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Assessment on the Incidence of Fusarium Wilt of Marigold Caused by *Fusarium oxysporum* f. sp. Callistephi (FOC) in Major Marigold Growing Areas of Dindugul District, India

Jeyaprabha J [a*], Mudhalvan S [a] and Sathish Kumar M [a]

[a] Department of Agriculture, Kalasalingam School of Agriculture and Horticulture, Srivilliputhur, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Marigold (*Tagetes erecta* L.), one of the commercially exploited ornamental flower crops belonging to the family Asteraceae is naturalized in tropical and subtropical regions worldwide.It is affected by several fungal, bacterial and viral diseases. Among these Fusarium wilt caused by the fungus Fusarium *oxysporum f*.sp. callistephi causes 30-40% yield loss. A survey was conducted to investigate the incidence and severity of Fusarium wilt incited by *Fusarium oxysporum* f.sp. callistephi in ten major marigold growing areas of Dindugul district. The occurrence of wilt disease incidence ranged from 20 % to 49% was noticed. Plant showing typical symptoms were taken from 10 fields and identified based on symptom appearance as well as morphological characteristics. The result of the survey revealed that wide range of infection and severity of wilt disease were occurred in the major marigold growing areas in Dindugul district. Foc3 recorded the maximum wilt

**Corresponding author: E-mail: j.jeyaprabha@klu.ac.in;*

incidence followed by Foc7 and the minimum wilt incidence was recorded by Foc8. The pathogenicity of the fungal pathogen was also proved after artificial inoculation of the marigold seedlings.

Keywords: Survey; marigold; fusarium wilt; Fusarium oxysporum f.sp; callistephi.

1. INTRODUCTION

"Marigold (*Tagetes erecta* L.), a popular ornamental flower crop from the Asteraceae family, is widely naturalized in tropical and subtropical regions around the globe. It is cultivated as an annual herbaceous border plant. Originating from Mexico, marigold is broadly categorized into two groups: African marigold (*Tagetes erecta* L.) and French marigold (*Tagetes patula* L.). Its characteristics, including prolific flowering, quick production of marketable flowers, diverse colors, shapes, sizes, and good shelf life, have garnered attention from flower growers. In India, marigold is commercially grown in Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Gujarat, and Madhya Pradesh" [1]. "The total area under marigold cultivation in India is 28,825 hectares, with a production of 2.0 lakh tonnes. Karnataka leads in cultivation with an area of 6,725 hectares and an annual production of 64,025 tonnes, followed by Tamil Nadu. Tamil Nadu's total flower production is 31,500 tonnes, with a productivity of 11.9 tonnes per hectare. Marigold cultivation in Tamil Nadu primarily occurs in Erode, Dindigul, Madurai, Theni, Trichy, and Thanjavur districts" [2]. The prevalent diseases affecting marigold include Cercospora leaf spot caused by Cercospora [3], Septoria leaf spot caused by Septoria tageticola [4], Alternaria leaf spot caused by Alternaria tagetis [5], Fusarium wilt caused by Fusarium oxysporum f.sp. callistephi, and bacterial leaf spot caused by Pseudomonas tagetis (Hellmers, 1955). "The initial symptoms manifest within 48 hours after the pathogens' entry. Infected plants exhibit yellowing of leaves, followed by leaf shedding on one or both sides of the shoot" (Mui-Yun, [6]. "The fungus obstructs xylem vessels by invading vascular tissues, leading to reduced water movement and severe wilting. Characteristic symptoms include lengthwise brown streaks or vascular discoloration, especially noticeable upon cutting open the infected stem" [7]. "Light vein clearing of young leaves followed by epinasty of old leaves has been reported in infected plants" [8,9]. "Other key symptoms include yellowing of lower leaves, browning of vascular tissues, plant wilting, stunting, and eventual death. In humid conditions, a white or pink fungal growth may be observed in the stem" [9]. "Advanced stages may present browning of the vascular system, xylem transport blockage, water movement disruption, and severe wilting" [10]. "Leaf yellowing may initially occur on one side of the plant and gradually progress to affect most leaves, leading to wilting" [11].

2. MATERIALS AND METHODS

2.1 Disease Survey

A survey was conducted to evaluate the incidence of Fusarium wilt in marigold within the Dindigul district of Tamil Nadu. The wilt incidence was assessed by randomly selecting 100 plants from each field, and the number of infected plants was counted. The mean wilt incidence was expressed as a percentage. The percent disease incidence was calculated using the formula described by Mayee and Datar [12]. Samples of wilted plants were collected from these areas for further analysis.

> Per cent disease incidence = Number of infected plants / Total number of plants observed * 100

2.1.1 Isolation and Identification of pathogen

The wilt-causing pathogen in marigold was isolated from the samples using the tissue segment method. Infected tissue segments underwent surface sterilization with 0.1% mercuric chloride, followed by rinsing with three changes of sterile water. These surface-sterilized tissues were then plated on potato dextrose agar in sterile Petri plates and incubated at room temperature (28˚C) for seven days. The fungus was subsequently purified through single spore isolation and maintained on PDA [13]. Identification of the pathogen was based on colony characteristics, conidial production, and spore morphology.

2.1.2 Morphological and cultural characters of *Fusarium oxysporum* f.sp. *callistephi*

"Ten isolates of Fusarium spp. were collected and analyzed for variations in morphological and cultural characteristics on solid medium. Each isolate's ten-day-old culture was individually inoculated and incubated at 28 ± 2°C for seven days. Following the incubation period, various parameters such as fungal radial growth, micro and macro conidia population, colony characteristics, sporulation, and the size of micro, macroconidia, and chlamydospores were measured". These characteristics were compared to the descriptions provided by Booth [14].

2.2 Pathogenicity

2.2.1 Pathogenicity *In vitro*

A five-mm culture disc of Fusarium oxysporum f. sp. callistephi was positioned near the collar region of 20-day-old healthy marigold seedlings, which were rinsed prior to the experiment. These seedlings were placed in 150-mm-diameter Petri dishes over a layer of moist filter paper. As a control, a five-mm disc of potato dextrose agar (PDA) without the fungus was used. Three replications were conducted, and the Petri dishes were then incubated at room temperature (28 ± 2°C). The seedlings were regularly monitored for symptom expression.

2.2.2 Pathogenicity in glasshouse

The pathogenicity of the fungus was confirmed using Koch's postulates. Earthen pots with a diameter of 30 cm were filled with five kg of pot mixture consisting of red soil, sand, and farmyard manure (in a ratio of 1:1:1 w/w/w). This pot mixture was sterilized at 1.4 kg/cm^2 pressure for two hours on two successive days. Subsequently, each pot was inoculated by mixing 10 g of the fungus inoculum, which was multiplied on sand maize medium. Twenty-day-old marigold seedlings were then planted in the inoculated pots along with appropriate controls. The pots were placed in a glasshouse and maintained with uniform and judicious watering. The plants were regularly observed for the development of disease symptoms.

3. RESULTS AND DISCUSSION

An extensive survey was conducted in major marigold growing areas of Dindugul district across different locations. The age of the crops ranged from three to six months, with six-month-old plants exhibiting the highest disease incidence of Fusarium wilt, ranging from 23% to 67% (Table 1). Among the surveyed locations in Dindugul district, Nilakkottai recorded the highest incidence of Fusarium wilt (67.90%), followed by Ammapatti (60.93%), Silukkuvarpatti (51.55%), and the lowest incidence (23.75%) was recorded in Pallapatti. Similar to this study, Jayanta et al. (2018) conducted a survey in four districts of North Eastern Karnataka, where wilt incidence ranged from 8.33% to 38.66%, attributed to specific varieties. Fusarium oxysporum f. sp. callistephi was isolated from diseased marigold samples on fresh PDA plates. The isolates showed variations in morphological features. Among the ten isolates studied for their cultural characters, three isolates exhibited fast growth. The colony colors varied from white to shades of yellow, and the density of isolates varied from dense to sparse (Table 2). Rajendran et al. [15] reported that "the pathogen produced different colony colors, including light yellow, dark brown, pink, dark pink, creamy white, and pale white with pink, with mycelial growth patterns showing adherent smooth and fluffy growths". "All isolates of Fusarium oxysporum f. sp. callistephi varied in their ability to produce micro and macroconidia on PDA. For instance, the isolate F. oxysporum f. sp. callistephi (Foc3) produced the maximum conidia population of 2.6 × 10^6/ml, while the minimum conidial population of 0.5 × 10^6/ml was produced by the isolate Foc8 from Pallapatti (Table 2). In this study, isolates produced micro and macroconidia with populations ranging from 0.5 × 10^6 to 2.7 × 10^6 conidia/ml" [16]. The minimum and maximum lengths and widths of micro and macroconidia were also recorded. Additionally, the isolates varied in their mycelial dry weight production, with the minimum weight (120.10 mg) produced by the isolate Foc8 (Table 3). Furthermore, the isolates produced microconidia with one septation and macroconidia with an average of 3-4 septations. Different isolates showed varying degrees of variation in parameters such as size of microconidia, macroconidia, and chlamydospores. Previous studies have shown that the aerial mycelium of F. oxysporum generally appears white, subsequently changing to colors ranging from grey to violet and dark purple depending on the strain or special form (Smith et al., [17]. In F. oxysporum f. sp. lycopersici, macroconidia varied in size, with most being short with three septa, and they produced a large number of unicellular, elliptical

microconidia [18]. *In vitro* and in vivo pathogenicity tests produced symptoms similar to those observed in the field. Ahamad (2007) observed similar results in carnation Fusarium wilt. The data revealed varied levels of pathogenicity among isolates (Tables 4 and 5). Among the ten isolates collected from different marigold growing areas of Dindugul district, the isolate Foc3 from Nilakkottai was found to be the most virulent, with a maximum incidence of 69.23%, followed by Foc7 (65.52%) from Ammapatti. The isolate Foc8 from Pallapatti was the least virulent, recording a minimum Fusarium wilt disease incidence of 29.49%. Similarly, Rajendran et al. [15] noted that F. oxysporum f. sp. lycopersici isolates produced significant symptoms from 47 days after transplanting, with wilt incidence ranging from 48% to 100% among isolates. Among the ten isolates of F. oxysporum f. sp. callistephi, isolate Foc3 exhibited fast growth and was used for in vitro pathogenicity tests. The results of pathogenicity tests using 20-day-old seedlings are presented in Table 5. Symptoms were not observed until the sixth day after inoculation, with lesion length increasing over time. Seedlings inoculated with the fungus on pinpricked areas exhibited longer lesion lengths compared to those without pinpricks. For instance, at seven days after inoculation, lesion length was 80.0 mm in pinpricked seedlings compared to 74.0 mm in seedlings without pinpricks. Similar studies have been supported by Houssien et al. [19,20,21].

Table 1. Survey on the incidence of *Fusarium* wilt of marigolg caused by *Fusarium oxysporum* f.sp. *callistephi* (Foc) in major marigold growing areas of Dindugul district

S.No	Isolate	Location	Variety	Age of the crop	Disease Percentage (%)*
1	Foc1	Chatrapatti	Local	6	43.30^{f}(41.37)
2	Foc2	T.Vadugapatti	Local	5	33.48^{h}(35.29)
3	Foc3	Nilakkottai	MDU1	6	67.90^{a}(55.49)
4	Foc4	Kodairoad	MDU1	5	50.51^{d}(45.28)
5	Foc5	Gandhigram	Local	4	47.27^{e}(43.39)
6	Foc6	Silukkuvarpatti	MDU1	4	51.11^{c}(48.85)
7	Foc7	Ammapatti	MDU1	5	60.93^{b}(51.29)
8	Foc8	Pallapatti	Local	6	23.75^{j}(29.13)
9	Foc9	S.Vadipatti	Local	4	30.93^{i}(33.71)
10	Foc10	Salaiputhur	Local	5	41.00^{g}(39.81)

Mean of three publications

** In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)*

Table 2. Isolation and cultural characteristics of various isolates of *Fusarium oxysporum* f. sp. c*allistephi* (Foc)

S.No	Isolate	Location	Colony type	Colour of the culture medium	Mycelial Growth (mm)	Conidial population/ml (×10^{6})
1	Foc1	Chatrapatti	Compact	Light brown	77.20^{f}	1.6
2	Foc2	T.Vadugapatti	Sparse	Dark brown	73.00^{h}	1.0
3	Foc3	Nilakkottai	Fluffy	Light brown	89.00^{a}	2.6
4	Foc4	Kodairoad	Compact	Light yellow	83.12^{d}	2.1
5	Foc5	Gandhigram	Fluffy	Dark brown	79.87^{e}	1.9
6	Foc6	Silukkuvarpatti	Sparse	Light brown	85.23^{c}	2.1
7	Foc7	Ammapatti	Fluffy	Light yellow	86.76^{b}	2.4
8	Foc8	Pallapatti	Compact	Pink	70.56^{j}	0.5
9	Foc9	S.Vadipatti	Fluffy	Dark brown	72.87^{i}	0.8
10	Foc10	Salaiputhur	Compact	Light brown	74.35^{g}	1.3

Mean of three publications

** In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)*

Table 3. Mycelial dry weight and conidial characters of different isolates of *Fusarium oxysporum* f. sp. *callistephi* (Foc)

S.No	Isolate	Mycelial dry weight (mm)	Macro conidia		Micro conidia	
			Septation	Size (µm)	Septation	Size (µm)
1	Foc1	242.6^{e}	2-3	23.49×3.16	0	7.33×2.25
2	Foc2	244.0^{d}	2-3	22.48×3.05	0	7.81×2.33
3	Foc3	287.3^{a}	3-4	27.35×3.55	0	8.21×2.55
4	Foc4	182.0^{f}	2-3	24.43×3.21	0	7.23×2.31
5	Foc5	170.6^{g}	2-3	23.79×2.45	0	7.94×2.58
6	Foc6	260.3^{b}	3-4	25.82×2.45	0	7.61×2.31
7	Foc7	265.6^{c}	3-4	25.82×2.91	0	8.15×2.52
8	Foc8	120.1^{j}	2-3	20.41×2.12	0	7.18×2.31
9	Foc9	140.4^{h}	2-3	22.18×3.52	0	7.81×2.12
10	Foc10	132.6^{i}	2-3	22.35×2.61	0	7.79×2.53

Mean of three publications

** In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)*

Table 4. Effect of *Fusarium oxysporum f.sp. callistephi* on the incidence of marigold Fusarium wilt (Pot Culture)

S.No	Isolates	Diseases incidence(%)
1	Foc1	47.54^{f}(45.24)
2	Foc2	36.71^{h}(35.29)
3	Foc3	69.23^{a}(55.04)
4	Foc4	51.23^{d}(44.55)
5	Foc5	49.49^{e}(45.52
6	Foc6	56.52^{c}(49.87)
7	Foc7	65.52^{b}(52.46)
8	Foc8	29.49^{j}(32.89)
9	Foc9	33.25^{i}(31.12)
10	Foc10	45.49^{g}(40.55)

Mean of three publications

** In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)*

Table 5. Pathogenicity of *F.o.*f.sp.*callistephi* on marigold seedlings *In vitro*

Days after incubation	Lesion length(mm)			
	Control		Inoculated	
	Pin prick	Without pinprick	Pin prick	Without pinprick
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	35.0	28.0
8	-	-	52.0	41.0
9	-	-	71.0	63.0
10	-	-	80.0	74.0

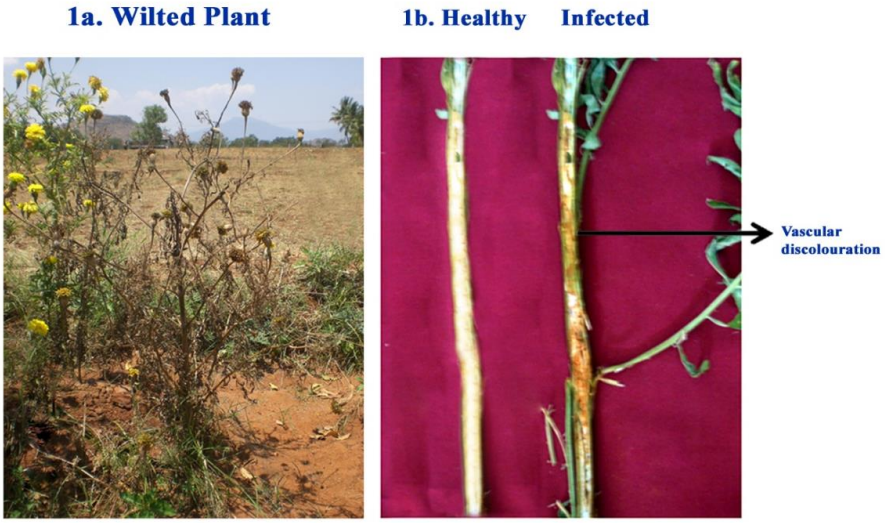

Plate 1. Symptoms of fusarium wilt

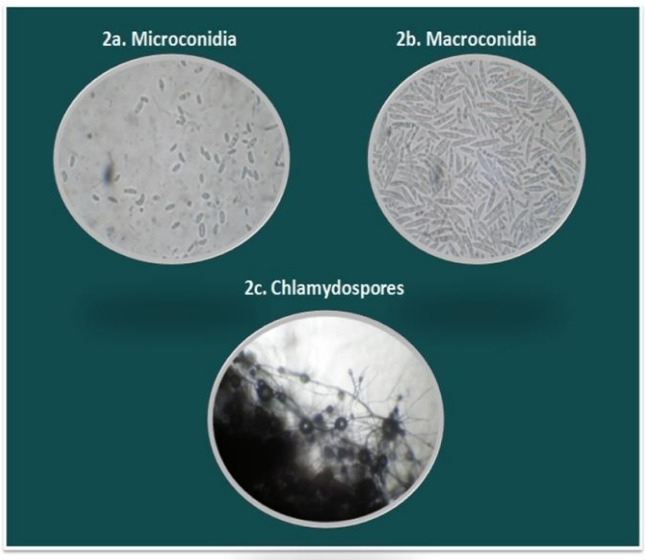

Plate 2. Spores of *Fusarium oxysporum* f sp *callistephi*

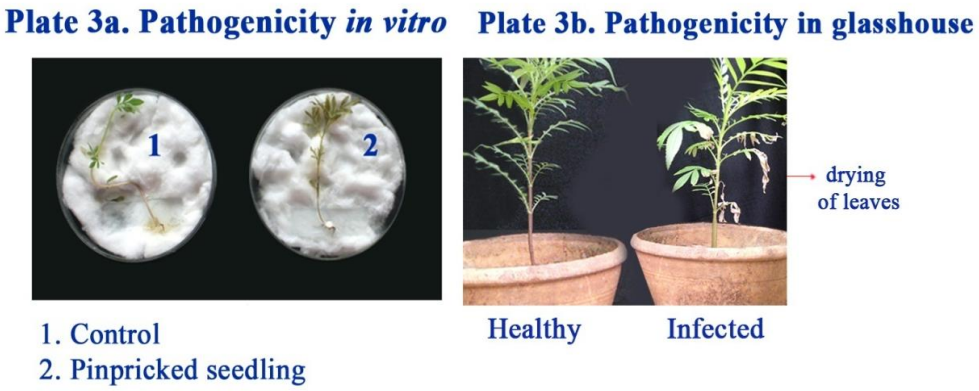

Plate 3. Pathogenecity of *Fusarium oxysporum* f sp *callistephi*

4. CONCLUSION

It is concluded that wide range of infection and severity of wilt disease were occurred in the major marigold growing areas in Dindugul district. Foc3 recorded the maximum wilt incidence followed by Foc7 and the minimum wilt incidence was recorded by Foc8. The pathogenicity of the fungal pathogen was also proved after artificial inoculation of the marigold seedlings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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