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RESEARCH ARTICLE

The impact of three rootstock types on *Fusarium* sp. wilt resistance in melons plants (*Cucumis melo* L.) in Can Tho city (Vietnam)

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Abstract

Growing melon (*Cucumis melo* L.) still faces many difficulties due to vine death disease caused by *Fusarium* sp. In the present study, melon plants with wilt disease symptoms were collected from Phong Dien (PD) and Cai Rang (CR) districts, Can Tho city (Vietnam). Three strains of *Fusarium* sp. (CRI, CR8.1 and PD) were isolated based on morphological characteristics such as the color of the fungus, spore sepals, branching patterns from the fungal hyphae, crescent-shaped megaspores measuring 24.7 - 27.34 x 3.66 - 3.68 µm; thin-walled spherical mantle spores. Among them, *Fusarium* isolate CRI had the highest virulence with a pathogenicity index of 96.8 % and a disease incidence of 100 % after 15 days of inoculation. The research results showed that the two rootstocks, pumpkin and luffa, had the lowest mortality rate (0.0 %) at all survey times and were highly resistant to string rot disease on melon caused by *Fusarium* sp.

Keywords: disease resistance; *Fusarium oxysporum*; wilt disease

Introduction

In recent years, melon (*Cucumis melon* L.) has become familiar to Vietnamese. Melon has a short growing period (about 60 days), providing high economic efficiency suitable for crop rotation regime on rice land in the Mekong Delta (1, 2). Melon has high nutritional value with quite high sugar content (8-12 %), vitamin C (31-37 mg/100 g of fresh weight), vitamin A (9.03 µg/100 g of fresh weight)), a quite high content of cellulose and minerals (Magie and Natri) (3, 4). Although market demand is quite large, melon production still faces many difficulties due to death disease caused by the *Fusarium* sp. General symptoms of wilt disease caused by *Fusarium* sp. The cause is slow growth, brown, dry and non-viscous vascular bundles that can be seen inside the stem as brown lines or dots. Others are white, pink or orange fungal growths on the outside of diseased tree stumps, especially in wet conditions, root rot or diseased tree trunks can wilt and die very quickly in hot weather, typically Notably, the yellowing of leaves occurs at the base first and gradually yellows up to the top of the stem. Severe disease is evident until the entire plant wilts, leading to low productivity and quality of fruit and short storage time.

One of the measures that can reduce the harmful effects of soil-borne diseases is to use grafted roots to increase the resistance of trees, which is widely used in the world (5, 6). The use of grafted roots is one of the effective technical measures in disease resistance that has been studied on cucumber (7, 8) and watermelon (9). In the Mekong Delta of Vietnam research into finding a strong grafted root and improving disease resistance of melon caused by *Fusarium* sp. is extremely necessary. Therefore, the study was conducted to determine the disease resistance effectiveness of three types of grafted roots against *Fusarium* on melon with the aim of limiting economic losses and improving productivity for farmers.

Material and Methods

Experiment plant materials

Melon plants with wilt disease symptoms were collected from districts in Phong Dien and Cai Răng districts, Can Tho city (Vietnam). Samples were placed in paper bags clearly stating location and time information; after that, transferred to laboratory at An Giang University, Vietnam University in Ho Chi Minh city (Vietnam) within 2.5-3 hrs.

PDA medium (Potato Dextrose Agar): 200 g potatoes, 20 g

dextrose, 20 g agar, 1000 mL water store

Methods for fungus isolation

Take the base of the plant showing disease symptoms, cut a section about 10 cm long while still fresh, wash thoroughly with water and then surface disinfect with 70 % (v/v) ethanol and 1 % sodium hypochlorite, respectively; after that, rinsed with distilled water. Samples were left to dry on sterilized absorbent papers. Using a sterile tool (a scalpel), the infected tissues were cut in small spieces (approximately 3-5 mm of length) (10 numbers) and were incubated on PDA medium about 2-3 days at 28±2 °C until the appearance of mycelium. The fungal isolates were subcultured four times on PDA until pure colonies were obtained, then observe the morphological characteristics of the colonies, including color, size, shape and hyphal texture (11).

Pure fungal samples were propagated in PDA medium until the colony diameter was 5-6 mm; after that, the spores were in sterilized distilled water to create a suspension solution. Spore counting was performed using a Kruss microscope (MLB2000, Germany) and a Neubauer-Improved hemacytometer counting chamber (Germany). When the spore density was $5x10^5$ cfu/mL, inoculation was performed.

The isolated fungal samples were artificially infected with the melon plant to confirm the pathogens according to Koch's rules.

Koch's method for artificial infection

The melon seeds were soaked in warm water for 1 hr and incubated until the seeds germinated (about two days). The germinating seeds were sown in plastic pots (containing pasteurized substrate). The pots were placed with plenty of light for seedings to grow. When the seedings had two true leaves, artificial infection was preceded. 10 mL of *Fusarium* sp. solution was watered around the roots of the melon plants for inoculation treatment (four plants). For the control treatment (four plants) without disease, use 10 mL of distilled water for irrigation (12).

Determination of morphology and identification of fungus

Fusarium was identified according to Lombard et al. method (13). Pure *Fusarium* was cultured into the concave slide (with sterilized PDA) and covered by cover glass. The slides were placed in petri plate and incubated at 25 °C for 2-5 days. Morphological identification of *Fusarium* was performed using a Biological microscope (connected to camera) (CX3, Olympus, Japan).

Characteristics of the colony: The color is pink to light purple; cottony shape with many aerial mycelium; the margin is irregular.

Conidiophores: Branched vertically and densely, with thin walls. The sporophyte consists of a short, smooth-surfaced stalk, bearing whorls at the apex and sized from 4-10 x 4-5 µm.

Macroconidia: Crescent-shaped, curved towards the back, smooth and thin walls; 1-5 septate conidia with size of 21-26 x 3-5 µm, 20-27 x 3-5 µm, 22-32 x 3-5 µm, 30-35 x 4-5 µm and 35-38 x 5-6 µm, respectively.

Microconidia: Elliptical or crescent shape; smooth and thin walls; 0-1 septate with size 4-11 x 2-4 µm and 13-16 x 2-4 µm, respectively.

Chlamydospore: Globose shape with 5-10 µm in diameter, formed terminally or intercalarily.

Determination of the ability of isolated *Fusarium* to cause death on melon

Similar to Koch's method, when the melon plants have two true leaves, the isolated *Fusarium* determines their death. 10 mL of *Fusarium* solution (with 10^6 cfu/mL of spore density) was artificially inoculated by watering around the roots of the melon plants. The experiment was arranged in a completely randomized design with four repetitions and 10 plants per repetition. The experiment was carried out the greenhouse in the Experimental-Practical Area of An Giang University (Vietnam) (with 27-33 °C and 70-91% humidity). Rate and index of the disease were monitored during 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} day intervals after the isolated *Fusarium* incubation on the melon plants.

Rate and index of the disease

Rate and index of the disease were calculated using equations 1 and 2 (14).

$$\text{Rate of disease (\%)} = \frac{N0}{N} \times 100 \quad (1)$$

Where, No is total of trees infected with *Fusarium*

N is total of trees in the treatment

$$\text{Disease index} = \frac{(N1x1)+(N2x2)+(N3x3)+(N4x4)}{Nx4} \quad (2)$$

Where, N1 is total of trees infected with Fusarium at level 1 (healthy tree (Fig. 1A))

N2 is total of trees infected with Fusarium at level 2 (the lower leaves were slightly necrotic and the tree is slightly wilted (Fig. 1B))

N3 is total of trees infected with Fusarium at level 3 (the lower leaves became necotic and fell off, while upper leaves turned yellow (Fig. 1C))

N4 is total of trees infected with Fusarium at level 4 (dead trees (Fig. 1D))

N is total of trees in the treatment

4 is the highest disease level on the scale

Data analysis methods

Data were collected and processed by SPSS 22 software with Duncan test and Microsoft Excel.

Results and Discussion

Isolation and morphology of *Fusarium* isolates

Based on the characteristic symptoms of the dead cord disease, which include the development of white, pink, or orange fungal growth on the exterior of the affected plants, as well as yellowing and wilting of the leaves, samples from diseased plants were collected in two districts, Phong Điền

Fig. 1. Disease index scale of an (A) healthy tree; (B) (the lower leaves are slightly necrotic and the tree is somewhat wilted; (C) the lower leaves become necrotic and fall off, while upper leaves turn yellow and (D) dead tree.

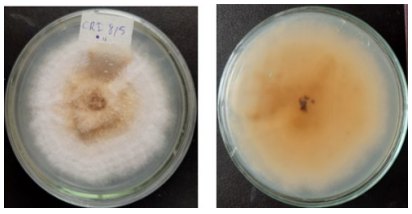
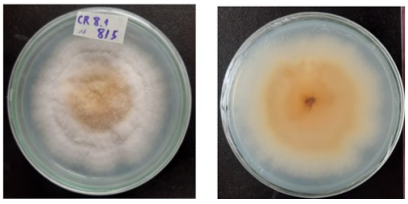
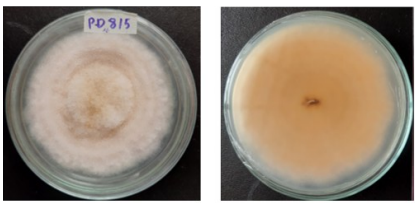
and Cai Rang, in Can Tho City, Vietnam. After collection, the diseased samples were transferred to the laboratory for isolation and identification of the causal agent. The results led to the isolation of three fungal strains with distinctive hyphal characteristics of the genus *Fusarium*, designated as CR I, CR8.1 and PĐ. The names of the fungal strains are abbreviated using the first letter of the name of the sampling location. These fungal strains were purified and cultured on a PDA medium. The characteristics of the isolated fungal strains showed variations in morphology, coloration and growth capabilities on the PDA medium (10) (Table 1).

Regarding the characteristics of the sepals and spores of three isolates (Fig. 2), they all have a crescent shape with septate conidia for the macroconidia. Microconidia are elliptical in shape (0-1 of septate conidia), colorless and with thin walls. This result is similar to other studies (15, 16), where microconidia is elliptical shape, zero or 1 of septate conidia and macroconidia is crescent-shaped, curved towards the back, three or four of septate conidia, transparent, smooth and thin-walled.

Chlamydospore of the isolates are spherical to platelet -shaped or oval; thick walls and formed terminally or intercalarily of the mycelium. This result is similar to a previous report (17) which suggest that chlamydospore are formed singly or in pairs from normal mycelium that have undergone growth and thickened their cell walls. According to the classification of Lombard et al. (13), the conidiophores grow directly from the hyphae, hyaline, branch vertically and densely which consist of a short, smooth and thin walls; and bear apical whorls.

In fact, CR1, CR8.1 and PD isolates were tested according to the Koch's method (Fig. 3) and all have similar characteristic of morphology, size, shape and chlamydospore with that of *Fusarium SP* (13, 15-18).

Table 1. Characteristics of morphology, color and growth ability of the isolates on PDA

Isolates		Characteristics
CRI		The center of colony is slightly protruding. The clear white mycelium is smooth, spongy and spreads out to the surrounding. When old, the mycelium turns light orange; the surface is light brown. The colony grows unevenly, densely and mycelium closely follows the culture medium.
CR8.1		The center of colony is slightly protruding, with a light pink circle surrounding it. The underside of the colony is cream. Colony grows unevenly. The mycelium is smooth, spongy, dense, intertwined and adheres closely to the culture medium.
PD		The center of colony is slightly protruding and white; the underside of it is cream. Circles appear on the surface of colony. Colony grows unevenly. The mycelium is smooth, spongy, dense, intertwined and adheres closely to the culture medium.

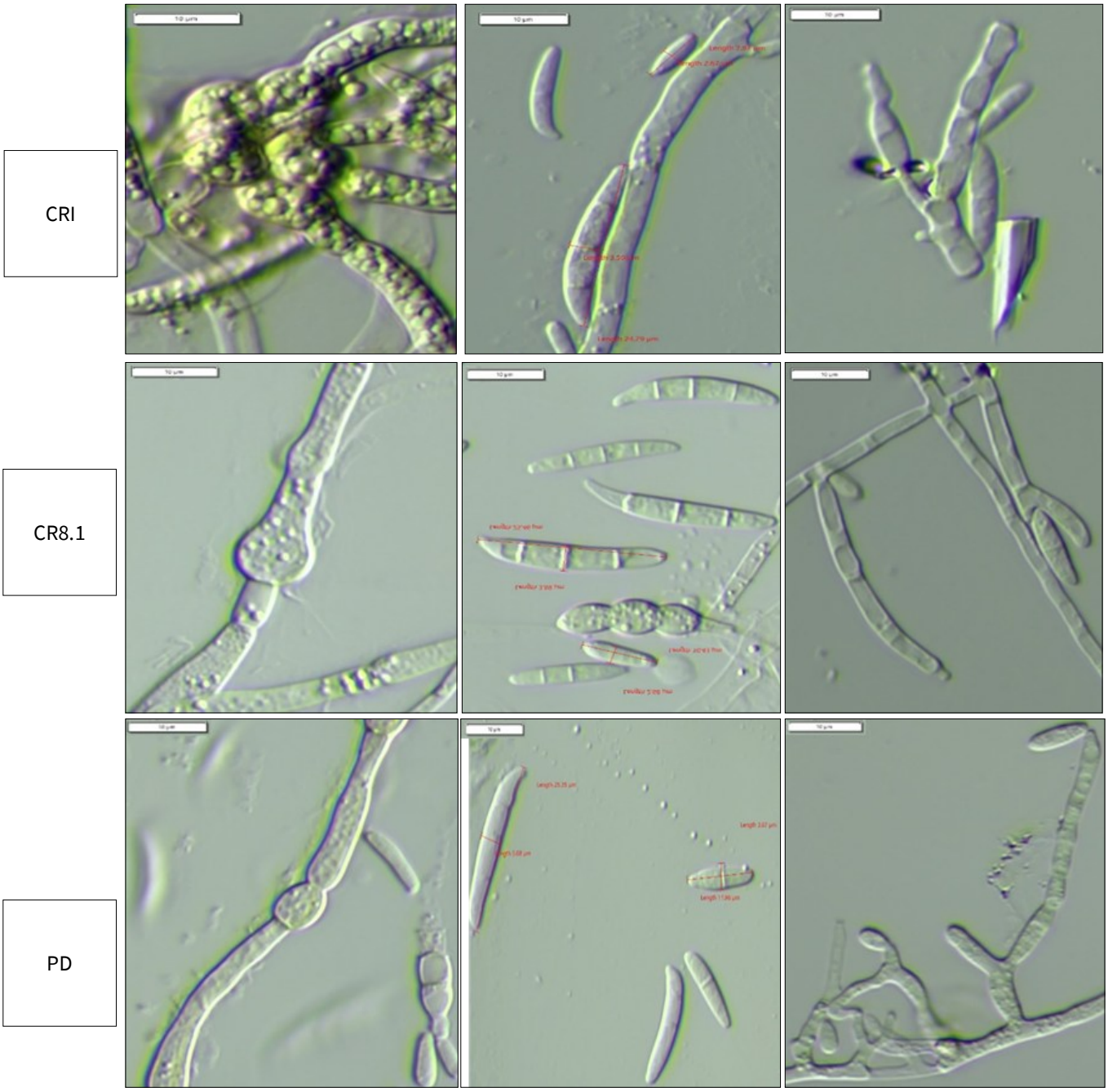


Fig. 2. Macroconidia, microconidia, chlamydospore and conidiophore of fungal isolates (CR1, CR8.1 and PD) causing death disease in melon plants.

(a) CR1 treated melon plant

(b) CR8.1 treated melon plant

(c) PD treated melon plant

(d) Control melon plant

Fig. 3. Disease symptoms of the *Fusarium* isolates (CR1, CR8.1 and PD) observed on melon plants during Koch's method and the control plant with no *Fusarium* incubation.

Table 2. Index disease of isolated *Fusarium* on melon under greenhouse at survey time

Isolates	Index disease at survey time (days)				
	7	9	11	13	15
PD	25.0^{a}	31.2^{bc}	40.6^{b}	56.2^{b}	68.7^{b}
CR8.1	25.0^{a}	34.3^{ab}	43.7^{b}	53.1^{b}	65.6^{b}
CR1	28.1^{a}	40.6^{a}	53.1^{a}	71.8^{a}	96.8^{a}
Control (no *Fusarium* incubation)	25.0^{a}	25.0^{c}	25.0^{c}	25.0^{c}	25.0^{c}
Level of signification	*ns*	*	**	**	**
CV (%)	12.1	16.7	10.2	15.6	18.6

Values are expressed as means of four tests. Values with a different superscript in each column are signification different from Duncan test. (ns) P>0.05, (*) P<0.05 and (**) P<0.01; PD and CR are the abbreviations for Phong Dien and Cai Rang districts, respectively.

Table 3. Rate disease (%) of *Fusarium* isolates on melon under greenhouse at survey time

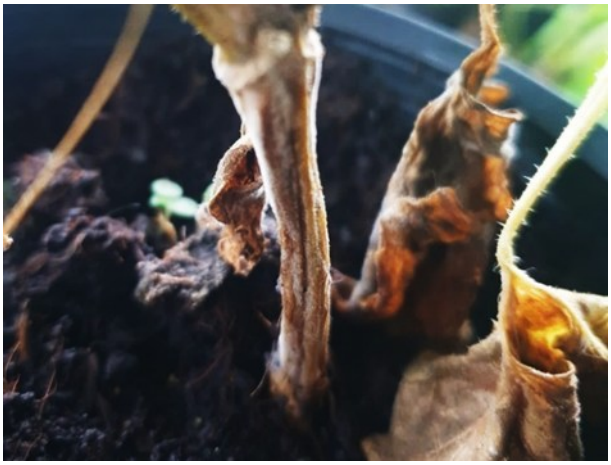
Isolates	Rate disease (%) at survey time (days)				
	7	9	11	13	15
PD	0.0^{b}	25.0^{c}	62.5^{b}	62.5^{b}	62.5^{b}
CR8.1	0.0^{b}	37.5^{b}	62.5^{b}	62.5^{b}	62.5^{b}
CR1	12.5^{a}	62.5^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Control (no *Fusarium* incubation)	0.0^{b}	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{c}
Level of signification	**	**	**	**	**
CV (%)	33.3	7.0	2.4	2.4	2.4

The ability check of the *Fusarium* isolates causing death on melon plants

The index and rate disease of the isolated Fusarium isolates to cause death on melon plants at the survey time are shown in Table 2 and 3.

Values are expressed as means of four tests. Values with a different superscript in each column are statistically significantly difference. '**' indicates 99% statistically significant; PD and CR are the abbreviation for Phong Dien and Cai Rang district, respectively.

The results in Table 2 showed that the disease appeared on the melon plant on the 7th day after artificial infection of CR1. The melon plants showed signs of wilting



Mycelium

Necrotic leaf

Fig. 4. Symptoms of disease on melon plant caused by CR1 after 11 days of artificial infection.

and brown spots at the roots. At 11th and 15th days after artificial infection, index disease was 53.1 % and 96.8 %, respectively (Fig. 4). The PD caused the lowest disease with 56.2 % after 15 days of artificial infection.

Similarly, CR1 also showed the highest rate disease after 15 days of artificial infection (100 %), while rate disease of PD and CR8.1 was 62.5 %.

Conclusion

In laboratory conditions, three fungal strains were isolated based on colony color: pale pink, light brown and cream. Macroconidia sizes were measured for strains CR1 (26.64 x 3.68 µm), CR8.1 (24.7 x 3.66 µm) and PD (27.34 x 3.6 µm), all of which were crescent-shaped with short, branched conidiophores, confirming the species *Fusariu*m sp, which causes vine wilt in muskmelons in Phong Dien and Cai Rang, Can Tho City; *Fusarium* sp CR1 was identified as the most pathogenic strain. In greenhouse trials, *Fusarium* sp CR1 showed high virulence with a disease index of 96.8 % and an infection rate of 100 % and disease resistance was tested on three rootstocks: pumpkin, wax gourd and luffa, with results showing that pumpkin and luffa had a disease index of 25.0 % and a mortality rate of 0.0%, indicating their high resistance to wilt caused by *Fusarium* sp CR1. CR1 was the one with the strongest ability to cause death and is used for further research. However, this is a preliminary study that relies solely on the morphological characteristics of the isolated strains. We will continue our research and proceed with molecular identification at the species level for these fungal strains to facilitate more in-depth studies in the next phase.

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Authors' contributions

TVK designed, carried out the experiment, analysed data, wrote, reviewed and edited. NTNG and NTMC carried out the experiment and analysed data. VTBT designed the experiment, wrote, reviewed and edited.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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