Original Research Article

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

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 byseveral 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*inten majormarigold 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 morphologicalcharacteristics. 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 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.*

Introduction

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 grown as an annual herbaceous border. Marigold which is broadly divided into two groups *viz*., African marigold (*Tagetes erecta*L.) and French marigold (*Tagetes patula*L.) owe their origin to Mexico. Its habit of free flowering, short duration to produce marketable flowers, wide spectrum of attractive colours, shape, size and good keeping quality has attracted the attention of

flower growers. In India marigold is grown as a commercial crop in Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Gujarat and Madhya Pradesh (Bhattachaarjee*et al*., 1994). The area under marigold cultivation in India is 28,825 ha with a production of 2.0 lakh tonnes . Karnataka ranks first occupying an area of 6,725 ha with an annual production of 64,025 tonnes followed by Tamil Nadu. Total flower production in Tamil Nadu is 31,500 tonnes with a productivity of 11.9 tonnes/ha. In the State, marigold is grown in Erode, Dindigul, Madurai, Theni, Trichy and Thanjavur districts (Anonymus, 2011).

The important diseases of marigold are Cercospora leaf spot caused by *Cercosporatageticola*(Ellis and Everh, 1902), Septoria leaf spot caused by *Septoria tageticola*(Changsri and Weber, 1958), Alternaria leaf spot caused by *Alternaria tagetis* (Hotchkiss and Baxter, 1983), Fusarium wilt caused by *Fusarium oxysporum*f.sp.*callistephi*, and bactreial leaf spot caused by *Pseudomonas tagetis*(Hellmers,1955).

The earliest symptoms appearwith in 48 h after the entry of the pathogens. In the infectedplants the leaves becomes yellow followed by dropping ofleaves which occurs may be on one side of the plant or onboth the sides of shoot (Mui-Yun, 2003b).The fungus blocks the xylem vessels by invading thevascular tissues and reduces the movement of water andcauses severe wilting. A lengthwise brown streaks orvascular discolouration may be seen when the infected stemis cut open. This is the characteristic symptom and used forthe identification of disease (Mui-Yun, 2003a). Thisdiscolouration often extends far up the stem and is especially noticeable in a petiole scar. Sally *et al*. (2006) reported that the light vein clearing of young leaves followed by epinasty of old leaves appear in infected plants. The main symptoms of the disease include yellowing of lower leaves, browning of vascular tissues, wilting of plant, stunting and eventually death. A white or pink colour fungal growth may be noticed in the stem especially in the wet conditions (Ajigbola and Babalola, 2013). Browning of the vascular system, blocking xylem transport and movement of water and severe wilting could be seen in advance stage (Decal *et al*. 2000).Leaf yellowing can occur on one side of the plant and gradually most leaves turn yellow and wilt (Anil Kumar *et al*. 2015).

Material and Methods

Disease Survey

A survey was conducted to assess the incidence of Fusarium wilt of marigold in Dindigul districts of Tamil Nadu. The incidence of marigold wilt randomly100 plants were selected from each field and the numbers of infected plants were counted and the mean wilt incidence was expressed in percentage. The percent disease incidence was calculated by using the formula (Mayeeand Datar1986) . Samples of wilted plants were collected from these areas.

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Isolation and Identification of pathogen

The pathogen inciting wilt in marigold was isolated from the samples by tissue segment method. The infected tissue pieces were surface sterilized with 0.1% mercuric chloride and rinsed with three changes of sterile water. The surface sterilized tissues were plated on potato dextrose agar in sterile Petri plates and incubated at room temperature (28˚C) for seven days. The fungus was further purified by single spore isolation and maintained on PDA(Rangaswami, 1972). The pathogen was identified based on colony character, conidial production and spore morphology.

Morphological andcultural characters of *Fusariumoxysporum*f.sp. *callistephi*

Ten isolates of *Fusarium* spp.obtained were comparedfor variation in respect of morphological and culturalcharacters on solid medium. Ten days old culture of eachisolate was separately inoculated andincubated at28 ± 2°Cfor seven days. After the incubation period, fungal radialgrowth, micro &macro conidia population, colony characters,sporulation and size of micro, macroconidia andchlamydospores were measured. The characters werecompared with those described byBooth(1971).

Pathogenicity

Pathogenicity *in vitro*

Afive- mm culture disc of *F.o* f. sp. *callistephi*was placed closer to the collar region of 20-day-old healthy, rinsed marigold seedling and kept in 150- mm-dia Petri dish over a layer of moist filter paper. A five-mm disc of PDA without the fungus served as control. Three

replications were maintained and the plates were incubated at room temperature (28 ± 2^0C). The seedlings were monitored for symptom expression.

Pathogenicity in glasshouse

The pathogenicity of the fungus was confirmed by Koch's postulates. Earthen pots of 30-cm-dia were filled with five kg of pot mixture (redsoil : sand : FYM at 1:1:1 w/w/w) was sterilized at 1.4 kg cm^{-2} for two h on two successive days and inoculated by mixing 10 g inoculum of the fungus and multiplied on sand maize medium. Twenty-day-old, marigold seedlings were planted in pots with proper control. The pots were maintained in glasshouse by uniform and judicious watering. The plants were observed for development of disease symptom.

Result and Discussion

An extensive survey conducted in major marigoldgrowing areas of Dinduguldistrict in different locations. The age of the crop varied from three to six months and the six-month old plants showed maximum disease incidence, the endemic nature of thedisease with *Fusarium* wilt incidence ranging from 23 to 67 % (Table 1).Among the different locations of Dinduguldistrict surveyed for marigold*Fusarium* wilt incidence, Nilakkottai recorded the maximum incidence of the disease (67.90 %)followed by Ammapatti(60.93%), Silukkuvarpatti(51.55%) and the minimum *Fusarium* wilt incidence of(23.75%) was recorded in Pallapatti. Similarto the present study Jayanta*etal*. (2018) conducted a survey infour districts of North Eastern Karnataka and the wiltincidence was noticed in all locations surveyed with arange of8.33to 38.66 percent attributed by specific variety.*Fusarium oxysporum*f.sp. *callistephi*was isolated from the diseased samples of marigold plants in fresh PDA plates. The isolates of *F. oxysporum*f.sp. *callistephi*showedvariation with respect to phenotypic characters. In the present study, the isolates of*F.o.*f.sp.*callistephi*varied in morphological features. Of the ten isolates studied for their cultural characters, three isolates were fast in growth. The colony colour of the isolates varied from white to shades of yellow. The isolates were either dense or sparse (Table 2). Rajendran *etal*. (2018) reported that the pathogen produced differentcolony colors *viz*., Lightyellow,dark brown, Pink, Dark pink, Creamywhite, pale white with pink and the mycelial growth patternshowed two different pattern namely adherent smooth andfluffy growths.All the *F.*

*oxysporum*f.sp. *callistepfi*isolates varied intheir ability to produce micro and macro conidia on PDA.The isolate *F. oxysporum*f.sp. *callistephi*(Foc3) producedthe maximum conidia population of 2.6/ ml ($\times 10^6$). Theminimum conidial population of 0.5/ml ($\times 10^6$) was producedby the isolate Foc8 isolated from Pallapatti(Table 2). In the present studies the isolates produced micro andmacro conidia with populations ranging from 0.5 x 10^6 to2.7x 10^6 conidia ml^{-1}. The minimum length and width ofmicro and macro conidia observed was 20.41×2.12µm,7.18×2.31µm respectively. The same isolate Foc3 recordedthe maximum length and width of micro and macro conidia with 27.35×3.55µm and 8.21×2.55µmrespectively. The minimum mycelial dryweight (120.10mg) was produced by the isolate Foc8 (Table 3).The isolates produced the micro conidiawith 1 septation and macro conidia producedwith an average 3-4 septations. The different isolates showedsmaller to high degree of variation within differentparameters like size of microconidia, Macroconidia andChlamydospores. In past studies, the aerial mycelium of *F.oxysporum* generally appears white subsequently changing in colour ranging from grey to violet and dark purple depending on the strain or special form (Smith *et al*., 1988). In *F.o.*f.sp.*lycopersici,* macroconidia varied in size and most were short with three septa. They produced a large number of unicellular, elliptical, microconidia (Ignjatov*et al*., 2012). In the pathogenicity tests carried *in vitro* as well as *in vivo* symptoms were produced similar to those observed in the field. Similar results have been observed by Ahamad (2007) in carnation Fusarium wilt.The data depicted in table 4and 5 revealed that varied levelsof pathogenicity with difference in isolates. Among the tenisolates of *F. oxysporum*f.sp. *callistepi*collected fromdifferent marigold growing areas of Dinduguldistrict, theisolate (Foc3) collected from Nilakkotaiwas found to bemore virulent and recorded the maximum incidence of 69.23per cen followed by Foc7 (65.52%) collected fromAmmapatti. The isolate Foc8 collected from Pallapattiwasthe least virulent which recorded the minimum (29.49%)*Fusarium* wilt disease incidence. Similarly Rajendran *et al*.(2018) mentioned that the *F. oxysporum*f.sp. *lycopersici*isolates produced significant symptoms from 47 days aftertransplanting. The percent wilt incidence ranged from 48 to100 between the isolates. Among ten isolates of *F.o.*f .sp.*callistephi,* the isolate Foc3 was fast in growth and this isolate was used for pathogenicity *in vitro*.Theresults on pathogenicity of *F.o.*f. sp.*callistephi*using 20-day-old seedling are furnished in (Table5). Till sixth day, symptoms were not noticed in seedlings inoculated with or without pinprick. Symptoms developed 7 days after inoculation (DAI) and the lesion length increased

over time. Lesion length was more when the inoculum of the fungus was placed on the pinpricked seedlings than the seedlings without pinprick. At seven DAI, the lesion length was 80.0 mm in pin pricked seedlings while it was 74.0 mm in seedlings that were not pinpricked.This type of study was supportedby Houssien*et al*. (2010).

Table 1Survey 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.*callistephi* (Foc)

S.No	Isolate	Location	Colony type	Colour of the culture medium	Mycelial Growth (mm)	Conidial population/ml ($\times 10^6$)

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)

Table3 Mycelial dry weight and conidial characters of different isolates of*Fusarium oxysporum*f.sp.c*allistephi* (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 oxysporumf.sp. callistephi* on the incidence of marigold Fusarium wilt (Pot Culture)

S.No	Isolates	Diseases incidence(%)
1	Foc1	47.54[i] (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

Plate1.Symptoms of Fusarium wilt

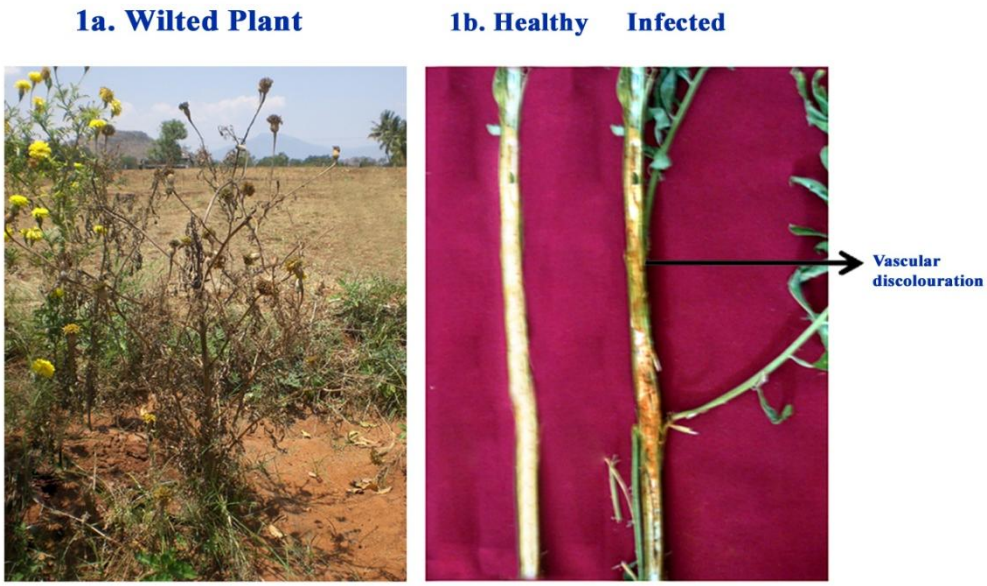

Plate 2.Spores of *Fusarium oxysporum* f sp *callistephi*

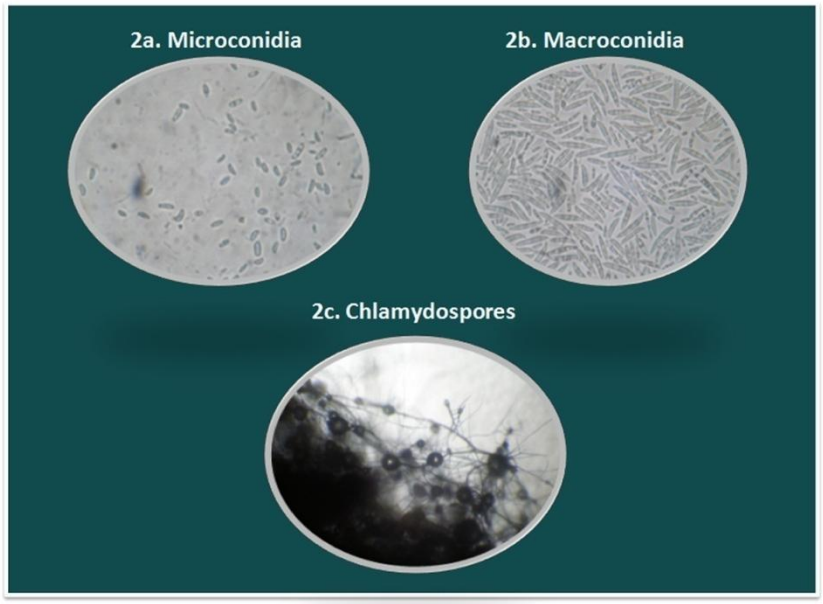

Plate 3.Pathogenecity of *Fusarium oxysporum* fsp*callistephi*

Plate 3a. Pathogenicity *in vitro*

1. Control
2. Pinpricked seedling

Plate 3b. Pathogenicity in glasshouse

Healthy Infected

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