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Research Article

Effect of disease complex of *Meloidogyne incognita* and *Fusarium solani* on fungus root rot incidence, nematode reproduction, and enzyme activities involved in defense mechanisms of grafted cucurbit hybrids

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Abstract

This study investigated the interplay between the root-knot nematode, *Meloidogyne incognita* (Mi), and the fungal pathogen, *Fusarium solani* (Fs) in grafted cucurbit hybrids (watermelon and sweet melon). Results revealed that Fs root rot incidence was exacerbated when combined with Mi. Nonetheless, the existence of Fs caused a noteworthy decline in Mi reproduction, affecting hosts that were both resistant and susceptible. Additionally, plant growth suffered more from combined infection than from single pathogens, with the susceptible sweet melon showing greater growth reduction. The study further explored the impact of the disease complex on antioxidant defence mechanisms. Leaf tissues from both grafted hybrids displayed elevated levels of lipid peroxidation (MDA) and antioxidant enzyme activities (SOD and APX) upon infection with Fs alone, Mi alone, or both. Interestingly, the susceptible sweet melon exhibited a stronger increase in these defence responses compared to the resistant watermelon. Notably, combined Fs and Mi infection led to the highest levels of MDA, SOD, and APX activity. These findings highlight the complex interplay between Fs and Mi in cucurbit hybrids. While Fs virulence increased with coinfection, Mi reproduction was suppressed. Furthermore, the study suggests that susceptible plants may have a more pronounced activation of antioxidant defence mechanisms when confronted with the combined stress of these pathogens.

Keywords: cucurbit hybrids; disease complex; enzyme activities; *Fusarium solani*; *Meloidogyne incognita*; nematode development

Introduction

Numerous research endeavours have been undertaken to explore the associations between *Fusarium* root rots and root-knot nematodes on various cucurbitaceous crops, comprising summer squash (*Cucurbita pepo*), muskmelon (*Cucumis melo*), and watermelon (*Citrullus lanatus* var. *lanatus*) (Bergeson, 1975; Caperton

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et al., 1986). Taking into account these factors pertaining to the interplay of *Fusarium* root rots and the RKN infestation, it is highly likely that intricate diseases may arise in Egyptian greenhouse crops of the cucurbitaceous varieties. Nevertheless, limited research has been carried out on the disease complex within the cucurbitaceous crops, especially in continuous greenhouse cropping (CGC). In the cucumber rhizosphere, Sharma *et al*. (1995) identified root-knot nematode, *M. incognita*, and *Fusarium* root rot, *Fusarium oxysporum* f.sp. *cucumerinum* as highly detrimental soil-borne diseases.

Moreover, grafted transplants were employed in managing soil-borne diseases like *Fusarium* root rot in melon, watermelon, and cucumber (Lee, 1994; Traka-Mavrona*et al*., 2000; Cohen *et al*., 2002; Pavlou *et al*., 2002; Davis *et al*., 2008). Lee and Oda (2003) identified fig leaf gourd as a widely used rootstock for controlling *Fusarium* root rot, root-knot nematode, and increased cucumber's resilience to low soil temperatures. Furthermore, the utilization of Cucurbita rootstocks for grafting watermelon not only effectively managed soil-borne diseases but also stimulated vigorous vegetative growth and root formation. This resulted in earlier and higher crop yields, along with enhanced fruit quality (Davis *et al*., 2008).

Liu *et al*. (2015) screened a wild *Cucumis pustulatus* as a viable rootstock for melon, cucumber, and watermelon scions (53 accessions from 16 species) for the resistance against root-knot nematode, *M. incognita*, and *Fusarium* root rot. Root-knot nematode resistance was observed in five accessions, while 12 accessions exhibited resistance to *Fusarium* root rot. They reported that *C. pustulatus* was an appropriate rootstock for melon, cucumber, and watermelon, offering dual resistance toward root-knot nematode and *Fusarium* root rot. This makes it promising for low-input sustainable horticulture.

Smith *et al*. (2019) tested 7 watermelons, *Citrullus lanatus* var. *citroides* (L.H. Bailey) rootstocks resistant to *Fusarium* root rot for their susceptibility to reniform nematode (*Rotylenchulus reniformis*) and root-knot nematodes (RKNs; *Meloidogyne* spp.). They reported that rootstocks ('Carolina Strongback', USVL-360, USVL252-FR2, and USVL246-FR2) and 'SP-6' (a commercially accessible pollination variety) demonstrated resilience against plant-parasitic nematodes in comparison to the susceptible hybrid rootstock Carnivor [*Cucurbita maxima* (Duchesne) × *C. moschata* (Duchesne)]. Comparatively, USVL-482351 rootstock exhibited partial resistance, resulting in a reduced presence of *R. reniformis* and *Meloidogyne* spp. within the root tissue. The findings suggested that in a grafted production system for cucurbits, rootstocks could potentially be utilized to control both *Fusarium* root rot and RKN.

Therefore, the present study is dedicated to exploring the interplay between the root-knot nematode (*M. incognita*) and the root rot fungus (*F. solani*) on grafted cucurbit hybrids. Specifically, the research examined how this combined infection affects root rot incidence, nematode reproduction, plant growth, and the antioxidant enzyme activity in both nematode-resistant and susceptible grafted plants.

Materials and Methods

Propagation of root-knot nematode, Meloidogyne incognita

A pure stock culture of the identified root-knot nematode, *M. incognita* (Kofoid and White, 1919). Chitwood (1949) was obtained from an isolate cultivated on tomato plants (*Solanum lycopersicum* Mill) cv. Super Strain B in a greenhouse of Nematology Division, Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University.

Propagation of root rot disease, Fusarium solani

Isolation of *Fusarium solani*

Cantaloupe plants exhibiting indications of *Fusarium* root rot disease were obtained from naturally infected fields in El Ayyat, Giza governorates, and sent to the laboratory for further analysis. The pathogen was

isolated using Water Agar (WA) with a concentration of 20 g L^{-1}, sourced from Oxoid Ltd., Basingstoke, Hampshire, England. The infected tissues received initial treatment with a 1% sodium hypochlorite (NaOCl) solution for approximately 60 seconds to eliminate surface contaminants. The tissue pieces were thoroughly dried and then placed individually on a water agar plate. The plates were positioned within an incubator for a week. Afterward, a pure culture was obtained by transferring the growing colonies to Potato Dextrose Agar (PDA) (39 g L^{-1}; Oxoid, Basingstoke, UK).

Identification of *F. solani*

The morphological characteristics were examined using a pure culture of the fungus that had been grown for 12 days. The fungus was identified by assessing its growth rate, culture color, and the shape of its mycelia, conidia, and sporulating structures, as outlined in the works of Barnett and Hunter (1986) and Leslie and Summerell (2006).

Molecular characterization

Approximately 100 mg of fungal mycelia were collected by scraping the colony's surface and grinding it into a fine powder using liquid nitrogen. The homogenized samples were then transferred to a sterile 1.5 ml Eppendorf tube. Next, 500 µl of preheated CTAB extraction buffer encompassing 0.2% β-mercaptoethanol was added and thoroughly mixed. The mixture was incubated at 65 °C for 30 minutes. Following this, 500 µl of PCI (phenol:chloroform: isoamyl alcohol, 25:24:1) was introduced and vigorously admixed, and subsequently centrifuged at 14,000 rpm for 15 min. The liquid portion was moved to a 1.5 ml sterile Eppendorf tube. A total of 500 µl of Isopropanol was mixed by pipetting. The resulting mixture was then placed on ice for 3 minutes, after which it underwent 10-minute centrifugation at 14,000 rpm. The liquid portion was taken out, and the DNA pellet was rinsed with 500 µl ethanol (70%) and spun at a speed of 10,000 rpm for 5 min. The precipitated DNA pellet underwent air-drying. Then, the pellet was dissolved within 75 µl of Milli-Q water and stored at -20 °C until it was needed (Doyle 1987).

The ITS4/ITS5 IT5f, 5'-GGAAGTAAAAGTCGTAACAAGG-3'/ITS4r 5'-TCCTCCGCTTATTGATATGC-3' (White *et al*., 1990) primers were employed for the ITS region amplification. The PCR was performed using a 25 µl reaction mixture consisting of 1 µl of fungal DNA extract (containing 40 ng of total DNA), 2 mM $MgCl_2$, 2.5 µl of 10× PCR buffer, 2.5 µl of 10 mM dNTPs, 1.5 µl of 10 µM of each primer, 0.3 µl of 5U Taq DNA Polymerase, and the remaining volume was made up to 25 µl with Nuclease-free water. The PCR experiment was performed using the ESCO Swift Maxi Thermal Cycler. PCR was conducted with an initial 2-minute denaturation step at 95 °C. Subsequently, 35 cycles were carried out, each consisting of three steps: denaturation at 95 °C, annealing at 52 °C, and extension at 72 °C, each lasting for 30 seconds. Finally, a polymerization cycle was carried out at 72 °C for 10 min. The QIAquick® PCR Purification Kit (Cat. No. 28106) was employed for the PCR product purification, adhering to the manufacturer's guidelines. These PCR products underwent sequencing in both directions by Macrogen, Korea.

Preparation of F. solani inoculum

Under aseptic conditions within a laminar flow hood, the fungus was introduced into the PDA and subsequently incubated for two weeks at room temperature (26 °C). Barley meal-sand medium (barley grains (75 g): sand (25 g): sucrose (2 g): yeast (0.1 g): 40 ml tap water) sterilized at 121 °C for 20 min within an autoclave was employed to prepare *F. solani* inocula. To prepare the fungal inocula, mycelial plugs from *F. solani* cultures cultivated on PDA for 7 days at 25 °C were introduced into the Barley meal medium and underwent 15 days of incubation at 25 °C. Then, the cultural medium (CM) underwent 15 days of incubation at 25 °C (Song *et al*., 2014; Seo and Kim, 2017).

Nematode-Fusarium interactions

To study the impact of the fungal root rot disease, *F. solani* (Fs), and *M. incognita* (Mi) disease complex on cucurbitaceous plants, 2 weeks seedlings with uniform size of 2 grafting cucurbits hybrids, Aswan F1/6001 F1 and FaransawyF1/6001 F1 were transplanted in clay pots of 20-cm diameter filled with sandy loam soil (1:1, v/v) that had been sterilized using steam and inoculated with either Fs, Mi inocula, or both. To perform Fs inoculation, the topsoils at a depth of 5 cm of each seedling were inoculated with 30 g CM/pot of barley meal-sand Fs cultures. To perform Mi inoculation, a nematode water suspension containing 4000 J2 nematodes was poured into 3-4 holes created around the root system of each seedling. Subsequently, the holes were covered with sterilized soil. All treatments were repeated 6 times. The seedlings in the pots were cultivated in an entirely randomized design within a greenhouse clean bench at 25±2 °C. The seedlings were watered daily until reaching field capacity.

Four weeks post-inoculation, disease severity caused by Fs was assessed by observing symptoms of root rot or stem rot. The severity was rated on a scale of 0 to 5, known as the disease index (DI) adhering to Bletsos (2005) as the following scale: 0=no symptom; 1=underground stem yellow-brownish discoloration; 2=<30% aboveground stem brownish discoloration; 3= decay in the stem bottom region; 4= dark discoloration and splitting of the stem; 5=complete plant death. Similar disease indices were used to assess symptoms of root or stem rot.

For the Mi-induced disease, according to Hooper *et al.* (2005) the number of galls, embedded stages, nematode soil population, nematode accumulation, and eggs/root were examined 90 days following inoculation.

Effects of individual and combined pathogen infections were assessed by comparing criteria for synergistic effects.

Plant oxidant and antioxidant enzymes analysis

Subsamples of fresh leaves of each grafted cucurbit hybrid were chemically analysed at the Central Chemical lab., Faculty of Agriculture, Cairo University as follows:

Preparation of enzyme extracts

Samples of 1 g were homogenized in 3 ml of 50 mM phosphate buffer of pH 7.0 containing 1.0 N NaCl, 1% PVP (Sigma), 1 mM ascorbate (Sigma) at 4 °C. After centrifugation at 15,000 × g for 15 min, the supernatant was collected.

Assay of protein content

Protein was determined by the method of (Bradford, 1976) with standard curves prepared using bovine serum albumin.

Determination of oxidative burst (Lipid peroxidation, MDA) contents

Thiobarbituric acid reaction (TBA) was estimated as described by Heath and Packer (1968). Fresh mass (200 mg) from culture was homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation at 12,000 × g for 20 min. The supernatant (1 ml) obtained was mixed with an equal volume of TCA (10%) containing 0.5% (w/v) TBA or no TBA as the blank, and heated at 95 °C for 30 min and then cooled in ice. The reaction product was centrifuged at 12,000 × g for 15 min and the supernatant absorbance was measured at 400, 532, and 600 nm. The MDA equivalent was derived from the absorbance according to Hodges *et al.* (1999).

Determination of antioxidant defence enzymes (Superoxide dismutase, SOD) and (Ascorbate peroxidise, APX) activity

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of (Beauchamp and Fridovich, 1971). The 3 ml reaction mixture contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA, and 20 µl enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was read. The volume of enzyme extract corresponding to 50% inhibition of the reaction was considered as one enzyme unit.

Ascorbate peroxidase (APX) activity was determined spectro-photometrically by a decrease in the absorbance at 265 nm ($e = 13.7mM^{-1}\ cm^{-1}$) using the method of (Nakano *et al*., 1981). The reaction mixture contained 50 mM potassium phosphate buffer pH 7.0, 5 mM ascorbate, 0.5 mM H_2O_2, and enzyme extract. Addition started the reaction. The rates were corrected for non-enzymatic oxidation of ascorbate by the inclusion of a reaction mixture without enzyme extract.

Statistical analysis

The obtained data were statistically analysed according to the SPSS software package version 12 (Plume, 2003). The differences between means were tested by multiple ranges Duncan test (1955) at the 5% significance level.

Results

Isolation and identification of Fusarium solani

Plants revealing root rot disease symptoms were collected, and morphological characteristics revealed the production of microconidia and macroconidia by the culture 8 days post-incubation, as well as chlamydospores after twelve days. Further confirmation of the predicted F. *solani* isolates was conducted where the ITS1, 5.8S rRNA gene, and ITS2 regions were among the PCR results that produced a 700 bp PCR fragment. Next, the sequences obtained from the PCR product were compared to those found in the GenBank database. After a 100% match with *F. solani* was found using BLAST, the sequence was submitted to GenBank with accession number PP801243.

Meloidogyne incognita reproduction and Fusarium solani incidence as affected by disease complex

Because of the coexistence of *Fusarium* root rot and phytopathogenic nematodes on plant feeder roots, this experiment was accomplished to explore the mutual influences of Mi and Fs on their activities on highly resistant and resistant cucurbit grafted hybrids (watermelon and sweet melon).

The grafted seedlings of Aswan F1/6001 F1 (HR) and FaransawyF1/6001 F1(R) were inoculated either with 30 g CM/pot of barley meal-sand Fs cultures, 4000 newly hatched J2 of Mi alone or in combination. Ninety days after inoculation, seedlings were lifted out, and the disease index (DI) of Fs and nematode criteria were estimated.

In general, Data presented in Table 1 reveal that DI of Fs and Mi development and reproduction were highly influenced by host type. *Fusarium* root rot symptoms were significantly higher on the sweet melon (scion) hybrid, FaransawyF1 grafted on 6001 F1, than watermelon (scion) hybrid, Aswan F1 grafted on 6001 F1, achieving the top rate of DI on the former. Fs Virulence based on DI was affected positively by the presence of Mi, where the DI was doubled on Aswan F1/6001 F1. Meanwhile, on FaransawyF1/6001 F1, Mi seemed to

have no synergetic effects on Fs root rot incidence. No significant differences were noticed between Fs alone or coinhabited with Mi. It is worthy to mention that Fs was initially isolated from the cantaloupe field.

Table 1. The dual existence of *M. incognita* and *F. solani* infecting grafted cucurbit hybrids

Cucurbit hybrids	Treatment	DI	Galls/ Root	Embedded stages	Final population	Pf/Pi	Eggs/ Egg mass
Aswan F1 /6001 F1 (HR)	Fs	2 c	-	-	-	-	-
	Mi	-	335 b	546 b	10610 b	2.65 b	169 a
	Fs+Mi	4 ab	152 c	248 c	6362 d	1.59 d	77 b
FaransawyF1/6001 F1 (R)	Fs	5 a	-	-	-	-	-
	Mi	-	678 a	985 a	12630 a	3.16 a	199 a
	Fs+Mi	5 a	308 b	584 b	9120 c	2.28 c	105 b
SE±		0.496	58.034	79.732	710.203	0.178	15.244

Means tailed by similar letter(s) within a column are insignificantly different (P ≤ 0.05) based on Duncans' multiple range tests. DI=Disease index. Embedded stages= Developmental stages + Egg masses. Final population= Embedded stages + Soil population. SE±= Standard error.

In the view of Mi development and reproduction, they were affected by host type and Fs presence. *Fusarium* presence significantly suppressed nematode penetration, gall numbers, development, root embedded stages, and final population, which was interpreted to significantly low values of reproduction (egg masses and eggs/egg mass) when compared with Mi alone treatment. Deterioration in Mi population, build-up, and reproduction in combined treatment was more or less half of that obtained from its single treatment on Aswan F1/6001 F1. Nematode attitude on FaransawyF1/6001 F1 followed the same trend recorded on Aswan F1/6001 F1 regardless of the host resistance. It is interesting to notice that the higher inoculation rate of nematode inocula and prolonged exposure time change the host category of resistance.

When nematode and fungus were introduced individually or combined, they induced considerable reductions in the plant's fresh weight. On Aswan F1/6001 F1, Fs alone or coexisted with Mi achieved (-52 and -60%), respectively; however, Mi alone recorded the lowest reduction (-6 %). On the other hand, Fs+Mi together recorded the highest reduction (-67%) followed by Fs alone (-64%) and then Mi alone (-20%) on FaransawyF1/6001 F1 when compared to the check (Figure 1). In the comparison of both grafted hybrids, the visual damage that occurred on plant growth exhibited that FaransawyF1/6001 F1 was more damaged than Aswan F1/6001 F1 by more than 60% reduction.

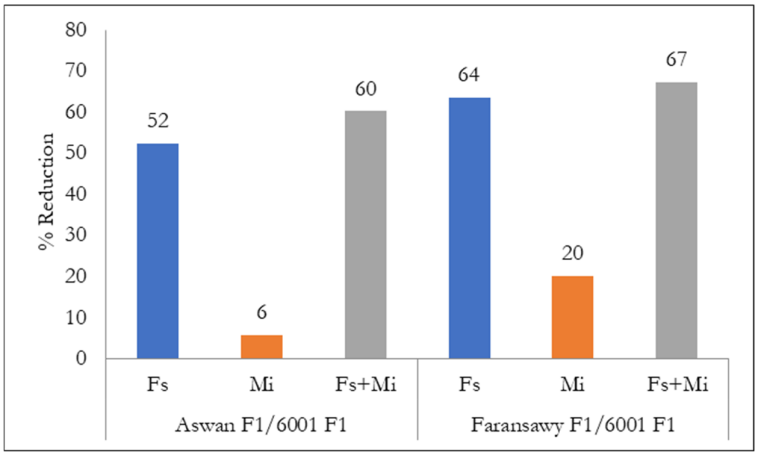

Figure 1. The percentages of reduction in plant fresh weights of grafted cucurbit hybrids as impacted by *M. incognita* and *F. solani* infection

The oxidant and antioxidant substances and enzyme activities involved in defence mechanisms as affected by disease complex

Regarding alterations in plant enzymes associated with defence mechanisms against plant pathogens, Table 2 data indicate that lipid peroxidase (MDA) in leaves of healthy grafted seedlings of Aswan F1/6001 F1 (HR) and FaransawyF1/6001 F1(R) were found to be the lowest and exhibited significant variations in those infected with Fs, Mi or Fs+Mi coexistence when compared with healthy plants. No significant differences were detected between single treatments of Fs and Mi either on Aswan F1/6001 F1 or FaransawyF1/6001 F1.

Table 2. Effect of *M. incognita* and *F. solani* on grafted cucurbit hybrids leaf contents of oxidant (MDA) and antioxidant enzymes (APX and SOD)

Cucurbit hybrids	Treatment	MDA (µ mol/g FW)	APX (unit/mg protein)	SOD (unit/mg protein)
Aswan F1/ 6001 F1 (HR)	Fs	4.5 d	71.7 f	20.7 e
	Mi	4.8 d	60.0 g	14.5 fg
	Fs+Mi	12.7 a	80.8 e	24.0 d
	Healthy	2.6 e	51.0 h	12.8 g
FaransawyF1/ 6001 F1 (R)	Fs	12.5 ab	150.0 b	29.6 b
	Mi	11.4 b	109.0 c	27.1 c
	Fs+Mi	7.2 c	210.3 a	38.6 a
	Healthy	6.3 c	90.6 d	15.8 f
SE±		0.776	10.448	1.720

Means tailed by similar letter(s) within a column are insignificantly different (P ≤ 0.05) based on Duncans' multiple range tests. MDA= Lipid peroxidation. APX= Ascorbate peroxidase. SOD= Superoxide dismutase. SE±= Standard error.

The highest rise in MDA contents of Aswan F1/6001 F1 leaves was recorded with infected seedlings with dual treatments, followed by Fs alone and Mi alone. MDA increased almost 5 times when compared with healthy treatment on Aswan F1/6001 F1, but there was a slight and insignificant increase between dual and healthy treatments on FaransawyF1/6001 F1. In general, the MDA contents in the grafted sweet melon seedlings' leaves were more significant than those in the grafted watermelon seedlings' leaves.

The leaves of Aswan F1/6001 F1 exhibited the highest percentage increase when infected with Fs+Mi together (388%), followed by those in leaves of FaransawyF1/6001 F1 infected with Fs alone (98%) then in leaves of both grafted hybrids infected with Mi alone (85 and 81%) respectively and the lowest percentage (14%) was noticed in leaves of FaransawyF1/6001 F1 infected with Fs+Mi as compared to those in healthy plants (Figure 2).

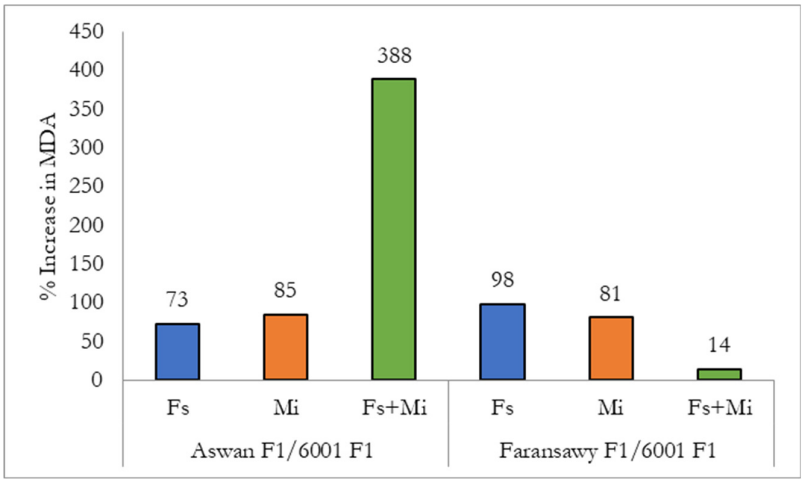

Figure 2. The percentages of increases in lipid peroxidase (MDA) in leaves of grafted cucurbit hybrids as influenced by *M. incognita* and *F. solani* infection

The activities of antioxidant enzymes, ascorbate oxidase (APX), and superoxide dismutase (SOD) were found to be at their lowest levels in healthy plants of both grafted hybrids with significant differences. Enormous increases due to the pathogens infection in APX were observed as a result of Fs, Mi, or coinfection in leaves of FaransawyF1/6001 F1 and achieved the highest activity followed by Fs alone then Mi alone with significant differences among treatments and healthy plants. The enzyme activities in leaves of Aswan F1/6001 F1 followed the same trend with the same treatments. The leaves of FaransawyF1/6001 F1 exhibited the highest increase percentage when infected with Fs+Mi together (132%), followed by Fs alone (66%) and then Mi alone (20%). In leaves of Aswan F1/6001 F1, Fs+Mi coexistence, Fs alone and Mi alone recorded significant percentages of increase by (58, 41, and 18%) respectively when compared with healthy plants (Figure 3).

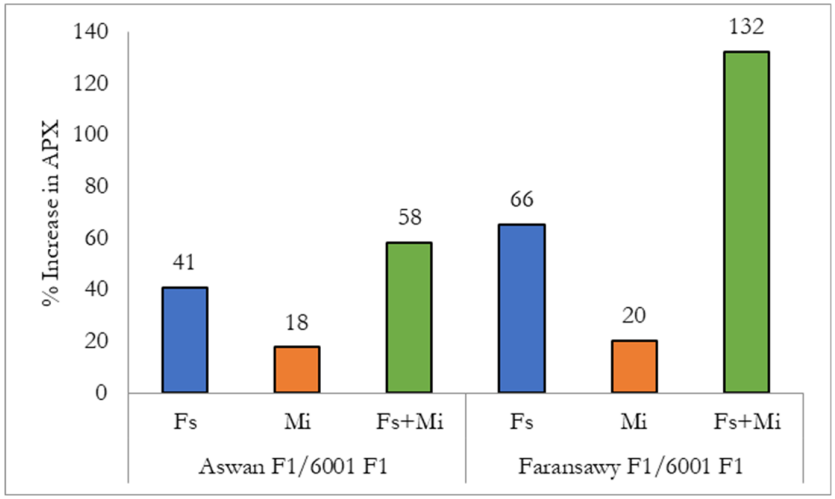

Figure 3. The percentages of increases in ascorbate oxidase (APX) in leaves of grafted cucurbit hybrids as influenced by *M. incognita* and *F. solani* infection

SOD activity in leaves of both grafted hybrids was likely increased as in APX in the same treatments. The FaransawyF1/6001 F1 leaves exhibited the highest increase percentage when infected with Fs+Mi together (144%), followed by Fs alone (87%) and then Mi alone (72%). In leaves of Aswan F1/6001 F1, the

increased percentage recorded 88, 62, and 13% with Fs+Mi coexisted, Fs alone and Mi alone, respectively, when compared to those in healthy plants (Figure 4).

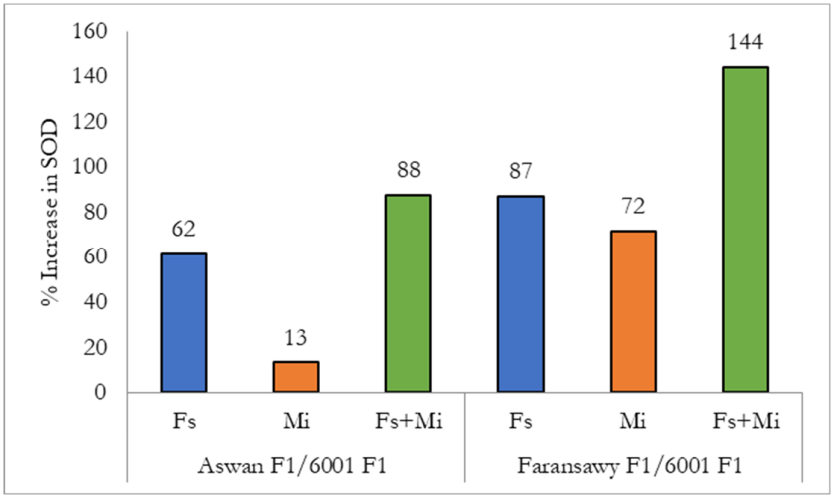

Figure 4. The percentages of increases in superoxide dismutase (SOD) in leaves of grafted cucurbit hybrids upon *M. incognita* and *F. solani* infection

Mostly, the infection of both grafted cucurbit hybrids with a concomitant of Fs+Mi induced higher and significant rates of increase of oxidants and antioxidants when compared to single infections of either Fs or Mi regardless of the type of the scion.

Discussion

Pathological criteria and interrelationships of soil-borne diseases on 2 grafted cucurbit hybrids (Aswan F1/6001 F1 and FaransawyF1/6001 F1) caused by Fs and Mi were examined. The DI assessment revealed high Fs virulence to both grafted cucurbits, without differential interactions between Fs and cucurbit hybrids tested. Moreover, the presence of Mi coinfection did not induce any noteworthy alterations in the higher-order virulence of Fs, signifying that the Fs virulence may be differentially impacted by the Mi infection, host characteristics, and their interactions. Insignificant increases in DI as a result of Mi coinfection were apparent in both grafted cucurbits tested. This indicates that the DI rise in the disease complex (caused by Mi coinfection) is likely dependent on cucurbit hybrids resistance, Fs isolate, and the density of the inoculum. These factors collectively affect the occurrence and intensity of the disease (Agrios, 2004).

When examining DI in cucurbits affected by Fs, it was observed that FaransawyF1/6001 F1 exhibited a higher susceptibility to Fs compared to Aswan F1/6001 F1. Aswan F1/6001 F1, which is typically utilized as a rootstock for grafting cucurbits to ensure their propagation, is known for its resistance against *Fusarium* root rot (Lee, 1994). This specifies that the existing occurrence of *Fusarium* root rot in greenhouses cultivating sweet melons might stem from the growers' misidentification of *Fusarium* diseases induced by counterfeit *Fusarium* species like *F. solani* that is responsible for the decay of various plant components, encompassing stems and roots (Seo and Kim, 2017).

The invasion of *Fusarium* root rot pathogens initiates when they penetrate the surfaces of roots and invade the vascular tissue within the stele. The progression of the disease is influenced by the timing of defence structure formation and their respective quantities (Crews *et al*., 2003; Egel and Martyn, 2007). However, DI has significantly increased Mi coinfection, resulting in a noticeable reduction in defence structures, associated

with the creation of enlarged cells that obstruct xylem vessels, intensifying water scarcity in the aerial sections of the plant (Jones, 1981; Shepherd and Huck, 1989; Sijmons*et al*., 1994; Mitkowski and Abawi, 2003). A substantial rise in the severities of *Fusarium* disease due to Mi coinfection was also noticed in Aswan F1/6001 F1 infected by Fs; nevertheless, the escalation of DI could potentially be attributed to the widespread devastation of vascular tissues by the Fs+Mi coinfection compared to the Fs infection lonely.

Both grafted cucurbits examined appeared to be highly susceptible responses to Mi infection (Sasser *et al*., 1984; Roberts *et al*., 1990). The most significant damage was shown in FaransawyF1/6001 F1 and the lowest in Aswan F1/6001 F1. Numerous research has been carried out on the concurrent existence of microbial pathogens and plant-parasitic nematodes, leading to the development of complex diseases among various crops, such as alfalfa (Griffin and Thyr, 1986) beans (France and Abawi, 1994), tomatoes (Abawi and Barker, 1984; Suleman *et al*., 1997), bananas (Jonathan and Rajendran, 1998), peas (Siddiqui *et al*., 1999), and coffee (Bertrand *et al*., 2000). The nutrient-rich giant cells produced by Mi could act as a substrate for fungal pathogens, facilitating their growth and advancement, thereby intensifying the severity of the disease. It's worth noting that Mi infection alone does not induce any symptoms of root rot or decay (Meléndez and Powell, 1970; Khan and Muller, 1982; McLean and Lawrence, 1993; Abawi and Chen, 1998; Seo and Kim, 2017). This implies that the severity of *Fusarium* diseases in cucurbits infected by Fs may be intensified due to Mi coinfection. However, the presence of Fs coinfection hinders the formation of giant cells and accelerates their deterioration, resulting in depleted cytoplasmic contents. Consequently, fungal disease severity is reduced due to the diminished contribution of Mi infection to the exacerbation of DI. Moreover, the Mi infection suppressed the development of defence structures triggered by the fungal infection, weakening the plant's resistance response to Fs infection (Beckman, 1987; Hall *et al*., 2011; Inch *et al*., 2012). These factors indicate that the interactions between Mi and *Fusarium* isolates, leading to disease synergism, are contingent upon the virulence of the fungal pathogen, the potential of the inoculum, the aggressiveness of the nematode, and the susceptibility and appropriateness of the host for pathogen growth and progression.

Based on the results of inoculation tests, Mi and *Fusarium* pathogens do not mutually derive advantages from each other. Instead, the fungal pathogens tend to benefit more from the occurrence of Mi. The fungal pathogen's role as a necrotrophic parasite can be attributed to its utilization of the decaying cells and tissues resulting from nematode infection. However, the reverse does not hold true as the fungal infection commonly worsens the conditions and nutritional resources for the biotrophic parasite, Mi. Hence, our results offer valuable insights into comprehending the pathological traits of soil-borne diseases and the underlying principles of their interrelationships in cucurbits cultivated under greenhouse conditions in Egypt. This knowledge can be harnessed to develop effective strategies for controlling these diseases.

Our results revealed that the contents of MDA in leaves of both scions, Aswan F1 and FaransawyF1 grafted on the rootstock, 6001 F1 increased significantly as a response to infection with Fs alone, Mi alone, or Fs+Mi coexistence compared to healthy ones attributed to the defence mechanism countering the invasion of both pathogens. In addition, the speed of activation and the levels of activity of defence enzymes exhibit differences relating to the specific plant genotypes or the interactions between plants and pathogens (Yan *et al*., 2009; Kesba and El-Beltagi, 2012). The rates of rise in MDA rely upon the nature of the scion, rootstock, fungus isolate, nematode density, and exposure time. The compounds produced post-infection play a more influential role in expressing incompatibility to nematodes compared to constitutive plant products, involving active mechanisms (Kaplan and Keen, 1980). According to this perspective, plants promptly initiate defence mechanisms upon invasion by nematodes and fungi. Commonly, the reactive oxygen species (ROS) generation arises from the incompatible resistant interactions between pathogens and plants, constituting the majority of these defence mechanisms (Montes *et al*., 2004; Murgia *et al*., 2004; Bakker *et al*., 2006). Following pathogen invasion, the induction of ROS triggers lipid peroxidation, which is responsible for cell death. According to Zacheo *et al*. (1993), Zacheo and Bleve-Zacheo (1988), Davis *et al*. (2000), and Huang *et al*. (2004), the susceptible cultivars exhibit an initial response that resembles that of resistant hosts, possibly attributed to the

secretion of nematodes into plant tissues. Also, MDA has been considered one of the principal forms of ROS and a crucial biochemical marker indicating oxidative harm in plants when confronted with pathogen infection (Alcalá-García *et al*., 2002; Mellersh*et al*., 2002; Peltzer *et al*., 2002; Gill and Tuteja, 2010; Mandal *et al*., 2011; Chavan *et al*., 2013). Debona*et al*. (2012) indicated the potential triggering of ROS-mediated cell death in plants due to the escalation of oxidative damage, as evidenced by elevated MDA levels following pathogen infection.

The infection with both pathogens caused a remarkable rise in SOD and APX in Aswan F1/6001 F1 and Faransawy/6001 F1. The most significant rise occurred in the leaves of Faransawy/6001 F1. The lower levels of both antioxidants recorded in healthy leaves of Aswan F1/6001 F1 may be attributed to its consumption in scavenging higher levels of peroxidases produced. Arrigoni *et al*. (1979) demonstrated the ascorbic acid significance in plant defence mechanisms against nematodes. Their findings indicated that the depletion of ascorbic acid in plants weakened tissue resistance against nematode infections. They assumed that plants could utilize ascorbic acid to synthesize mitochondrial hydroxyproline proteins that regulate the establishment of cyanide-resistant respiration. In addition, they noted that the resistant plants exhibited an enhanced ascorbic acid synthesis, suggesting that the activation of biological defence mechanisms necessitates cyanide-resistant respiration (CCR), which is typically linked to wound responses. The synthesis of hydroxyproline-containing protein, which relies on ascorbic acid, is related to mitochondria-associated CCR. Through peroxidase activity, mitochondria-associated CCR produces hydrogen peroxide, subsequently leading to superoxide generation. Superoxides possess a high level of toxicity and permeate through cells, oxidizing functional groups of enzymes and phospholipids, reducing S-S bonds, and causing injuries to macromolecules and membranes. In some instances, the down-regulation of APX has been observed to be linked to the manifestation of resistance rather than susceptibility (El-Zahaby, 1995; Vanacker *et al*., 1998). Also, it is demonstrated that the activity of ascorbate peroxidase exhibits an improvement in pathogens-infected plants like apricot, wheat, and mung bean (Asada and Takahashi, 1987; Asada, 1992; Hernández *et al*., 2001; Chen *et al*., 2015; Farahani and Taghavi, 2016).

The plant's adaptive response to abiotic and biotic stresses involves an elevation in the SOD and peroxidase activities, which serves as a protective mechanism (Guida *et al*., 1992). Susceptible plants revealed a rise in the SOD protective activity, whereas resistant plants displayed a decline in its effectiveness (Zacheo *et al*., 1993; Maninder *et al*., 2013). Accordingly, in M. incognita-infected roots, SOD activity was substantially improved compared to uninfected controls (Zacheo and Bleve-Zacheo, 1988; Sgherri *et al*., 2013). The differential regulation of antioxidants, including APX and SOD, in wheat plants due to *Fusarium* infection was highlighted by Gherbawy *et al*. (2012). Our results revealed an increase in SOD and ascorbate oxidase (APX) following nematode infection, with the rate of increment varying based on scions, fungus isolate, nematode density, and rootstock-scion compatibility.

Conclusions

The presence of both Fs and Mi significantly impacted the grafted cucurbit hybrids. Fs virulence was significantly affected by Mi coinfection. Fs infection suppressed nematode development and reproduction in both hybrids. The combined infection caused substantial reductions in plant fresh weight. The antioxidant enzymes (APX and SOD) activities and the MDA content (an indicator of oxidative stress) were significantly increased in response to infection with either Fs, Mi, or both pathogens. These findings suggest that the inter-relations between Fs and Mi depend on factors such as the scion type, fungal isolate virulence, and nematode aggressiveness. The results provide valuable information for understanding disease development in cucurbit crops grown in greenhouses and can be used to develop control strategies.

Authors' Contributions

Conception and design, acquisition of data, analysis, and interpretation of data, and drafting the article: HHK; Acquisition of data and analysis and interpretation of data: SME-G.; Analysis and interpretation of data, drafting the article, and critical review of important intellectual content: AHHA; Acquisition of data and analysis and interpretation of data: NMMH. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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