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S Nandhini
PG Scholar, Department of Plant Pathology, AC & RI, Madurai, Tamil Nadu, India

M Theradimani
Professor and Head, Department of Plant Pathology AC & RI, Madurai, Tamil Nadu, India

S Harish
Assistant Professor, Centre for Plant Protection Studies, AC & RI, TNAU, Coimbatore, Tamil Nadu, India

C Rajamanickam
Assistant Professor, Department of Horticulture, AC & RI, Madurai, Tamil Nadu, India

Corresponding Author:
S Nandhini
PG Scholar, Department of Plant Pathology, AC & RI, Madurai, Tamil Nadu, India

Effect of different media on the mycelial growth of *Fusarium oxysporum*, the causative agent of bitter gourd wilt

S Nandhini, M Theradimani, S Harish and C Rajamanickam

Abstract
Bitter gourd (*Momordica charantia*) is economically predominant cucurbit crop. It is widely cultivated in all over the world with high nutritive and medicinal value. Cultivation of bitter gourd is often affected by many diseases, *Fusarium* wilt is one of the devastating disease incited by *Fusarium oxysporum*. Survey was carried out for the isolation of ten different *Fusarium* spp. from various localities of Tamil Nadu. The virulent isolate (BGFI2) which proved in pathogenicity test and molecularly identified as *Fusarium oxysporum* was taken to evaluate the mycelial growth on the nine different solid and liquid media. The result of this experiment is the diameter of the pathogen mycelial growth was observed as best in the Czapek's dox agar in both solid and liquid media. Least mycelial growth of solid media was observed in Asthana and Hawker's agar and least mycelial dry weight of liquid media was observed in Malt extract broth.

Keywords: Fusarium wilt, bitter gourd, pathogenicity, solid media and liquid media

Introduction
Bitter gourd (*Momordica charantia* L.) is a commonly consumable vegetable crop in cucurbitaceous family (Win *et.al.*, 2014). This crop has been cultivated all over India during warm season (Satkar *et al*., 2013) [9]. Bitter melon contains more amount of nutritive value than other cucurbits (Desai and Musmade, 1998; Miniraj *et al*., 1993) [3, 5]. Bitter gourd has been susceptible by number of diseases among those, *Fusarium* wilt is an important disease causing huge yield losses (Tamilselvi, 2014). It is a common soil borne pathogen and saprophyte and has the ability to survive in soil for long days (Snyder and Hansen, 1940) [10]. Initial symptoms appear as chlorosis and distortion of the lower leaves that leads to stunting which become more pronounced as the disease progresses. Wilting occurs on the affected side of the plant, followed by vascular discoloration and stem necrosis. The entire plant wilts and dies in severe stage of infection (Bowers and Locke, 2000) [4]. The objective of this study, to evaluate the mycelial growth habits of *Fusarium oxysporum* on different solid and liquid media.

Materials and Methods
Survey and collection of sample
Survey was conducted to collection of wilt pathogen at various locations of bitter gourd cultivating areas of Madurai and Theni districts. In each field, wilted plants were identified based on the symptoms of *Fusarium* wilt. The stem and root portions were cut out from infected plants and taken for isolation of *Fusarium* spp. under laboratory conditions.

Isolation of the pathogen
Totally, ten *Fusarium* spp. were isolated by tissues segment method (Rangaswamy and Mahadevan, 1999) [8]. PDA media was made for pathogen isolation. After three days of incubations pathogen grows actively and covered entire Petri plates at tenth day of inoculation. The growth habit was dissimilar in each isolates. Cultural and morphological characters of all the isolates were identified (Booth, 1971) [1]. The pure culture was obtained by hyphal tip method and subculture was done from that for further studies.

Proving of pathogenicity
All the isolates were taken for proving its virulence by pathogenicity test according to the Koch's Postulates. After three weeks of inoculation, plants showed typical symptom of *Fusarium* wilt and the wilted plants were pulled out and the pathogen was re-isolated to confirmation of Koch postulates.

From pathogenicity test, isolate BGFI2 selected as virulent isolate which express the symptom earlier and molecularly recognized as *Fusarium oxysporum*.

Growth on different solid media

Growth of *Fusarium oxysporum* was evaluated in different solid media. A total of nine media *viz*., Richard's agar, Malt extract agar, Potato dextrose agar, Carrot dextrose agar, Czapek's dox agar, Sabouraud dextrose agar, Nutrient agar, Asthana and Hawker's agar and Rose Bengal agar were used. Media was prepared and sterilized in an autoclave at 121° C for 20 minutes. 15 ml of sterilized medium was poured into sterile Petri plate and allowed to solidify. After solidification, a 9 mm pathogen disc from ten days old culture was placed in the center of plates under *in vitro* condition. For each medium three replications were maintained and incubated under room temperature (28º±2º) for ten days. The radial growth of the mycelium was measured when the mycelium covered the entire Petri plate in any one of the treatments.

Growth on different liquid media

Altogether nine liquid media were prepared in 250 ml conical flask without adding agar *viz.*, Richard's agar, Malt extract agar, Potato dextrose agar, Carrot dextrose agar, Czapek's dox agar, Sabouraud dextrose agar, Nutrient agar, Asthana and Hawker's agar and Rose Bengal agar. The prepared liquid media were taken for sterilization in an autoclave at 121° C for 20 minutes. A nine mm disc of ten days old culture was inoculated in conical flask containing liquid medium. After inoculation, the conical flask was incubated at room temperature (28º±2º C). Totally, three replications were taken for each treatment. The mycelial mat was filtered through a pre weighed Whatman No.1 filter paper in each case and dried until a constant weight was obtained. The mycelial dry weight was obtained separately by subtracting the weight of the filter paper alone from the total weight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) at significant levels (P<0.05) and means were compared by least significant difference.

Result

Effect of different solid media

Totally, the nine different solid media were taken for the experiment. In each media, *Fusarium oxysporum* were inoculated and incubated for ten days. The pathogen was covered entire plates containing Czapek's dox agar media at 7th DAI. Once the growth of the pathogen was reached the entire plates containing Czapek's dox agar media, all the plates were taken for measurements. So, the result of this experiment is the best and fast mycelial growth was observed in Czapek's dox agar media and no growth was noticed in Asthana & hawker's agar media. (Table.1)

Effect of different liquid media

Different liquid media were prepared in the nine numbers and the growth of *Fusarium oxysporum* were evaluated. The dry weight of mycelial mat for each treatment were assessed after seven days of incubation. The highest dry weight of pathogen was observed in Czapek's dox broth and lowest mycelial dry weight was observed in malt extract broth. (Table. 2)

Discussion

The survey was conducted in various locations of Tamil Nadu. The pathogens were isolated from the diseased samples which collected from the infected field of bitter gourd.

Ten different isolates namely, BGFI1, BGFI2, BGFI3, BGFI4, BGFI5, BGFI6, BGFI7, BGFI8, BGFI9 and BGFI10 were obtained and morphologically identified based on their colony type, colony colour, conidial formation *i.e.* macro conidia, micro conidia, chlamydospore. (Pad Wick. 1939 and 1942; Chakravarty and Gupta, 1995) [6, 7, 2]. The isolate BGFI2 was proved as virulent isolate and molecularly recognized as *Fusarium oxysporum.*

In order to find out suitable solid media for the growth of the fungus, the pathogen was grown on nine different solid and liquid media. From the result, it was found that Czapek's dox agar medium supported the best and fast growth of the pathogen in solid media. No growth of fungus was found in Asthana and Hawker's agar media. In the case of different liquid media experiment, the more mycelial dry weight was recorded in Czapek's dox broth when compared to other liquid media and very low dry weight of mycelial growth was obtained in malt extract broth.

This study will facilitate the identification of different growth habits, sporulations, conidial formation and other morphological characters of the pathogen.

Table 1: Effect of different solid media on the mycelial growth of *Fusarium oxysporum*

S. No.	Solid media	Diameter of mycelial growth at 7 DAI (cm)*
1.	Richard's agar media	5.2
2.	Malt extract agar media	5.5
3.	Potato dextrose agar media	7.5
4.	Carrot dextrose agar media	3.9
5.	Czapek' s agar media	9.0
6.	Saboraud's agar media	4.4
7.	Nutrient agar media	3.1
8.	Asthana & hawker's agar media	0.0
9.	Rose bengal agar media	7.0
	CD (P=0.05)	0.37

*Mean of three replications
DAI- Days After Inoculation

Table 2: Effect of different liquid media on the mycelial growth of *Fusarium oxysporum*

S. No.	Liquid media	Mycelial dry weight (g) at 7 DAI*
1.	Richard's broth	0.15
2.	Malt extract broth	0.09
3.	Potato dextrose broth	0.33
4.	Carrot dextrose broth	0.12
5.	Czapek's dox broth	0.40
6.	Saboraud's broth	0.13
7.	Nutrient broth	0.30
8.	Asthana & hawker's broth	0.14
9.	Rose bengal broth	0.20
	CD (P=0.05)	0.026

*Mean of three replications
DAI – Days After Inoculation

References

1. Booth C. The genus Fusarium. (Commonwealth Mycological Institue: Kew 1971.
2. Chakraborty A, Gupta PKJ. Factors affecting chlamydospore formation by *Fusarium udum*, the causal organism of wilt of pigeionpea. J Mycopath Res 1995;33(2):71-75.
3. Desai UT, Musmade AM. Pumpkins, Squashes and Gourds. In: Handbook of Vegetable Science and Technology- Production, Composition, Storage and

Processing (Eds. Salunkhe, D.K. and S.S. Kadam). Marcel Dekker Inc., New York 1998, 273-297.
4. John H. Bowers and James. C. Locke. Effect of botanical extracts on the populations density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the green house. National Arboretum, Floral & Nursery Plants Research Unit Rm 2000, 238.
5. Miniraj N, Prsanna KP, Peter KV. Bitter Gourd Momordica spp. In: Genetic Improvement of Vegetable Plants (Eds. Kalloo, G. and Bergh, B.O.). Pergamon Press, Oxford, UK 1993, 239-246.
6. Padwick GW. The genus *Fusarium* I, own occurrence in India. Indian Agric. Sci 1939, 171-184
7. Padwick GW. Some problems on control of soil borne fungal disease in plants. Anniv. Vol. R. Bot. Gdn. Calcutta 1942, 213-220
8. Rangaswamy G, Mahadevan A. Diseases of crop plants in India. 4th ed. New Delhi, Prentice Hall of India Pvt. Ltd 1999, 607.
9. Satkar KP, Kulthe AA, Chalke PR. Bioscan 2013;8:115-117.
10. Snyder and Hansen. The Species Concept in Fusarium**.** American Journal of Botany 1940, 64-67.