INTERNATIONAL JOURNAL OF PLANT PROTECTION | VOLUME 6 | ISSUE 2 | OCTOBER, 2013 | 457-461

IJPP

RESEARCH ARTICLE

Evaluation of different culture media for *Alternaria helianthi* causing blight in sunflower

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ARITCLE INFO

Received : 12.08.2013
Revised : 10.10.2013
Accepted : 21.10.2013

Key **W**ords :
Leaf blight, Symptoms, Isolation, Pathogenecity, Culture media

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ABSTRACT

Symptoms of Alternaria leaf spot/blight were observed in the form of small scattered brown spots on the leaf surface. Further, these spots covered larger leaf area with dark brown margin and yellow halo with indistinct zonations. Linear necrotic lesions were observed on the stem, petioles, capitulum, sepals, and petals at different stages of plant growth. Isolation of the fungus from the infected leaf sample collected from the field yielded *Alternaria helianthi* and pathogenicity was proved on KBSH 44 which expressed the symptoms in 8-9 days after inoculation under laboratory condition. The pathogenicity studies showed the external symptoms as small scattered brown spots on the leaf surface. Later, these spots increased in size covering larger area with dark brown margin and yellow halo with indistinct zonations. Linear necrotic lesions also appeared on stems, petioles and sepals. Cultural studies revealed that potato dextrose agar (82.80 mm) was the best followed by Sunflower leaf extract agar (71.54 mm) and Richard's agar (63.64 mm) for growth of fungus. Whereas, the growth of *A. helianthi* was not observed in water agar. In case of liquid media, potato dextrose broth yielded maximum dry mycelial weight (282.79 mg) followed by sunflower leaf extract broth (241.51 mg).

How to view point the article : Basarigid, Kalamesh, Karuna, K., Jagadish, K.S., Shadakshari, Y.G. and Geetha, K.N. (2013). Evaluation of different culture media for *Alternaria helianthi* causing blight in sunflower. *Internat. J. Plant Protec.*, **6**(2) : 457-461.

INTRODUCTION

Alternaria blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been considered as a potentially destructive disease in many parts of the sunflower growing countries and in Northern Karnataka (Shankergoud *et al.*, 2006) in India. The disease has been known to cause reduction in flower size, number of seeds per head, seed yield per plant, seed weight and also oil content (Balasubramanyam and Kolte, 1980). The loss in yield varied from 11.30 to 73.33 per cent depending on the extent of infection (Reddy and Gupta, 1977). In Northern Karnataka, Alternaria leaf spot is known to cause more than 80 per cent of the yield loss under severe epiphytotic conditions (Amaresh, 1997).

MATERIAL AND METHODS

Isolation of the pathogen :

The leaves of sunflower infected by *Alternaria helianthi* showing typical dark brown to black, circular to irregular spots were collected from the field in *Kharif* 2010 and brought to the laboratory for isolation of the causal agent. The leaf spot infected specimen was microscopically examined for confirmation of the fungus. Sections of the diseased leaves were cut with the help of a sharp blade on a clean glass slide having a drop of lactophenol. The slide was then covered with a cover slip and observed under microscope. After confirmation of fungal spores, isolation was done in the laminar air flow chamber under aseptic conditions by following the standard tissue isolation procedure. Infected leaves exhibiting typical Alternaria leaf spot symptoms were selected and pathogen was isolated by following the standard tissue isolation method. The infected leaf bits along with healthy leaf tissue measuring about 2 mm were washed well in running tap water. These bits were surface sterilized with 0.1 per cent mercuric chloride solution for one minute. The bits were then

washed thoroughly in sterile distilled water three times to remove the traces of mercuric chloride and then aseptically transferred to sterile Petri plates containing Potato dextrose agar (PDA) (3 pieces/dish).

These plates were incubated at 27±1^0 C. After 7-8 days, the growth of the fungus was observed to know the association of the fungus with the leaf spot. A loopful of fungal culture developed on potato dextrose agar medium in the Petri plates was taken on a glass slide and observed under the microscope for the presence of conidia. From such Petri plates the growing hyphal tip portion was transferred to PDA in Petri plates under aseptic condition and incubated at 27±1 ^{0}C for a week. The pure culture of the fungus was made by transferring spores of *A. helianthi* to PDA slants.

Maintenance of culture :

The maintained pure culture was sub-cultured on potato dextrose agar slants and allowed to grow for one week at 27±1º C. Such slants were stored in a refrigerator at 4 º C and again sub-cultured once in a month during the course of investigation under aseptic conditions to maintain the viability of the pathogen.

Proving of the pathogenicity :

Pathogenicity test was carried out to establish the fungus isolated is capable of producing typical symptoms of leaf spot under artificial inoculation condition on sunflower and also to prove Koch's postulates of the pathogen. Sunflower seeds (KBSH 44) were surface sterilized with 0.1% mercuric chloride and sown in earthen pots containing sterilized soil and they were allowed to grow for a month. The seedlings (30 days old) were inoculated with spore suspension of 106 spores/ml of the fungus from 9 days old culture grown on PDA. The conidial suspension was sprayed uniformly on the leaves. Control plants were also maintained by spraying with sterilized water. The inoculated plants were watered and covered with plastic cover to maintain humidity for 1-2 days. Observation was made at regular intervals for the symptom development after 7 days of inoculation.The symptoms appeared 8-9 days, after inoculation. The organism was re-isolated from these artificial infected leaves showing leaf spot symptoms and the culture obtained was compared with the original culture for confirmation.

Cultural studies :

The culture of *A. helianthi* was grown on various solid and in liquid media *viz.,* Sunflower leaf extract medium, Czapek's agar, Potato dextrose agar, Richard's agar and Water agar. For liquid media, agar was not added. The composition of different media used was according to Tuite (1969) as follows :

Preparation of media :

Richard's agar medium **:**

Magnesium sulphate (MgSO4)	2.5 g
Ferric chloride (FeCl2)	0.02 g
Potassium dihydrogen phosphate (KH2PO4)	5.00 g
Potassium nitrate (KNO3)	10.0 g
Sucrose	50.0 g
Agar	20 g
Distilled water	upto 1000 ml
pH	7.2

All the ingredients were dissolved in 500 ml distilled water in a flask kept on hot plate. The agar melted in another 500 ml of distilled water and both were mixed thoroughly and sterilized.

Czapek's medium **:**

Sodium nitrate (NaNO3)	20 g
Potassium dihydrogen phosphate (KH2PO4)	0.50 g
Magnesium sulphate (MgSO4.7H2O)	0.50 g
Potassium chloride (KCL)	0.50 g
Ferrous sulphate (FeSO4.7H2O)	0.01 g
Agar	20 g
Distilled water	upto 1000 ml
pH	7.2

Agar was melted in 500 ml distilled water. The other ingredients were dissolved in remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto one litre and sterilized.

Potato dextrose agar medium **:**

Peeled potato	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	upto 1000 ml
pH	7.2

Peeled potato pieces were boiled in 500 ml distilled water for half an hour and extract was filtered through a muslin cloth. Agar was melted in 500 ml of distilled water separately and dextrose was added to extract collected after filtering through muslin cloth. The solutions were mixed thoroughly and volume was made upto one litre by using distilled waterand sterilized.

Sunflower leaf extract medium **:**

Sunflower leaves	40 g
Agar	20 g
Distilled water	upto 1000 ml
pH	7.2

Sunflower leaf bits were boiled in 500 ml of distilled water for half an hour and extract was filtered through a muslin cloth. Agar was melted separately and extract was collected after filtering through muslin cloth. The solutions were mixed thoroughly and volume was made upto one litre by using distilled water and sterilized.

Growth on solid media :

Approximately 20 millilitres each of the sterilized media were poured into 90 mm diameter sterilized Petri plates, under aseptic conditions and allowed to solidify. Five mm disc of inoculums cut from the periphery of ten days old culture were used for inoculation. The disc was placed at the centre of the Petri plate on solidified surface. Then the inoculated Petri plates were incubated at 27 ± 1^0 C. The colony diameter on each of the medium was recorded by taking an average of linear growth of colony in two directions at right angles of each plate.

Growth in liquid media :

The composition and preparation of different liquid media used were same as that of solid media except that agar was not added. Twenty millilitre of different liquid media was added separately into each of 100 ml conical flasks. These flasks were then sterilized at 15 lbs pressure for 15 min. The flasks were then inoculated with five mm mycelial disc of *Alternaria helianthi*. Each medium was replicated five times. Then these flasks were incubated at 27 ± 1^0 C. for 12 days. The fungal growth was harvested on 12th day after inoculation and filtered through whatman No.42 filter paper of 9 cm diameter. The weight of dried filter paper was recorded before using for filtration (W_1). Filter paper along with the mycelial mats were dried in an electric oven at 60^0C for 48 hours, cooled in a dessicator and weighed in an electronic balance (W_2). The dry weight of mycelial mat (W) was calculated by deducting the freshweight of filter paper (W_1) from the mycelial mat + filter paper (W_2) *i.e.* $W=W_2-W_1$.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following sub heads :

Symptomatology :

Symptoms on the leaf surface were observed under field condition in the form of small scattered brown spots, further these spots covered larger leaf area with dark brown margin and yellow halo, with indistinct zonations. Linear necrotic lesions were observed on the stem, petioles, capitulum, sepals and petals at different stages of plant growth (Fig. 1and 2). These results were similar to the symptomatology studied by Tubaki and Nishihara (1969), Narain and Saksena (1973) and Kolte and Mukhopadhyay (1973).

Fig. 1 : Symptoms of Alternaria leaf spot on leaf surface

Fig. 2 : Symptoms of Alternaria leaf spot on stem

Isolation of the pathogen :

The colonies of *Alternaria helianthi* on PDA were dark green coloured, with olivaceous green mycelium, conidiophores were septate, smooth and branched. The fungus produced cylindrical to long ellipsoidal conidia. This showed that this fungus is similar to the one described by Narasimha Rao and Rajagopalan (1977) who reported the morphology of *Alternaria helianthi*, as mycelial colonies on potato dextrose agar were dark, profusely branched and frequently septate. The conidiophores were cylindrical, often branched, septate and difficult to be distinguished from the mycelium. Conidia were golden yellow or dark brown, ellipsoidal having tapered apex. Tubaki and Nishihara (1969) described the morphology of the pathogen. Mycelium was olivaceous green, septate, smooth and branched. The conidiophores were cylindrical, scattered or gregarious, pale grey yellow, upto five septa, geniculate, single or branched.

Pathogenicity test :

The pathogenicity studies revealed the external symptoms, as small scattered brown spots on the leaf surface. Later the spots increased in size, covering larger area with dark brown margin and yellow halo with indistinct zonations.

Linear necrotic lesions also appeared on stem, petioles and sepals. The reisolated culture was similar to the original culture. Similarly Prathibha (2005) reported the pathogenicity on sunflower by inoculating spore suspension of *Alternaria helianthi* (106 spores/ml) grown on PDA. The symptoms appeared 8-9 days after inoculation.

Cultural studies :

Growth on solid media **:**

Growth of *A. helianthi* was studied on five different solid media and presented in the (Table 1 and Fig. 3) which, revealed that there was significant difference among the media requirement of *A. helianthi*. The maximum mean colony diameter was noticed in potato dextrose agar (82.80 mm) followed by sunflower leaf extract agar (71.54 mm) and Richard's agar (63.64 mm). The least mean colony diameter was noticed in Czapek's agar (55.12 mm). Since, maximum colony diameter of *A. helianthi* was recorded in potato dextrose agar; the culture of *A.helianthi* was maintained on potato dextrose agar.

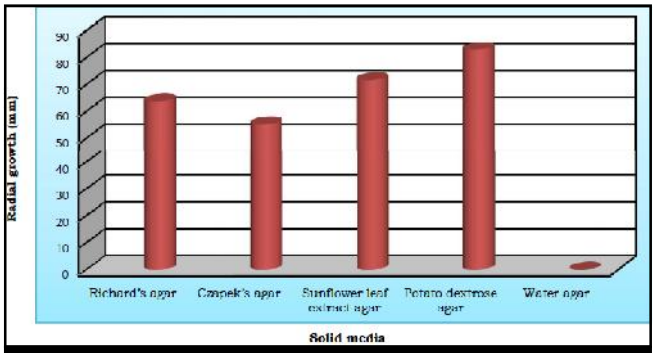

Fig. 3 : Redial growth of *A. helianthi* on different solid media

Growth in liquid media **:**

Alternaria helianthi was grown in five different liquid medium for detection of best liquid medium for the growth of fungus and isolation of the toxin. Results on average dry mycelial weight in different liquid media on 12th day after inoculation are presented in (Table 2 and Fig. 4).The results revealed that, there was a significant difference in the mycelial weight in different broths used. *Alternaria helianthi* produced excellent growth on Potato dextrose broth and Sunflower leaf extract broth (Fig. 4).

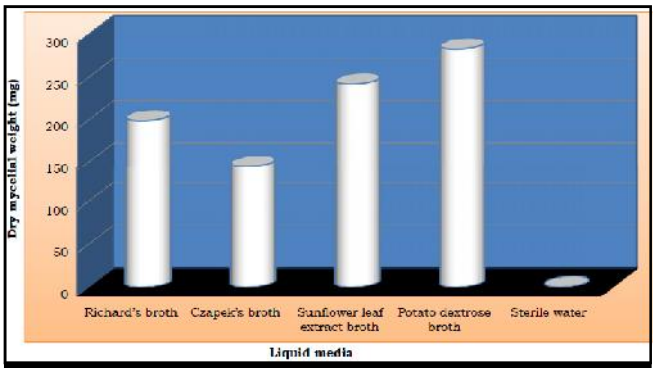

Fig. 4 : Dry mycelial weight of *A. helianthi* in different liquid media

The maximum dry mycelial weight of 282.79 mg was observed in Potato dextrose broth followed by Sunflower leaf extract broth (241.51 mg). In Richard's broth, *Alternaria helianthi* yielded dry mycelial weight of 198.49 mg and in Czapek's broth minimum dry mycelial weight of 144.23 mg was recorded. In distilled water which served as control, for growth of *Alternaria helianthi* was not observed. Among the

Table 1: Radial growth of *Alternaria helianthi* on different solid media

Sr. No.	Medium	Mean colony diameter (mm)
1.	Richard's agar	63.64
2.	Czapek's agar	55.12
3.	Sunflower leaf extract agar	71.54
4.	Potato dextrose agar	82.80
5.	Water agar	0.00
	S.E. ±	0.89
	CD (P=0.05)	2.63

Table 2 : Dry mycelial weight of *Alternaria helianthi* in different liquid media

Sr. No.	Medium	Mean dry mycelial weight (mg)
1.	Richard's brothar	198.49
2.	Czapek's broth	144.23
3.	Sunflower leaf extract broth	241.51
4.	Potato dextrose broth	282.79
5.	Water broth	0.00
	S. Em ±	2.53
	CD (P=0.05)	7.4

different liquid media tested *A. helianthi*, potato dextrose broth yielded maximum dry mycelial weight.

Reddy and Gupta (1981) found that potato dextrose agar media was the best for the growth of *Alternaria helianthi*. Similarly, Sitarama Raju and Mehta (1982) reported that among different media tried, potato dextrose agar medium supported best growth in both solid and liquid media followed by Richard's and Czapek's solution. Narasimha Rao and Rajagopalan (1977) also observed that growth of *Alternaria helianthi* in PDA and Miller's medium was outstandingly superior and significantly inferior in Czapek's solution. Prathibha (2005) recorded potato dextrose agar supported best growth of *Alternaria helianthi* in solid media and reported maximum growth of *Alternaria helianthi* on Richard's broth followed by potato dextrose broth.

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