International Journal of Biological Research, 4 (1) (2016) 42-45

International Journal of Biological Research

Website: www.sciencepubco.com/index.php/IJBR
doi: 10.14419/ijbr.v4i1.5901
Research paper

Influence of temperature and different culture media on growth of fusarium udum (Butler), causal organism of wilt of pigeonpea

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Abstract

The conditions suitable for growth of Fusarium udum causing wilt of Pigeon pea were analysed. Pigeon pea seed agar medium and Czapek Dox Agar medium was found to be most suitable growth medium for mycelial growth and sporulation of Fusarium udum. The optimum temperature for growth of Fusarium udum was found to be 28±2°C.

Keywords: *Pigeon Pea Wilt; Fusarium Udum; Temperature; Culture Media.*

1. Introduction

Pigeon pea is an important food crop in India. It is a very demanding and always in need food crop in India. India holds a major contribution of 90% of total world production. India engages an area of 3.85 million hectare with an annual production of 2.68 million tonnes (Anonymous, 2002). The crop is prone to many serious diseases caused by fugal, bacterial viral and nematode infections. High sensitivity of the crop to the attack of insect-pests and diseases appears to be the main reason for such low yields. Pigeonpea is known to be affected by more than hundred pathogens (Nene et al., 1989). However, only a few of them cause economic losses. The diseases of considerable economic importance at present are sterility mosaic (SM), Fusarium wilt, Phytophthora blight (PB), Macrophomina root rot, stem canker and Alternaria blight.

Wilt caused by Fusarium udum Butler is the most important disease caused to this crop (Kannaiyan et al., 1984). Fusarium udum is a soil born pathogen and also the pigeonpea specific wilt pathogen (Kannaiyan et al., 1985). It causes huge loss to the crop as well as economic loss to the farmers. The fungus has an ability to survive in the infected crop debris for 3 – 4 years and cause serious economic loss, sometimes upto 100% in the susceptible cultivars (Kiprop et al., 2002). The loss caused by Fusarium udum in India alone is US $ 71 million (Reddy et al. 1993). The economic loss caused by Fusarium wilt in Pigeon pea is 470,000 tonnes in India (Joshi et al., 2001). Besides these, the members of the Fusarium have been reported to produce type A and B trichothecene mycotoxins that cause toxicosis in humans and animals (Mali et al., 2015).

2. Material and methods

During present investigation, infected plant parts from field were collected and brought to the laboratory. After collection isolation of pathogen was made. To isolate the causal agent, tissue samples were excised from symptomatic leaves, sterilized by sequential dipping in 70% ethanol for 30 sec. and in 0.1% $HgCl_2$ for 1 min., rinsed in sterilized distilled water, and then cultured on Czapek Dox agar (CDA) amended with 25 mg/litre of streptomycin sulphate to avoid bacterial contamination (Patil et al., 2012). Plates were incubated at 25 ± 2°C for 6 days. A Fusarium sp. was consistently isolated from infected tissues, and was purified by single-spore culture (Mali et al, 2015)*****. The fungus was grown on CDA and cultures were maintained at 4°C for further studies. To evaluate the effect of temperature on the radial mycelia growth of pathogen, the cultures plates having 8 mm diameter mycelial disc of Fusarium udum were incubated at various temperature 0, 5, 10, 15, 20, 25, 30, 35, 35, 40 °C.

Different media were selected for the assessment of the growth of the Fusarium udum viz. Pigeon pea root Agar, Pigeon pea stem Agar, Pigeon pea leaf Agar, Pigeon pea seed Agar, Potato Dextrose Agar, Czapek Dox Agar, Sabouraud Agar, Corn meal Agar, Malt Extract Agar. Composition of growth media were listed in (Table 1. a and Table 1. b). The culture media were prepared (Ronald, 2006) by the extract obtained from plant parts of Pigeon pea. In order to investigate the effect of media composition on the growth of pathogen, different media as stated above were tested. Colony diameter was recorded by measuring the radial growth of the mycelium in mm after eight days of incubation at 28 ± 2 °C and number of spores /cm^3 were counted by using Haemocytometer. All the experiments were carried out in triplicates.

Table 1: (A) Composition of Different Fungal Media Containing Host Plant Tissue.

Nutrient Media			
Pigeon pea seed agar	Pigeon pea leaf agar	Pigeon pea stem agar	Pigeon pea root agar
Pigeon pea seed 200 g	Pigeon pea leaf 200 g	Pigeon pea stem 200 g	Pigeon pea root 200 g
Dextrose 20 g	Dextrose 20 g	Dextrose 20 g	Dextrose 20 g
Agar 20 g	Agar 20 g	Agar 20 g	Agar 20 g
Distilled Water 1000 ml	Distilled Water 1000 ml	Distilled Water 1000 ml	Distilled Water 1000 ml

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Table 1: (B) Czapek Dox Agar (CDA) Media Composition

Constituents	Concentration (g L^{-1})
NaNO3	2.00
KH2PO4	2.00
MgSO4. 7H2O	0.50
KCl	0.50
FeSO4. 7H2O	0.01
Sucrose	30.00
Agar- Agar	20.00
Distilled water	1000 ml

3. Result and discussion

In current investigation, the pathogen was exposed to different ranges of temperature (Table no.2). The optimum temperature for the growth and spore production of Fusarium udum in vitro was within range (25-30°C) (Table no. 2). In the current experiment, it was found that, mycelial growth was reduced at temperature below 15 °C and above 35 °C (Figure. 1). Among the different media tested, maximum growth of Fusarium udum was obtained on culture medium containing host plant tissue such as Pigeon pea seed agar followed by Pigeon pea leaf agar, Pigeon pea stem agar, Pigeon pea root agar as compared to Czapek Dox agar medium (Figure. 2). Effect of different culture media on radial growth of Fusarium udum at 28±2 °C for 8 days of incubation is shown in Fig no. 3 and Fig. 4 and graphically represented in Fig no. 5 and Fig no. 6.

Table 2: Mycelial Diameter (Cm) and Number of Spores/ Cm^3 of Fusarium Udum Cultured on Czapek Dox Agar at Various Temperature.

Sr. no.	Temperature °C	Radial mycelial growth (cm)	Spores number / cm^3
1	0	00	0
2	5	0.5	0
3	10	1	8
4	15	2.7	58
5	20	5	122
6	25	6.6	154
7	30	8.9	210
8	35	2.1	34
9	40	00	0

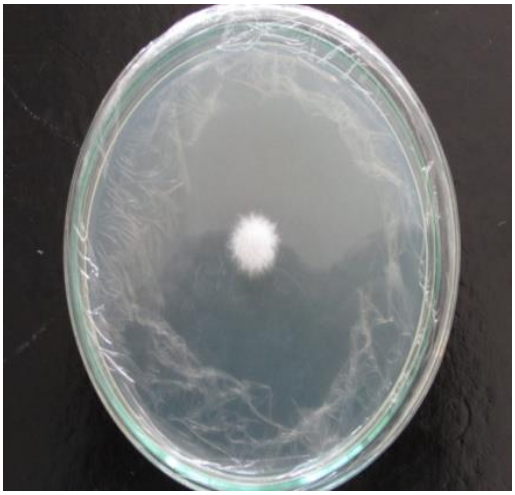

Fig .3: Radial Mycelial Growth at 10± 2°C.



Fig. 4: Radial Mycelial Growth at 28± 2°C.

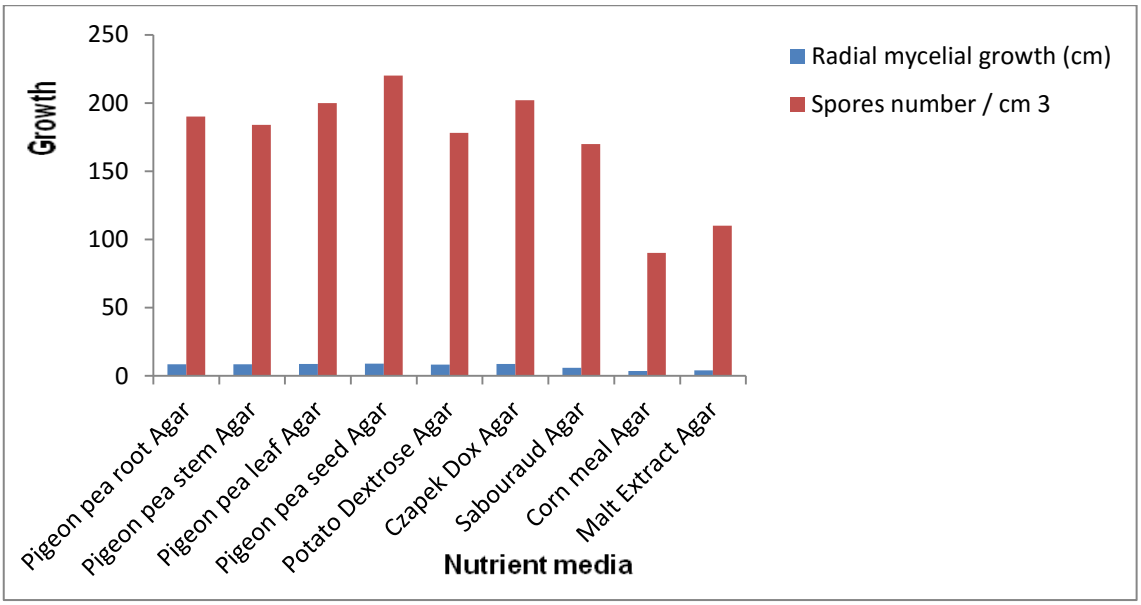

Fig. 5: Effect of Different Culture Media on Radial Growth of Fusarium Udum at 28±2 °C for 8 Days of Incubation.

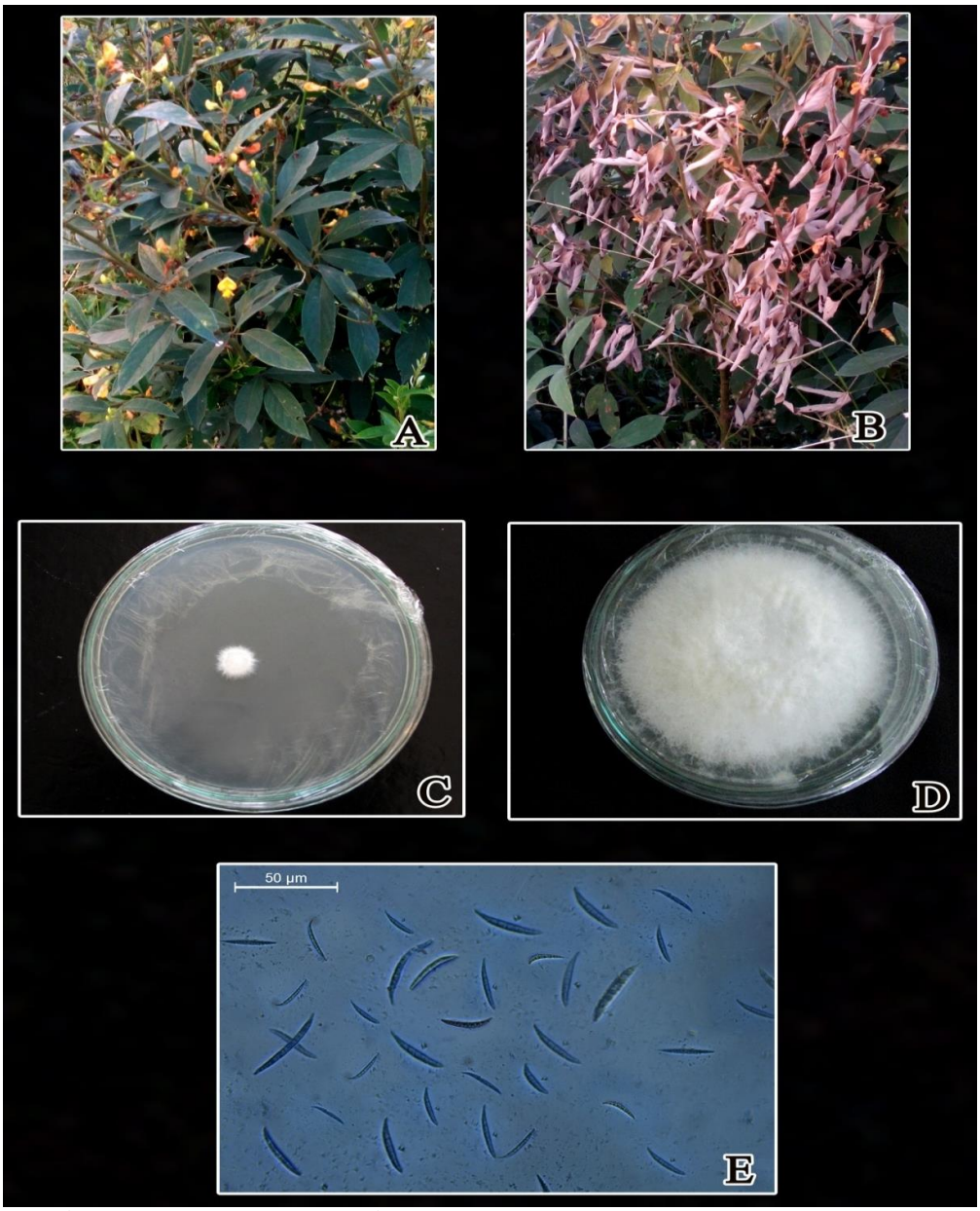

Fig. 6: A-Healthy Plant of Pigeon Pea, B Sympomatic Plant of Pigeon Pea, C - Radial Mycelial Growth at 10± 2°C, D - Radial Mycelial Growth at 28± 2°C, and E – Spores of Fusarium Udum.

4. Conclusion

After this investigation it is concluded that Pigeon pea seed agar and Czapek Dox Agar medium are an ideal medium for linear radial growth of Fusarium udum and 28 °C is the optimum temperature.

5. Acknowledgement

We are grateful to the Professor and Head, Department of Botany, Shivaji University, Kolhapur for providing the necessary research facilities.

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