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## SURVEY ON THE INCIDENCE OF FUSARIUM WILT IN WATERMELON (*CITRULLUS LANATUS*) CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* IN MAJOR WATERMELON-GROWING REGIONS OF TAMIL NADU, INDIA

B. Tamilselvan<sup>1</sup>, S. Sudhasha<sup>2\*</sup>, K. Sanjeevkumar<sup>3</sup>, S.M. Suresh Kumar<sup>2</sup>, A.P. Srinivasperumal<sup>2</sup>, G. Baradhan<sup>4</sup> and S. Kanaka<sup>2</sup>

<sup>1</sup>I.C.A.R. K.V.K., Karur sistrict - 621 313, Tamil Nadu, India.

<sup>2</sup>Rice Research Station, Tamil Nadu Agricultural University, Tirur, Tamil Nadu, India.

<sup>3</sup>Oilseed Research Station, Tindivanam, Tamil Nadu, India.

<sup>4</sup>Agricultural college and Research Institute, Vazhavachanur, Tamil Nadu India.

\*Corresponding author E-mail : [sudhashapat@gmail.com](mailto:sudhashapat@gmail.com)

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### ABSTRACT

Watermelon is susceptible to a range of diseases caused by various agents, including bacteria, viruses, nematodes, fungi and abiotic factors. Among these, Fusarium wilt, caused by *Fusarium* spp., is one of the most critical threats to watermelon cultivation, with yield losses of up to 40%. Chemical treatments used to control this disease often have negative environmental impacts. In the present study, 10 isolates of *Fusarium* spp. exhibited variability in cultural and morphological characteristics, as well as virulence. Of these, the Fon5 isolate from Siruvadi village was the most virulent, showing the highest disease incidence (41.24%). These *Fusarium* isolates produced a profuse, fluffy cotton-like growth with white to pink mycelium.

**Key words :** Survey, Watermelon, *Fusarium* wilt, *Fusarium oxysporum* f. sp. *niveum*.

### Introduction

Watermelon (*Citrullus lanatus*) is one of the most popular tropical and subtropical fruit crops cultivated globally, including in India (Giakhuong *et al.*, 2020). Belonging to the Cucurbitaceae family, it is considered a major horticultural crop worldwide. The total cultivation area of watermelon is approximately 3.5 million hectares, with an annual production of around 100 million tonnes (Xing Guangxie *et al.*, 2021). Watermelon seeds are highly nutritious, rich in proteins, vitamin C, and essential minerals such as magnesium, potassium, phosphorus, sodium, iron, zinc, manganese and copper, in addition to healthy fats and phytochemicals (Braide *et al.*, 2012). Watermelon is also an excellent source of L-citrulline, lycopene and carotenoids (Liu *et al.*, 2010). Composed of 6% sugar and 92% water by weight, it has various health benefits, including antimicrobial properties, antiprostatic hyperplasia

activity, antiangiogenic effects, antisecretory effects, antidiabetic activity, laxative effects, and anti-ulcerogenic properties (Erhirhie *et al.*, 2013).

According to the National Horticultural Board (2017-18), India is the second-largest producer of watermelon in Asia. Uttar Pradesh is the leading state in watermelon production, while Tamil Nadu ranks 6th in both area and production. The total area under watermelon cultivation in India is about 2.5 million hectares. Watermelon is believed to have originated from the Kalahari Desert in Africa. In India, it is primarily cultivated in Uttar Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan and Tamil Nadu. In Tamil Nadu, key growing regions include Villupuram, Cuddalore, Thanjavur, Coimbatore, Karur, Kancheepuram, Salem and Erode.

Watermelons are susceptible to several diseases such as anthracnose, *Fusarial* wilt, downy mildew, Gummy

stem blight, root knot nematode and viral diseases. Amongst these diseases, *Fusarial* wilt is a serious disease that attacks the Watermelon crop in continuous cropped soil (Zhou *et al.*, 2004). It is the most important disease of Watermelons throughout the world. It is a soil borne pathogen limiting the Watermelon production and it is very difficult to remove from the soil once it has been introduced (Waseem *et al.*, 2008). *Fusarium* wilt is a destructive soil borne disease caused by the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON) (Zhu *et al.*, 2020). This pathogen survives in soil for longer period as chlamydospores and also has the ability to colonizes on non-host plants (Harveson *et al.*, 2002). *Fusarium oxysporum* f. sp. *niveum* not only cause wilt, also causes damping off, cortical rot and stunting of Watermelon seedlings (Ning ling *et al.*, 2010). *Fusarium oxysporum* f. sp. *niveum* has 4 races like race 0,1,2,3. Race 3 is more virulent than race 2. *Fusarium oxysporum* f. sp. *niveum* race 1 is uniformly present in all growing regions, but race 2 is present in limited areas like Texas, Maryland and Delaware (Zhou *et al.*, 2010).

Generally, yield losses of Watermelon caused by *Fusarium*, varies between 10% to 40%. In severe cases, it will reach up to 80%. The Initial symptoms of *Fusarium* wilt include leaves turning a dull grey green color and wilting during the heat of the day (Egel *et al.*, 2007). Wilting of plants followed by necrosis (Martyn, 2014). In older plants, *Fusarium* wilt is characterized by wilting of individual Watermelon runners and the hallmark of *Fusarium* wilt is unilateral stem necrosis, which is easily visualized, when runners or stems are sectioned (Kleczewski *et al.*, 2011).

## Materials and Methods

### Survey for occurrence of *Fusarium* wilt of Watermelon in the major growing areas of Tamil Nadu

The field survey was conducted to assess the percent disease incidence (PDI) of Watermelon Wilt across various districts in Tamil Nadu during 2019. A total of fifteen locations from five different districts were selected for the survey. At each site, a 2 m × 2 m plot was designated, and the number of infected plants exhibiting disease symptoms was counted and recorded. Additionally, the total number of plants within each plot was determined. The percent disease incidence was calculated using the formula outlined by Mayee and Datar (1986).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Additionally, completely wilted plants were collected for pathogen isolation, along with rhizosphere soil to isolate antagonistic organisms. Information regarding the soil type in which the crop was grown and the variety of watermelon cultivated was also recorded in the respective survey fields

### Isolation and identification of *Fusarium oxysporum* f. sp. *niveum* pathogen

The infected root and stem portions were washed in tap water and tissues exhibiting vascular brown discoloration were cut into small pieces. The pathogen was isolated from the diseased watermelon tissues using the tissue segment method (Rangaswami, 1958). The infected plant portions were cut into small pieces, surface sterilized with 0.1% sodium hypochlorite for one minute, and then washed in three changes of sterile distilled water to remove any traces of sodium hypochlorite. The sterilized tissues were placed on previously poured and solidified Petri dishes containing Potato Dextrose Agar (PDA) medium. These plates were incubated at room temperature (28±2°C) for five to seven days and observed for fungal growth. Pure cultures of the pathogen were obtained using the hyphal tip method and transferred aseptically to PDA slants to maintain the pure culture. The pathogen *Fon* was identified according to the descriptions of Subramanyan (1970) and Booth (1971). A total of ten isolates were maintained, designated as Fon1 to Fon10.

### Cultural and morphological characteristics of *Fusarium oxysporum* f. sp. *niveum* (Naveenkumar *et al.*, 2017)

From seven-day-old culture plates, a 9 mm culture disc of the pathogen was aseptically cut using a sterilized cork borer and placed at the centre of each sterile Petri dish containing 15 ml of previously sterilized and solidified PDA medium. The growth and morphological characteristics of the isolates, including colony morphology, mycelial growth, colony color, conidia size, shape and septation were observed and measured under a Labomedtrinoocular microscope (magnification 400X, 40X × 10X). The number of conidia produced in both solid and liquid media by the pathogen in response to culture filtrates of fungal and bacterial antagonists was calculated using a haemocytometer (Sharma, 1996). Spores were collected from the solid and liquid media by flooding the culture with sterile saline containing 0.01% (v/v) Tween (BDH) and dislodging spores from the hyphae with a sterile glass spreader. The resulting solution was filtered through four consecutive sterile absorbent cotton wool plugs to remove any hyphal fragments. The

number of spores was counted using a haemocytometer, diluted to  $10^6$  spores/ml as a stock solution and stored at 4°C until use

The cell concentration is calculated as follows:

Cell concentration (in cells/ml) = average count  $\times$  2  $\times$  10,000  $\times$  dilution factor of original cells

### Evaluation of virulence and pathogenicity of *Fusarium oxysporum* f. sp. *niveum* under pot culture condition

Uniform-sized earthen pots with a 30 cm diameter were filled with 5 kg of garden soil. The soil was sterilized in an autoclave at 15 lbs pressure (1-4 kgs/cm<sup>2</sup>) for two consecutive days and then inoculated by mixing freshly prepared *Fusarium* inoculum (grown on sand-maize medium) at a rate of 50g/kg of soil (Muthusamy, 1972). Two watermelon seeds were sown in each pot, with three replications. The pots were maintained and observed for the development of disease symptoms. The percent disease incidence of each isolate was recorded 25 days after inoculation (Naveenkumar *et al.*, 2017).

### Scanning electron microscopy

An actively growing fungal culture was fixed overnight at 28°C in 0.05M phosphate buffer containing 4% glutaraldehyde. The following day, the fungal mat was washed three times with phosphate buffer, and the

sample was dehydrated using ethanol for 15 minutes. The fixed and dehydrated samples were then dried with CO<sub>2</sub> for 5 minutes, fixed onto aluminum stubs, and sputter-coated with carbon using a Polaron E-500 sputter coater. The samples were immediately observed under a scanning electron microscope at 15 kV. This work was carried out in the Department of Physics, Annamalai University.

## Results and Discussion

### Survey on the incidence of *Fusarium* wilt of Watermelon incited by *Fusarium oxysporum* f. sp. *niveum* in major Watermelon growing areas of Tamil Nadu

The data presented in Table 1 from the survey of various major watermelon-growing areas in Tamil Nadu highlight the endemic nature of *Fusarium* wilt in watermelon. Among the surveyed locations, Siruvadi village in Villupuram district recorded the highest disease incidence at 41.24%, followed by Elupatti in Thanjavur district with 38.17%, Marakkanam in Villupuram district with 34.79% and the lowest incidence of 5.24% at Pattanam in Villupuram district. A total of 15 locations were surveyed, with 10 locations exhibiting a considerable percentage of disease incidence, while the remaining five locations showed minimal disease incidence, particularly in sandy soil conditions. From these 15 locations, 10

**Table 1:** Survey on the incidence of *Fusarium* wilt of Watermelon incited by *Fusarium oxysporum* f. sp. *niveum* in major Watermelon growing areas of Tamilnadu.

S. no.	Village	District	Soil ty pe	Variety	Stage of the crop	Disease incidence (%)*
1.	Siruvadi	Villupuram	Sandy loam	SW 2208	Seedling	41.24 <sup>a</sup> (39.95)
2.	Marakkanam		Clay loam	NS 295	Seedling	34.79 <sup>c</sup> (36.14)
3.	Pattanam		Sandy loam	Sweety	Flowering	5.24 <sup>o</sup> (13.23)
4.	Sivapuri	Cuddalore	Sandy loam	Maharaja	Flowering	12.18 <sup>l</sup> (20.42)
5.	Ramapuram		Clay loam	Vikram	Seedling	31.83 <sup>d</sup> (34.34)
6.	Bhuvanagiri	Cuddalore	Sandy loam	Spic	Flowering	6.92 <sup>m</sup> (15.25)
7.	Mondipatti	Trichy	Clay loam	Dragon king	Seedling	27.18 <sup>e</sup> (31.42)
8.	Vellakal		Red sandy	NOVA46	Flowering	9.29 <sup>j</sup> (17.74)
9.	Vannankovil		Sandy loam	Sweety	Flowering	8.56 <sup>k</sup> (17.01)
10.	R.T. Malai	Karur	Red sandy	Apoorva	Flowering	14.42 <sup>b</sup> (22.31)
11.	Pathiripatti		Sandy loam	NS 295	Seedling	22.35 <sup>f</sup> (28.21)
12.	Kalladai		Sandy loam	Maharaja	Flowering	6.03 <sup>n</sup> (14.21)
13.	Elupatti	Thanjavur	Sandy loam	Maharaja	Seedling	38.17 <sup>b</sup> (38.15)
14.	Marungulam		Sandy loam	Dragon king	Flowering	17.53 <sup>g</sup> (24.75)
15.	Eachangkottai		Sandy loam	Senthura	Flowering	7.41 <sup>l</sup> (15.79)

\*Mean of three Replication.





**Plate 1 :** *Fusarium* wilt infected plant.



**Plate 2 :** Damping off symptom.



**Plate 3 :** Vascular brown dis-colouration in stem portion of the infected plant.

isolates were selected for further studies.

From Table 1, it was observed that sandy loam soil exhibited the highest disease incidence, followed by clay loam and red sandy soil types. Additionally, the Sweety variety of watermelon showed a lower disease incidence, despite being grown in sandy loam soil, suggesting that it is a more tolerant variety compared to others. Regarding

the crop's growth stage, it was noted that pathogen infection was highest at the seedling stage. However, at crop maturity (i.e., during flowering), the *Fusarium* sp. infection was lower compared to the younger stages. Similar findings were reported by Naveenkumar *et al.* (2017), who conducted a survey in 9 districts across 15 locations in Tamil Nadu, where wilt incidence varied from 8.00% to 40.21%. Jagrajsingh *et al.* (2018) also confirmed that sandy soils were more conducive to watermelon and tomato wilt incidence.

#### **Isolation and Cultural characteristics of various isolates of *Fusarium oxysporum* f. sp. *niveum* from major Watermelon growing areas of Tamil Nadu**

**Colony characters :** The isolates of *Fusarium* species exhibited variation in colony characteristics. The color of the isolates ranged from velvety white to cottony white and pinkish. Most isolates produced fluffy to moderately fluffy cottony white aerial mycelium (Table 2). Nathu *et al.* (2017) noted that the colony color of pathogenic *Fusarium* species varied from purplish white to cottony white.

**Mycelial growth :** Among the 10 *Fusarium* isolates, the maximum mycelial growth (90.00 mm) was recorded by the isolate Fon5, which was obtained from Siruvadi village in Villupuram district. The minimum mycelial growth (74.73 mm) was observed in isolate Fon6, which was isolated from Vellakal village in Trichy District (Table 2).

**Conidial population :** All the *Fusarium* species isolates varied in their ability to produce micro- and macro-conidia on Potato Dextrose Agar medium. The isolate Fon5 produced the highest conidial population of  $2.9 \times 10^6$  conidia/ml, while the lowest conidial population of  $0.6 \times 10^6$  conidia/ml was recorded by isolate Fon6, which was isolated from Vellakal village in Trichy District (Table 2).

#### **Mycelial dry weight and conidial characters of different isolates of *Fusarium oxysporum* f. sp. *niveum***

Among the 10 *Fusarium* isolates, the highest mycelial dry weight (237.19 mg) was recorded by isolate Fon5. This same isolate also exhibited the maximum length and width of microconidia, macroconidia, and chlamydospores, measuring  $11.51 \times 4.23 \mu\text{m}$ ,  $44.17 \times 5.16 \mu\text{m}$  and 7.70–7.86  $\mu\text{m}$ , respectively. The lowest mycelial dry weight (150.47 mg) was observed in isolate Fon6 (Table 3). It was noted that there was no positive correlation between mycelial dry weight and conidial production or their size. Sandra *et al.* (2020) reported that microconidia varied in shape from oval to kidney-shaped with 0-1 septa, while

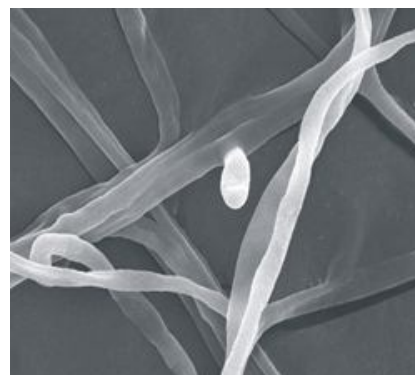
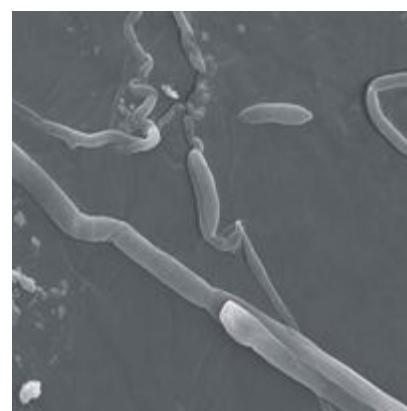
**Table 2 :** Isolation and Cultural characteristics of various isolates of *Fusarium oxysporum* f. sp. *niveum* collected from major Watermelon growing areas of Tamil Nadu.

S. no.	Isolates	Locality	Cultural characteristics	Mycelial growth (mm)*	Micro/macro conidia population/ml ( $\times 10^6$ )*
1.	Fon <sub>1</sub>	Sivapuri	Velvety white	75.97 <sup>e</sup>	0.8 <sup>i</sup>
2.	Fon <sub>2</sub>	Marakkanam	Creamy white	85.27 <sup>b</sup>	2.1 <sup>c</sup>
3.	Fon <sub>3</sub>	Mondipatti	Pale yellow with white	80.74 <sup>c</sup>	1.6 <sup>e</sup>
4.	Fon <sub>4</sub>	R.T.Malai	Velvety white	76.58 <sup>de</sup>	1.9 <sup>h</sup>
5.	Fon <sub>5</sub>	Siruvadi	Cottony white	90.00 <sup>a</sup>	2.9 <sup>a</sup>
6.	Fon <sub>6</sub>	Vellakal	Pinkish white	74.73 <sup>ef</sup>	0.6 <sup>j</sup>
7.	Fon <sub>7</sub>	Elupatti	Cottony white	88.96 <sup>ab</sup>	2.5 <sup>b</sup>
8.	Fon <sub>8</sub>	Pathiripatti	Pinkish white	79.06 <sup>cd</sup>	1.4 <sup>f</sup>
9.	Fon <sub>9</sub>	Ramapuram	Pale yellow with white	83.19 <sup>bc</sup>	1.0 <sup>d</sup>
10.	Fon <sub>10</sub>	Marungulam	Cottony white	77.92 <sup>d</sup>	1.1 <sup>g</sup>

\*Mean of Three Replications.

**Plate 4 :** Native isolates of *Fusarium oxysporum* f.sp. *niveum* collected from the different locations of Tamil Nadu.**Plate 5 :** Axenic culture of *Fusarium oxysporum* f.sp. *niveum*.

macroconidia were curved or sickle-shaped with 3-5 septa. Microconidia ranged in size from 6.14 to 9.75  $\mu\text{m}$ , and macroconidia ranged from 18.7 to 48.9  $\mu\text{m}$  in *Fusarium oxysporum* f. sp. *lycopersici*. These findings are consistent with the present study. Additionally, Sonakar *et al.* (2013) reported that chlamydospores, both smooth and rough-walled, were abundant and formed terminally or intercalary, with a diameter ranging from 7.5 to 8.4

**Plate 6 :** SEM view of Fon Micro conidia.**Plate 7 :** SEM view of Fon Macro conidia.

$\mu\text{m}$  (Sandra *et al.*, 2020).

Pathogenicity study for the *Fusarium oxysporum* f. sp. *niveum* showed that the symptoms were expressed after 25 days of inoculation in Watermelon variety Maharaja. All the isolates produced significant symptoms under pot culture studies and per cent disease incidence (PDI) ranging from 41.63 to 80.36 percent was noticed. Among the different isolates of Fon collected from

**Table 3 :** Mycelial dry weight and conidial characteristics of different isolates of *Fusarium oxysporum* f. sp. *niveum*.

S. no.	Isolates	Mycelial dry weight(mg)*	Micro conidia			Macro conidia			Chlamydospore size (µm)
			Size (µm)	Shape	Septation	Size (µm)	Shape	Septation	
1.	Fon <sub>1</sub>	165.22 <sup>g</sup>	6.91 × 2.72	Oval	1	17.74 × 3.26	Sickle	5	6.47- 6.57
2.	Fon <sub>2</sub>	225.28 <sup>b</sup>	10.04 × 3.67	Kidney	0	36.97 × 4.93	Sickle	6	7.20- 7.42
3.	Fon <sub>3</sub>	211.12 <sup>cd</sup>	9.29 × 3.09	Oval	1	29.69 × 4.61	Sickle	5	6.68 - 7.03
4.	Fon <sub>4</sub>	183.43 <sup>f</sup>	7.84 × 2.74	Fusiform	1	20.13 × 3.65	Sickle	4	6.59- 6.74
5.	Fon <sub>5</sub>	237.19 <sup>a</sup>	11.51 × 4.23	Oval	1	44.17 × 5.16	Sickle	7	7.70- 7.86
6.	Fon <sub>6</sub>	150.47 <sup>h</sup>	6.08 × 2.65	Kidney	0	15.24 × 2.97	Sickle	3	6.19- 6.43
7.	Fon <sub>7</sub>	230.72 <sup>ab</sup>	10.62 × 3.98	Oval	0	40.25 × 5.02	Sickle	5	7.50- 7.71
8.	Fon <sub>8</sub>	203.06 <sup>de</sup>	8.81 × 2.93	Kidney	1	25.71 × 4.35	Sickle	4	6.84- 6.98
9.	Fon <sub>9</sub>	217.85 <sup>bc</sup>	9.76 × 3.37	Oval	1	32.43 × 4.79	Sickle	5	6.97- 7.18
10.	Fon <sub>10</sub>	192.84 <sup>ef</sup>	8.49 × 2.81	Fusiform	1	22.66 × 4.17	Sickle	5	6.71 - 6.82

\*Mean of Three Replications

\*Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

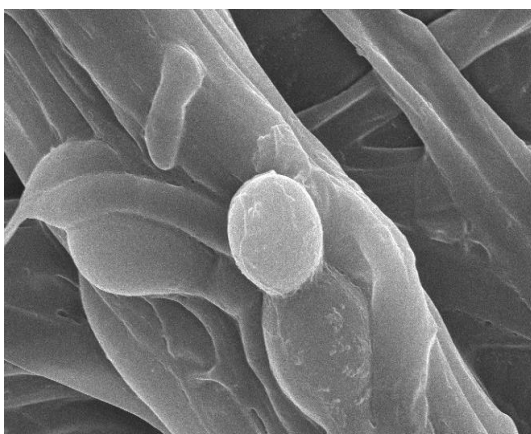
**Table 4 :** Evaluation of virulence and pathogenicity of different isolates of *Fusarium oxysporum* f. sp. *niveum*.

S. no.	Isolate	Symptom expression at DAS	Virulence Type	% Disease incidence*
1	Fon <sub>1</sub>	48	+	43.36 <sup>gh</sup> (41.18)
2	Fon <sub>2</sub>	30	+++	73.63 <sup>bc</sup> (59.10)
3	Fon <sub>3</sub>	39	++	66.39 <sup>d</sup> (54.56)
4	Fon <sub>4</sub>	52	++	47.33 <sup>fg</sup> (43.46)
5	Fon <sub>5</sub>	25	+++	80.36 <sup>a</sup> (63.69)
6	Fon <sub>6</sub>	60	+	41.63 <sup>h</sup> (40.18)
7	Fon <sub>7</sub>	28	+++	77.63 <sup>ab</sup> (61.77)
8	Fon <sub>8</sub>	56	++	55.69 <sup>e</sup> (48.26)
9	Fon <sub>9</sub>	35	+++	71.66 <sup>c</sup> (57.83)
10	Fon <sub>10</sub>	45	++	51.33 <sup>f</sup> (45.76)

\*Mean of Three Replications

\*Values in the column followed by same letters not differ significantly by DMRT(P=0.05)

+ Less virulent; ++ Moderately virulent; +++ Highly virulent

**DAS:** days after sowing.**Plate 8 :** SEM view of Fon chlamydospores.

Watermelon growing areas of Tamil Nadu, the isolate (Fon<sub>2</sub>) collected from Siruvadi village in Villupuram district was found to be more virulent with the maximum disease percent of 80.36 per cent followed by 77.63 per cent collected from Elupatti village in Thanjavur district in the decreasing order of merit. The isolate Fon<sub>6</sub> collected from Vellakal village in Trichy District was the least virulent (41.63%) with minimum *Fusarium* wilt incidence (Table 4). This table showed that the virulent strains caused infection at earlier stage which reflected on higher disease incidence percentage.

Zhou and Evert (2004) proved the pathogenicity of Fon in Watermelon in the university of Delaware. Ali *et al.* (2002) said that pathogen virulence Might be differed from place to place with the change of temperature, rainfall and humidity. Genotype of the plant determined the disease resistance or susceptibility of a particular plant.

## Conclusion

This study provides insights into the pathogen's biology, revealing that it thrives in sandy soils and is particularly virulent during the seedling stage of crops. While, *Fusarium* wilt affects watermelons worldwide, the pathogen's virulence varies depending on the macro- and microclimates of different regions. Effective disease management relies on maintaining the pathogen's virulence below the economic threshold. Since the pathogen is soil-borne and produces resting conidia to survive, it underscores the importance of enhancing soil biological activity. One effective approach is to enrich the soil with organic manures to promote a healthier soil ecosystem.



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