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Studies on Growth of *Fusarium oxysporum* f. sp. *vasinfectum* Isolates causing Okra Wilt on Different Culture Media

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A B S T R A C T

The present study was undertaken to find out the suitability of culture media for growth of twenty five isolates of *Fusarium oxysporum* f. sp. *vasinfectum* collected and isolated from different locations of Karnataka state using Czapek's dox agar (CDA), Potato dextrose agar (PDA), Richards Agar (RA) and Yeast Extract Agar (YEA). Significant variation recorded among twenty five isolates with respect to growth i.e colony diameter on different solid media. Isolates MYS-15 and MYS-16 showed fastest growth of 65.49 mm and 64.38 mm respectively after seven days in all the media tested. Richard's agar found to be the best for the growth of all the isolates tested with mean colony diameter of 61.78 mm. Twenty three isolates were exhibited fast colony growth and only two isolates were exhibited very fast colony growth. In liquid media, the growth of all the isolates was very good in Richard's medium with mean dry mycelial weight of 526.33 mg, whereas Yeast extract liquid media supported poorly for the growth of most of the isolates with mean dry mycelial weight of 321.27 mg. Twenty four isolates were categorized as fast growers with mean dry mycelial weight ranging from 300.1-450 mg and only one isolate (MYS-15) was exhibited very fast colony growth with mean dry mycelial weight of 451.18 mg.

Introduction

Okra (*Abelmoschusesculentus* L. (Moench)), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. *Fusarium oxysporum* f. sp. *vasinfectum* is one of the important pathogen reported to cause destructive disease like wilt.(Sultana *et al*., 1988). *F. oxysporum* is cosmopolitan in distribution and considered as one of the most important soil-borne fungi present in wide range of soils can infect root system and consequently invade the vascular tissues of the plants (Olivain and Alabouvette, 1997). The disease has been responsible for significant yield loss in all okra growing areas (Silva *et al.,* 2007a). Rough estimates indicated that Fusarium wilt causes loss around 10-15 per cent each year as regular feature. In the years of severe epidemics, crop losses will go as high as 60-70 per cent (Jalali and Harichand, 1992).

Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, have great influence on the growth of pathogen. Present work depicts the role of different media to growth of the pathogen which will be helpful in management strategy in the field.

Materials and Methods

Solid medium

The growth of the *F. oxysporum* f. sp. *vasinfectum* were studied on solid media *viz*., Czapek's dox agar, Richard's agar, Potato dextrose agar, and Yeast extract agar. Twenty ml of each medium listed above was poured into 90 mm diameter petriplates. After solidification, 5 mm discs of *F. oxysporum* f. sp. *vasinfectum* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petridish. Each set of experiment was replicated thrice and the plates were incubated at 28±1°C for 7 days. Based on the mean colony growth on different solid medium, isolates were categorized into four groups (Anon. 2006) *viz.*, I- Slow (<30 colony diameter), II-Medium (<30.1-45colony diameter), III-Fast (45.1-60 colony diameter), and IV-Very fast (60mm colony diameter).

Liquid Medium

The cultural characters of 25 isolates were studied on four liquid media viz., Czapek's dox solution, Richard's broth, Potato dextrose broth, Yeast extract broth. 100 ml of all respective broth were poured into 250 ml conical flask and sterilized. Seven day old five mm mycelial disc of the pathogen was inoculated separately into the conical flasks. Each treatment was replicated thrice and incubated at 28 ± 1°C for 7 days. Cultures were filtered through whatman's No.1 filter paper washed thoroughly with distilled water. It was dried at 40^0C for two days in hot air oven and weight was recorded. Based on the mean mycelial weight, isolates were classified into following four groups *viz.*, Slow (<150 mg), Medium (150.1-300 mg), Fast (300.1-450 mg) and Very fast (450.1-600 mg).

Results and Discussion

Growth on solid media

There was a significant variation recorded among twenty five isolates with respect to growth on different solid media and also in terms of colony diameter (mm) as shown in the Fig. 1. Similarly, there was marked difference among the media with each of the isolates. Results on interaction effect between media and the isolates were also found to be significant. It was found that, isolates MYS-15 and MYS-16 showed fastest growth of 65.49 mm and 64.38 mm respectively after seven days in all the media tested. Richard's agar found to be the best for the growth of all the isolates tested with mean colony diameter of 61.78 mm, which was statistically significant over all other media tested (Fig. 1).

On the basis of radial growth isolates were classified. Twenty three isolates were exhibited fast colony growth and only two isolates *viz*., MYS-15 and MYS-16were exhibited very fast colony growth. It was very interesting to note that none of the isolates were recorded under slow and medium colony growth groups (Table 1).

Growth on liquid media

Results in the Fig. 2 indicates that the growth of all the isolates was very good in Richard's medium with mean dry mycelial weight of 526.33 mg, whereas Yeast extract liquid media supported poorly for the growth of most of the isolates with mean dry mycelial weight of 321.27 mg. Among the twenty five

isolates studies, two isolates *viz.*, MYS-15 and MYS-16 recorded maximum dry mycelial weight in Richard's medium with 451.18 mg and 444.54 mg, respectively. Richard's medium found to be the best for the growth of all the isolates tested with mean dry mycelial weight of 526.33 mg, which was statistically significant over all other media tested. The Czapeck's and Potato dextrose liquid media supported the growth of all the 25 *F. oxysporum* f. sp. *vasinfectum* isolates with mean dry mycelial weight of 354.24 mg and 353.29 mg, respectively and both were found at par. However, Yeast extract agar medium recorded poorest growth of all the tested isolates with mean dry mycelial weight of 321.27 mg.

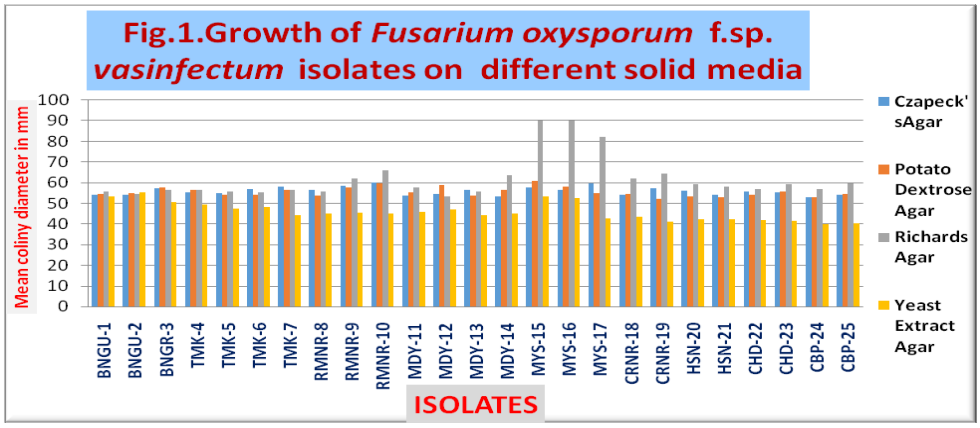
None of the isolates fell under group-I (Slow growers) and group II as shown in the Table 2. Twenty four isolates were categorized under group-III (Fast growers) with mean dry mycelial weight ranging from 300.1-450 mg and only one isolate (MYS-15) was exhibited very fast colony growth with mean dry mycelial weight of 451.18 mg.

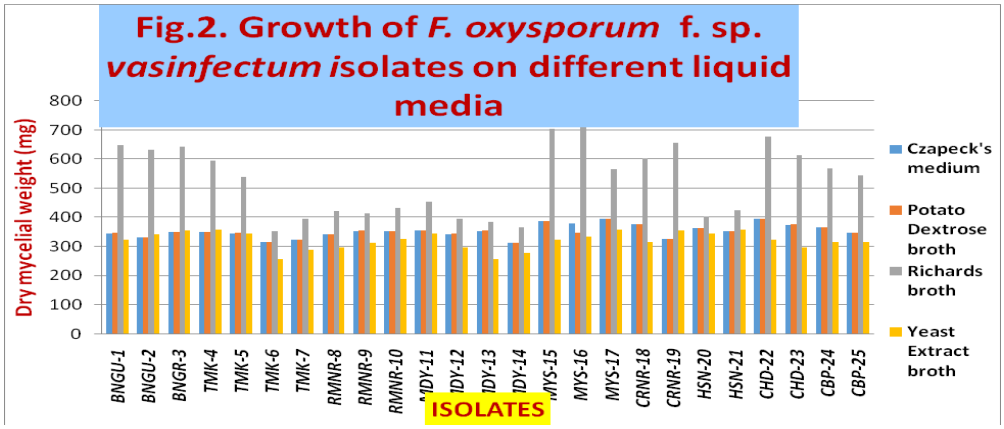
Table.1 Classification of *F. oxysporum* f.sp. *vasinfectum i*solates based on colony growth on different solid media

Group	Growth	Mean colony diameter (mm)	No. of isolates	Name of the isolates
I	Slow	<30	0	-
II	Medium	30.1-45	0	-
III	Fast	45.1-60	23	CRNR-19, HSN-21, CBP-24, TMK-5, TMK-6, RMNR-8, MDY-11, MDY-13, MYS-17, CRNR-18, HSN-20, CHD-22, CBP-25BNGU-1, BNGU-2, TMK-4, TMK-7, MDY-14, CHD-23, BNGR-3, RMNR-9, RMNR-10 and MDY-12
IV	Very fast	>60	02	MYS-15 and MYS-16,

Table.2 Classification of *F. oxysporum* f. sp. *vasinfectum isolates* based on mycelial weight on different Liquid media

Groups	Growth rate	Dry mycelial weight	No. of isolates	Name of the isolates
I-	Slow	<150 mg	-	-
II	Medium	150.1-300 mg	-	-
III	Fast	300.1-450 mg	24	BNGU-1, BNGU-2, BNGU-3, TMK-4, TMK-5, TMK-6, TMK-7, RMNR-8, RMNR-9, RMNR-10, MDY-11, MDY-12, MDY-13, MDY-14, MYS-16,MYS-17, CRNR-18, CRNR-19, HSN-20, HSN-21, CHD-22, CHD-23,CBP-24, CBP-25
IV	Very fast	450.1-600 mg	01	MYS-15





Nutrients are fundamental requirements of microorganisms for growth and development. Nutrient utilization and conversion of these into biomass was studied with respect to all twenty five isolates of *F. oxysporum* f. sp. *vasinfectum*. The isolates exhibited significant variation in respect of their ability to grow on various media which indicated the existence of variability among the isolates. The present study also indicated that Richard's agar was the best source of among four media used for the growth and development of *F. oxysporum* f. sp. *vasinfectum* isolates both in solid (61.78mm mean colony diameter) and liquid media (526.33 mg mean dry mycelial weight). These results were in confirmation with Ingole (1995) who reported that PDA and Richard's agar supported best mycelial growth of *Fusarium udum*. Jamaria (1972) also reported maximum growth and sporulation of *Fusarium oxysporum* f.sp. *vanillae* on Potato dextrose agar, Richard's agar and Czapek's Dox agar. Khilare*et al.*, (1975) reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's agar.These results were also similar with the findings of Major (1923) and Eshwarareddy and Basu choudary (1985), who studied the growth of *F. udum* in different liquid media and found that Richard's medium was the best medium for the growth of the fungus followed by Czapeck's medium. Anjaneya Reddy also observed maximum growth of *F. udum* on Richard's medium. Similarly, Mahesh (2004) and Mahesh (2008) also observed the maximum growth of *F. udum* on Richard's medium.

The isolates were categorized into four groups based on the colony diameter on solid media and mycelial weight in liquid media. These results were confirmed with Eshwarareddy and Basu Choudhary (1985), who grouped six

isolates of *F. udum* into three distinct groups based on radial growth and colony characters. Gupta *et al., (*1988) also grouped seven different strains of *F. udum* on the basis of growth rate.

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