from the median), and open circles represent outliers. Different letters indicate statistically significant differences according to Kruskal–Wallis test associated with Mann–Whitney U test ($P \leq 0.05$)

experiments (Figure 3). In the first experiment, *F. solani* was present in more than 92% (Figure 3a) plated fragments, but in the second crop cycle, its prevalence was 75% (Figure 3b). In this combination (Fs + Rs), *R. solani* prevalence was 7 and 25% in the first and second experiments, respectively (Figure 3a,b).

In the first experiment (Figure 3a), *Macrophomina phaseolina* showed higher prevalence in relation to Fs or Rs when these pathogens where paired. The presence of Mp was observed in 74% isolated fragments when combined with Fs and in 95% when combined with Rs (Figure 3a). In the second melon planting, Mp was the most prevalent pathogen, present in 71.4% fragments when combined with Rs but in only 40% when in combination with Fs (Figure 3b). *Macrophomina phaseolina* has been reported to be a very competitive and highly frequent pathogen in muskmelon fields of the northeastern Brazilian states (6). Occurrence of *M. phaseolina* might have been stimulated by *F. solani* and *R. solani*, probably due to its capability of competing with other pathogens, allied with favorable environmental conditions for this microorganism. As reported by Khan (19), *M. phaseolina* invades the host very rapidly, establishing itself in the host within 24 to 48 h, at the seedling infection stage, while the disease severity is influenced by drought and high temperatures; 30-35ºC is the favorable temperature range for disease expression (23). The incidence and the severity of diseases caused by soil-borne pathogens are influenced by environmental conditions, such as soil moisture and temperature. As reported by Aegerter et al. (1), temperature, irrigation and the type of soil greatly influence the severity of root rot because most microorganisms that cause root diseases are stimulated by high temperatures and excess moisture.

In both crop cycles, the fungus *R. solani* had the lowest prevalence in all combinations (Figure 3a), possibly due to the unfavorable environmental conditions for this pathogen, which is favored by excess moisture (13) and, therefore, potentially became uncompetitive, compared to the other pathogens in this study. Another hypothesis that might relate to the low prevalence of Rs is the inoculum potential, which might not have been high enough, since high *R. solani* inoculum densities produce greater disease severity in the crop, as reported by Andrade et al. (6). Low occurrence of Rs was found in the muskmelon fields of northeastern Brazil, and this pathogen was reported in only 40% assessed fields (6). Yildiz & Benlioğlu (32) also reported that *R. solani* is not as virulent as *M. phaseolina* in strawberry, but Rs caused greater disease severity than *F. solani* f. sp. *phaseoli* (30) in common beans. In the second experiment (Figure 3b), when the three pathogens were combined, *Fusarium* was present in 69.4% evaluated fragments, whereas *Macrophomina* and *Rhizoctonia* occurred in 24.2 and 6.4% fragments, respectively.

Even though Fs is one of the main pathogens that cause losses to melon crops, only 18.4% infections were caused by this pathogen alone and 81.6% in combination with other pathogens, such as *M. phaseolina*, *R. solani*, *M. cannonballus* and *Sclerotium rolfsii* (6). In relation to the development of aerial plant parts (Figure 4), treatment with Fs alone in the first experiment showed low fresh weight of aerial parts, equal only to that of the Fs + Mp treatment and different from all the other treatments ($P \leq 0.05$). This means that *Fusarium solani* was more successful in infecting plants than other pathogens and associations.

In the second experiment, the lowest fresh weight of muskmelon plants was found for the treatment with Fs alone, while Rs resulted in the greatest muskmelon fresh weight, showing a median close to 150 g. *Rhizoctonia solani* caused slight damage to melon plants, compared to the other evaluated pathogens. However, for other pathosystems and different environmental conditions (7; 24), *Rhizoctonia* have caused

major losses. For example, Tolêdo-Souza et al. (30) reported that the damage caused by *R. solani* alone to the development of common bean seedlings was higher than the damage caused by *F. solani* f. sp. *phaseoli* alone. However, these same authors reported that the damage caused by *F. solani* f. sp. *phaseoli* increased after combined infestation with *R. solani*, demonstrating results similar to the present findings.

Melon planting conditions, with or without crop rotation or fallow, in addition to the pathogens and their associations, evidence different behaviors from one crop cycle to the other. However, Fs and Mp were more competitive than Rs, suggesting that Rs can behave as a secondary pathogen in the presence of unfavorable environmental conditions for its development.

This study shows an interaction of the three soil-borne fungi *F. solani*, *M. phaseolina* and *R. solani* on the development of root rot in muskmelon. Further research is required to obtain information relating to the formulation of disease management strategies for root rot control.

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