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Morphological and cultural characteristics of *Fusarium oxysporum* f. sp. *dianthi* under laboratory condition

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Abstract
Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers in the world. Many factors may be attributed for the low yield of which, one of the important factors are pests and diseases. Among various diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Dianthi* being very serious disease contributing to yield loss. Keeping these points in view, efforts were made to study morphological and cultural characteristics of *Fusarium oxysporum* f. sp. *dianthi* under laboratorty conditions and the results revealed that, growth characters of *F. oxysporum* f. sp. *dianthi* studied in different solid media indicated that potato dextrose agar and Richards's agar recorded maximum growth of fungal colony. The results of the cultural studies on solid media indicated that the radial growth of *F. oxysporum* f. sp. *dianthi* was maximum on Potato dextrose agar (90mm).

Keywords: morphology, cultural, fusarium and laboratory

Introduction
Carnation (*Dianthus caryophyllus* L.) Or clove pink, is a species of *Dianthus*. It is probably native to the Mediterranean region but its exact range is unknown due to extensive cultivation for the last 2,000 years. It is a herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long. The flowers are produced singly or up to five together in a cyme; they are 3–5 cm diameter, and sweetly scented; the original natural flower colour is bright pinkish-purple, but cultivars of other colours, including red, white, yellow and green, have been developed. Carnation is preferred for export owing to its excellent keeping quality, wide range of forms, colours and ability to withstand long distance transportation. Cut carnations, roses and chrysanthemums contribute close to 50% of the world cut flower trade (Jawaharlal *et al*., 2009 [3].
In India annual production of carnation is 6 MT and 45 per cent production of carnation is from Himachal Pradesh (2.75 MT) followed by Uttarakand 1.25 MT. In Karnataka, production of carnation is 0.69 MT (Anon., 2015) [1]. The average yield level per hectare in Karnataka is very low; many factors may be attributed for the low yields, of which one of the important factors may be pests and diseases. Green house condition favours the luxuriant growth of carnation plants. A number of fungal diseases, such as, Fusarium wilt (*Fusarium oxysporum* f.sp. *dianthi*), bud rot (*Rhizoctonia solani*), rust (*Uromyces dianthi*), Grey mould (*Botrytis cinerea*), Stem and root rot (*Phytophthora* spp.), fairy ring spot (*Heterosporous mediolanum*). Fusarium wilt (*Fusarium oxysporum* f. sp. *dianthi*) is one of the major constraint worldwide in carnation cultivation. Keeping these points in view, efforts were made to study morphological and cultural characteristics of *Fusarium oxysporum* f. sp. *dianthi* under laboratorty conditions.

Material and Methods
Spore size and shape of *Fusarium oxysporum* f. sp *dianthi* were recorded by taking culture and mounting on a clean glass slide. Spores were mixed with lactophenol thoroughly in order to obtain a uniform spread, on which cover slip was placed. One hundred spores were measured under high power objective using Motic Images 2.0 Software. The average size of the spore was calculated. Microphotographs were taken to show the typical spore morphology of the pathogen
The cultural characters were studied on five non synthetic/semi-synthetic and two synthetic solid and liquid media. Non synthetic or semi synthetic media: Potato dextrose agar, Host leaf extract agar, Sabouraud's dextrose agar, Carrot agar, Corn meal agar, V8 juice agar and Oat meal agar. Synthetic media: Richard's agar and Czapek Dox agar All the media were sterilized

at 1.1 kg/cm^2 pressure for 15 min. To carryout the study, 20 ml of each of the medium was poured in 90 mm Petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1^{0}C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete petriplate in any one of the media. The colony diameter was recorded. The fungus colony colour and margin were also recorded. The data on radial growth was analyzed statistically. The liquid media used were same as that of solid media. The composition and preparations of different liquid media used were the same as that of solid media except that the agar-agar was not added. Hundred ml of the medium was added to each of 250 ml flask. All the flasks were sterilized at 1.1 kg /cm^2 pressure for 15 min. Inoculum disc of five mm size was inoculated to all flasks and incubated at 27±1^{0}C for 10 days. Each treatment was replicated thrice. The mycelial mat was harvested; dried in hot air oven at 60-65^{0}C and dry mycelial weight was recorded.

Results

Standard tissue isolation technique was followed to get culture of causal organism from the wilted plants, showing the symptoms. *Fusarium* was isolated consistently from carnation, which was showing wilting symptoms. Repeated subculturing was done to obtain pure culture on basal medium PDA. Hyphal tip method was used to obtain pure culture as detailed in material and methods. The pathogen was identified as based on their morphological and cultural characters as *F. oxysporum* f. sp. *dianthi* by reffering to Booth (1971) [2] and by its pathogenicity on carnation. Pathogenicity was proved by following Koch postulates.

Morphological studies: The spores of the pathogen were obtained from pure culture and temporary slide mounts were prepared in Lactophenol. Then they were observed under high power (45x) magnification. One hundred spores of pathogen were observed under microscope and measured using Motic Images 2.0 Software. The morphological characters of *Fusarium oxysporum* f. sp. *dianthi* are depicted in (Table.1, Plate1, 2 and 3). Microconidia were abundant, hyaline, continuous, or single, septate, ovoid and ovate and measured 4.0-8.0×2-3.5 µm (Average 6.0×2.75µm) (Plate 1a). Macroconidia were sparse, often lacking, fusoid and variable. 3 – septate, rarely 4-5 septate, measuring 18.5-29.5×3-4.5 µm (Average 24.0×3.75µm) (Plate 1b).Chlamydospores were hyaline, spherical with no septa (one celled), produced either singly or in chain and 3.0 – 8.5 µm in diameter (average µm) (Plate 1c). These characters are inagreement with Booth (1971) and Nelson *et al.* (1983) [4].

Cultural studies: Cultural characters were studied on nine different solid media. The radial growth of *F. oxysporum* f. sp. *dianthi* was measured when the maximum growth was attained in any of the media tested. The results of the cultural studies on solid media indicated that the radial growth of *F. oxysporum* f. sp. *dianthi* was maximum on Potato dextrose agar (90mm) and Richard's agar (89.33mm) both are on par with each other which were significantly superior over all other media. The minimum radial growth was obtained in V8 juice agar (50.33mm) and carrot agar (63.66 mm). Data on the study are presented in the Table 2. Growth characters of *F. oxysporum* f. sp. *dianthi* studied in different solid media indicated that potato dextrose agar and Richards's agar recorded maximum growth of fungal colony.

The growth of the fungus at at margins was irregular in Potato dextrose agar, Richards's agar and Sabouard's dextrose agar. In case of Oat meal agar, Czepeck's Dox agar and host extract agar the margin was smooth. Mycelium was whitish in all the media except in case of potato dextrose agar and carrot agar where mycelium was pink in colour (Plate 2a).In case of liquid media maximum dry mycelial weight of fungus was obtained in Oat meal broth (441.00 mg) which was significantly superior over all the media tested. This was followed by Richard's broth (360.00 mg), Potato dextrose broth (306.66 mg). Least mycelial weight was obtained in V8 juice broth (96.66 mg) (Plate: 3)

Table 1: Morphological characters of *Fusarium oxysporum* f. sp. *Dianthi*

Spore	Measurement L× B (µm)	
	Range	Average
Microconidia	4.0-8.0×2-3.5	6.0×2.75
Macroconidia	18.5-29.5×3-4.50	24.0×3.75
Chlamydospores	3.0-8.5	5.75

Table 2: Effect of different solid media on growthof *F. oxysporum* f. sp. *dianthi*

Sl. No	Media	Radial growth* (mm)	Mycelial character
Non synthetic / semi synthetic media			
1	Potato dextrose agar	90.00	Pinkish white cottony growth with irregular margin
2	Host leaf extract agar	70.33	Sparse white cottony growth
3	Sabouraud's dextrose agar	74.33	Pink pluffy growth with irregular margin
4	Oat meal agar	85.50	Sparse white cottony growth
5	Carrot agar	63.66	Pink cottony growth
6	Corn meal agar	80.66	Sparse white cottony growth
7	V8 Juice agar	50.33	White pluffy growth
Synthetic media			
8	Czapek's Dox agar	84.33	White cottony growth
9	Richard's agar	89.33	White cottony growth with irregular margin
S.Em±			1.21
CD at 1%			3.72

*Mean of three replication

Table 3: Effect of different liquid mediaon growth of *F. oxysporum* f. sp. *dianthi*

Sl.no	Liquid media	Dry mycelial weight* (mg)
Non synthetic / semi synthetic media		
1	Potato dextrose broth	306.66
2	Host leaf extract broth	158.33
3	Sabouraud's dextrose broth	266.33
4	Oat meal broth	441.00
5	Carrot broth	216.00
6	Corn meal broth	239.00
7	V8 Juice broth	96.66
Synthetic media		
8	Richard's broth	360.00
9	Czapek's Dox broth	294.00
S.Em±		4.86
CD at 1%		14.81

*Mean of three replication

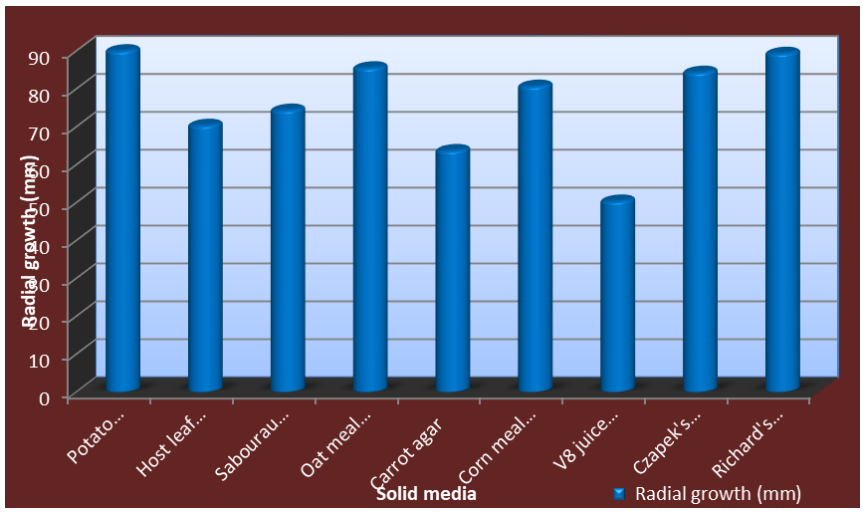

Fig 1: Effect of different solid media on growth of *F. oxysporum* f. sp. *dianthi*

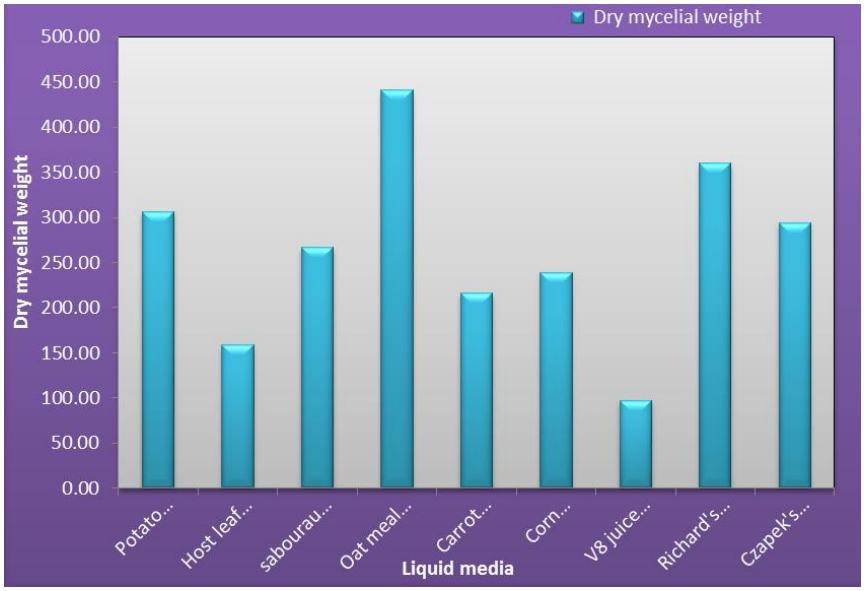

Fig 2: Effect of different liquid media on growth of *F. oxysporum* f. sp. *dianthi*

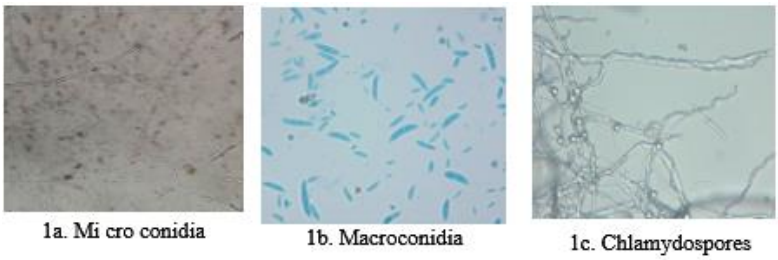

Plate 1: Spores of *Fusarium oxysporum* f. sp. *Dianthi*

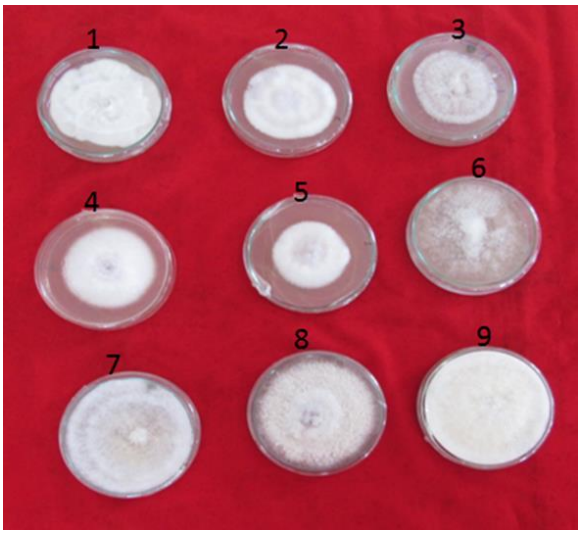

Plate 2: Effect of different solid media on growth of *F. oxysporum* f. sp. *dianthi*

1. Corn meal agar
2. Sabouraud's dextrose agar
3. Host leaf extract agar
4. Carrot agar
5. V8 juice agar
6. Oat meal agar
7. Potato dextrose agar
8. Czapek's Dox agar
9. Richard's agar

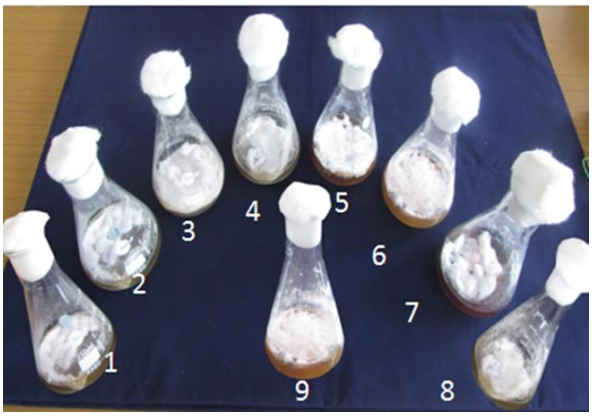

Plate 3: Effect of different liquid media on growth of *F. oxysporum* f. sp. *dianthi*

1. V8 juice broth
2. Host leaf extract broth
3. Oat meal broth
4. Carrot broth
5. Richard's broth
6. Czapek's Dox broth
7. Sabouraud's dextrose broth
8. Corn meal broth
9. Potato dextrose broth

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