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In vitro Studies on Physiology of Fungi Isolated from Stem pieces of *Cuscuta* in Pakistan

Iqbal M.Z., A.S. Shakir and S.T. Sahi
Department of Plant Pathology, University of Agriculture, Faisalabad-38040, Pakistan

Abstract

In vitro studies on physiology of four fungi namely *Alternaria alternata*, *Colltotrichum gloeosporioides*, *Curvularia lunata* and *Fusarium pallidoroseum* isolated from *Cuscuta* stem cuttings were conducted. All the fungi gave the best mycelial growth on universal medium. Out of these fungi *A. alternata* and *F. pallidoroseum* gave the best growth at 25°C and *C. gloeosporioides* gave the best mycelia! growth at 20°C while *C. gloeosporioides* required pH 6 for better mycelia! growth. 24 hours continuous light favoured the growth of *A. alternata* and *C. glpeosporioides* while *C. lunata* and *F. pallidoroseum* gave better results under 12 hours light and 12 hours darkness.

Introduction

Species of *Cuscuta* are widely distributed throughout the Pakistan which parasitize a variety of hosts, belonging to different botanical families. Citrus plantation specially khatti (*Citrus limon*) and hedges (*Clerodendrum inerme*) are severely infested by *Cuscuta* (Hafiz, 1986). In Pakistan, no attention has been paid to control this severe phanerogamia parasite which is urgent need of time.

Malik (1992) found that universal medium was proved to be the best medium for many fungi. Tariq *et al*. (1993) conducted an experiment on physiological studies of *Botrytis gladiolorum* and concluded that this fungus grew best on PEA, at 25°C, pH 7 and in continuous darkness.

Ehsan-ul-Haq *et al*. (1998) found universal medium to be the best for mycelial growth of *Botrytis cinerea*, *Curvularia lunata*, *Fusarium oxysporum* f. sp. *gladioli* and *Stemphylium botryosum* 25°C temperature was found better for all the fungi except *Curvularia lunata* which grew best at 30°C. A pH of 6 favoured the growth of *B. cinerea* and *S. botryosum* gave good mycelial growth under 24 hours continuous light while mycelial growth of other two fungi was observed under 12 hours alternate cycle of light and darkness.

Recently four fungi namely *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Curvularia lunata* and *Fusarium pallidoroseum* have been reported from *Cuscuta* stem (Shakir *et al*., 1999) but their biology is yet uninvestigated. A very little work has been done on biology of fungi associated with *Cuscuta*, therefore it was planned to study the effect of different media, temperature, light regimes and pH levels on growth of fungi associated with *Cuscuta* stem.

Materials and Methods

Pure cultures of *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Curvularia lunata* and *Fusarium pallidorseum* isolated from stem cuttings of *Cuscuta*, were collected from culture collection, Department of Plant Pathology, University of Agriculture, Faisalabad (Pakistan) to carried out physiological studies. Mycelial growth of fungus was observed on universal medium (Malt-extract = 30 gm, soya peptone = 3 gm, Agar = 15 gm, Distilled water = 1000 ml) Potato Dextrose Agar (Potato starch = 20 gm, Dextrose = 20 gm, Agar Agar = 20 gm, Distilled water = 1000 ml, corn meal agar (Corn meal = 17 gm, Agar Agar = 5 gm, Distilled water = 1000 ml) and on water Agar with *Cuscuta* stem were boiled in 500 ml of water for 30 minutes. Agar was dissolved in another 500 ml of water and both the solutions were mixed to make the volume of water one liter. The above mentioned four media were prepared separately and autoclaved for 15 minutes at 15 lbs pressure and 121°C temperature. Chloramphenicol was used as an antibacterial at the 0.05 g/liter of medium. About 20 ml of each of the sterilized medium was poured aseptically into each petri plate (9 cm). On solidification four plates of each medium were inoculated with 5 mm culture discs of each fungus in the centre, cut with a sterilized cork borer (5 mm) aseptically from fresh cultures of fungi namely *A. aftemata*, *C. gloeosporioides*, *C. lunata* and *F. pallidoroseum*. Inoculated petri plates were incubated at 25°C. Experiment was run in quadruplicate following factorial arrangement. To determine the most suitable temperature for the growth of above mentioned fungi, autoclaved universal medium was used, as it proved to be the best among different media tested for fungal growth. Petri plates thus inoculated with 5 mm discs of actively growing cultures of four fungi were incubated at 15, 20, 25, 30 and 35°C. Data regarding colony diameter of each fungus were recorded on eighth day of inoculation.

Mycelial growth of the fungi was also studied on pH levels of 5, 6, 7, 8, 9 and 10 pH, which were adjusted by the addition of N/10 HCl or (1N) NaOH solution. Medium for respective pH was autoclaved and adjusted again for its pH and poured aseptically in petri plates. Each petri plate was inoculated with fresh culture discs (5 mm) of different fungi in the centre and then placed at 25°C except *Curvularia lunata* which required 30°C. Data were recorded on eighth day of inoculations.

To study the effect of duration of light on mycelial growth of fungi, autoclaved universal medium was poured

aseptically in petri plate (9 cm) and inoculated with fresh culture discs (5 mm) of different fungi and placed at 25°C except *C. lunata* which required 30°C.

Following light regimes were analysed

1. 24 hours light
2. 16 hours light + 8 hours darkness
3. 12 hours light + 12 hours darkness
4. 8 hours light + 16 hours darkness
5. 24 hours darkness

Source of light was fluorescent tube at the distance of 41 cm from petri plates.

Results and Discussion

Effect of different culture media on mycelial growth: Universal medium proved to be the best medium for mycelial growth of *A. alternata*, *C. lunata*, *C. gloeosporioides* and *F. paffidoroseium* (Table 1). Malik (1992) also found the same medium to be the best for many fungi.

Effect of different temperatures on mycelial growth: Impact of temperature on the growth of different fungi was different (Table 2). The study revealed that 25°C temperature was the best for *A. alternata* and *F. pallidoroseum* and 20°C was the best temperature for

Table 1: Effect of different culture media on mycelial growth (mm) of different fungi isolated from stem cuttings of *Cuscuta*

	Name of fungi			
Media	*F. pallidoroseum*	*A. alternata*	*C. gloeosporioides*	*C. lunata*
1	75.63 a	58.94 of	65.19 cd	73.25 ab
2	69.63 bc	50.50 gh	60.88 de	66.44 c
3	55.31 fg	40.38 l	49.75 h	48.44 gh
4	60.88 de	39.75 l	46.94 h	41.19 l

Table 2: Effect of different temperatures on mycelial growth (mm) of different fungi isolated from stem cuttings of *Cuscuta*

	Name of fungi			
Temperature	*F. pallidoroseum*	*A. alternata*	*C. gloeosporioides*	*C. lunata*
15	42.13 g*	45.00 f	62.38 d	45.25 f
20	60.94 d	56.19 e	80.19 a	61.13 d
25	74.69 b	71.06 c	72.19 bc	70.69 c
30	62.31 d	71.44c	61.56 d	80.81 a

Any two figures sharing similar letter (s) do not differ significantly at p = 0.05

Table 3: Effect of different pH levels on mycelial growth (mm) of different fungi isolated from stem cuttings of *Cuscuta*

	Name of fungi			
pH levels	*F. pallidoroseum*	*A. alternata*	*C. gloeosporioides*	*C. lunata*
5	64.81 efg	63.00 g	63.44 fg	64.94 efg
6	76.94 c	74.94 cd	81.31 b	73.88 d
7	84.88 a	83.00 ab	77.25 c	85.31 a
8	66.88 e	73.31 d	73.63 d	67.00 e
9	55.19 h	63.50 fg	66.00 of	55.75 h
10	42.38 j	45.69 l	44.19 ij	36.19 k

Any two figures sharing similar letter (s) do not differ significantly at p = 0.05

Table 4: Effect of different light regimes on mycelial growth (mm) of different fungi isolated from stem cuttings of *Cuscuta*

	Name of fungi			
Light intensities	*F. pallidoroseum*	*A. alternata*	*C. gloeosporioides*	*C. lunata*
1	74.38 c*	79.13 b	82.44 a	66.2564
2	71.44 d	73.19 cd	73.63 cd	63.13 g
3	84.88 a	78.13 b	67.56 e	84.00 a
4	71.81 cd	66.25 ef	63.63 fg	57.44 h
5	65.25 efg	63.44 fg	53.88 l	55.38 h

1. 24 hours light, 2. 16 hours light + 8 hours darkness, 3. 12 hours light + 12 hours darkness, 4. 8 hours light + 16 hours darkness, 5. 24 hours darkness

C. gloeosporioides while *C. lunata* gave different response as compared to other fungi and preferred 30°C. McClellan and Marshall (1950) also concluded in his study that about 30°C was the best temperature for *C. lunata*.

Effect of pH levels on mycelial growth: pH 7 suitable for *A. alternata*, *C. lunata* and *F. pallidoroseum*, while pH 6 was proved to be the best for growth of *C. gloeosporioides* (Table 3).

Effect of different light regimes on mycelial growth: *C. lunata* and *F. pallidoroseum* gave good growth under 12 hours light and 12 hours darkness while *A. alternata* and *C. gloeosporioides* gave better mycelial growth under continuous light (Table 4). Our these results verifies the results of Tariq *et al*. (1993) and Ehsan-ul-Haq *et al*. (1998) who obtain the same results.

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