Cultural and physiological characterization of *Alternaria helianthi* causing Sunflower blight

ABHILASH AND K.KARUNA

J. Mycopathol, Res, 56(1) : 61-66, 2018;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***56(1)*** *: 61-66, 2018;* (ISSN 0971-3719)

Cultural and physiological characterization of *Alternaria helianthi* causing Sunflower blight

ABHILASH AND K.KARUNA*
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Bengaluru 560 065, Karnataka

Received : 05.01.2018 Accepted : 31.01.2018 Published : 30.04.2018

Effect of different media, carbon and nitrogen sources, pH levels, temperature and light intensity were tested against the growth of *Alternaria helianthi* under *in vitro* conditions. The results of experiment indicated that the growth of *A. Helianthi* was maximum in pH range of 6.00- 7.00 and temperature range of 25 - 30°C. Among synthetic media tested, Richard's agar medium and among non-synthetic media PDA supported maximum radial growth of the fungus which was on par with the host leaf extract media. Maltose and potassium nitrate were the best carbon and nitrogen sources respectively that showed maximum radial growth. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of the pathogencompared to continuous light and dark. Further, the cultural characters viz., colony diameter, margin, surface topography, luster, colony texture and sporulation varied among the different media tested.

Key words: *Alternaria helianthi,* sunflower, blight, cultural characters

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in the world and it ranks fourth in area after groundnut, rapeseed, mustard and soybean. Cultivated sunflower, a member of the family Asteraceae (Compositae) is believed to have been first domesticated in the central part of the United States. Sunflower oil is a combination of mono unsaturated and poly unsaturated fatty acids with low saturated fat levels.Presently, in India sunflower is cultivated over an area of 0.59 m ha with a production of 0.43 million tonnes and productivity of 736 kg/ha. Among the major sunflower growing states in the country, Karnataka occupies first position accounting to 3.84 lakh ha with a production of 2 lakh tonnes and productivity of 579 kg/ha. The full potential of the crop is far from being recorded and the yield levels of the country are lowest in world due to several biotic and abiotic stresses. Among the biotic stresses the diseases are one of the major constraints in successful sunflower production. Gulya and Masirevie (1991) listed 80 pathogens occurring on sunflower. In Karnataka, the major diseases of sunflower are necrosis virus, *Alternaria* blight, powdery mildew, rust, collar rot and downy mildew. Among these *Alternaria* blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been considered as a potentially destructive disease in many parts of sunflower growing countries Allen *et al*.(1983) and in north Karnataka Ramjegathesh and Ebenejer (2012). It has been reported from different parts of the world including India that 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 varies from 11.30 to 73.33 per cent depending on the extent of infection. In Northern Karnataka, *Alternaria* leaf spot is known to cause more than 80 per cent of the yield loss under epiphytotic conditions Hiremath *et al*. (1990). The disease severity is known to increase or decrease

*Corresponding author : kavalikaruna@yahoo.co.in

depending upon the change in environmental conditions during the crop growth period.

An understanding of the role of environmental conditions and its effect on infection and survival of the pathogen is necessary to develop cultural disease management practices. Therefore the objectives of this study included isolation, purification and identification of pathogenic fungus causing leaf blight disease of sunflowerand determine optimal conditions for the mycelial growth of the fungus including pH, temperature, light and type of media.

MATERIALS AND METHODS

Isolation, purification and identification of pathogen

The sunflower leaves infected by *Alternaria helianthi* showing typical dark brown to black, circular to irregular spots were collected from the field in *kharif* 2016and brought to the laboratory for isolation of thecausal agent. *Alternaria* infected leaves exhibiting typical *Alternaria* leaf spot symptoms were selected andpathogen was isolated by following standard tissue isolation method. The infected leaf bits measuring about 2 mm were disinfected with 1% NaOCl solutions for a minute and rinsed thrice with sterilized water to remove traces of disinfectant. These pieces were then transferred to petri plates containing Potato Dextrose Agar (PDA) (3 pieces/dish). These plates were incubated at 27 ± 1°C. After 10 days of incubation the growth of the fungus in association with the leaf spot was observed. Following hyphal-tip technique, test pathogen was transferred aseptically on the PDA slant in test tubes. Single spore isolation technique was followed for the purification of pathogen. The pathogen was identified up to species level based on Molecular characterization of ITS rDNA region.

Pathogenicity test

Ten days old culture of the organism was used for proving the pathogenicity by applying Koch's postulates. For this purpose, surface sterilized seeds of sunflower hybrid KBSH-44 which is susceptible to *Alternaria* blight (*A. helianthi*) were sown in the earthen pots filled with autoclaved potting mixture of soil: sand: FYM (2:1:1). Healthy growing sunflower seedlings were maintained, watered regularly and kept in the glass house for further inoculation. Conidial suspension (10^6 conidia /ml)of 10 days old test fungus were inoculated to 30 day old seedlings. Uninoculated seedlings of the same age sprayed with sterilized water served as control. After inoculation, the seedlings pots (both inoculated and uninoculated) were incubated in the glass house, where relative humidity (80 to 90%) and optimum temperature(27±1°C) were maintained for further development of *Alternaria* blight symptoms.

Environmental factors affecting to the growth of Alternaria helianthi

Cultural studies of *A. helianthi*

Effect of different media on growth and sporulation of *A. helianthi*

Petri plates containing 20 ml of each of following media, Oat meal agar, PDA+ $CaCO_3$, PDA, Host leaf extract agar, Rose Bengal agar, Richard's agar, Host extract + sucrose, Saboraud's agar, Peptone, Malt, Carrot agar, Host seed, Water agar were inoculated with One 5 mm disc of the actively growing culture of twelve days old fungus to the centre of each Petri plates and incubated at room temperature (27±1°C) for a period of 21 days. Colony diameter was recorded by averaging the linear growth of colony in two directions for each plate. Colony colour, surface elevation and sporulation were also noticed.

Effect of different carbon sources on the growth of A. helianthi

The effect of on the growth of the pathogen was studied followed the method described by Prathibha (2005) using PDA medium. Dextrose was replaced in PDA medium by different carbon sources viz., glucose, maltose, sucrose, fructose, pectin, mannitol, cellulose and starch . PDA devoid of dextrose served as control. The pathogen was inoculated to Petri plates containing different carbon sources and incubated at 27±1°C. Three replications were maintained per treatment. After 21 days of incubation the radial growth of the fungus and sporulation was recorded.

Effect of different nitrogen sources on the growth of A. helianthi

Various nitrogen sources incorporated in a basal

medium (Czapek'sDox). Thequantity of each nitrogen compound was determined on the basis of their molecular weight so as to provide equivalent amounts of nitrogen as that of potassium nitrate present in basal medium. The nitrogen sources used were potassium nitrate, ammonium chloride, ammonium nitrate, ammonium oxalate, ammonium phosphate, ammonium sulphate. The growth of the fungus was recorded as described under studies of different media.

Effect of hydrogen ion concentration on the growth of A. helianthi

The growth of *Alternaria helianthi* was tested at six pH levels *viz*., 5, 6, 7, 8, 9 and 10 respectively. Hydrogen ion (pH) concentration of the potato dextrose broth was determined by using pH meter. Adjustment of pH was done using 0.1 N alkali (Sodium hydroxide) and 0.1 N acid (Hydrochloric acid) and was sterilized in an autoclave at 121 ^{0}C for 15 minutes. To 100 ml flasks containing 50 ml of sterilized medium, one 5 mm fungal disc was inoculated and kept for incubation at 27±1^{0}C for 20 days. Each treatment was replicated thrice, the ideal pH for growth of the fungus was determined by harvesting mycelial mat that was filtered through Whatman filter paper and dry mycelial weight (mg) was recorded (Archana,2012).

Effect of temperature on the growth of A. helianthi

The growth of the fungus was tested at 15, 20, 25, 30 and 35 ^{0}C. Fifty ml of potato broth was dispensed and sterilized in 100 ml conical flask. Each flask was inoculated with 5 mm disc of the test fungus and incubated for 20 days at different temperature levels as mentioned above. Each treatment was replicated thrice. After incubation period, the dry mycelial weight (mg) was recorded and data were analyzed statistically (Archana,2012).

Effect of light on the growth of A. helianthi

The effect of light on growth and sporulation of *Alternaria helianthi* was studied by using potato dextrose medium. Five mm culture discs were inoculated into the flasks and were incubated at 27±1^{0}C for three weeks. The flasks were exposed to different light treatments *viz*., continuous light (24 h), dark, alternate cycles of light and dark (12 h each). Observations on the mycelial growth and sporulation were recorded. The data obtained was analyzed statistically.

Statistical ananlysis

All experiments were conducted with three replications and the data were statistically analysed. Suitable controls were maintained for all the experiments conducted. Completely randomized design (CRD) was used for analysing the data obtained on colony diameter.

RESULTS AND DISCUSSION

Isolation, purification and identification of pathogen

Isolated pathogen was identified and confirmed as *Alternaria helianthi* through sequencing of ITS 1 AND ITS 4 rDNA region of the pathogen and the same was deposited in NCBI with GenBank accession number SUB2900726 Alternaria_helianthi_AB1 MF563494.

Pathogenicity test

Initially eight days after inoculation the plants showed typical 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.Reisolation trails revealed that the isolated fungi from diseased seedlings are found to be identical with those used for artificial inoculation.

Effect of culture media

Every living being requires food for its growth and reproduction and fungi are not an exception to it Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life process. Neither all media are equally good for all fungi nor there can a universal substrates or artificial medium on which all fungi grow well. So different media including synthetic and non synthetic were tried for the growth of *A. helianthi.*

Among non-synthetic media PDA supported maximum radial growth (80.07 mm) of the fungus which was on par with the host leaf extract (80.03 mm)

Table 1: Effect of different media on growth and sporulation of *Alternaria helianthi*

Medium	Radial Colony Growth (mm)	Sporulation
Oat meal agar	69.30	+++
PDA+ $CaCO_3$	61.70	++++
PDA	80.70	+++
Host leaf extract agar	80.30	+++++
Rose Bengal agar	42.30	++
Richard's agar	85.00	+++
Host extract + sucrose	72.70	+++++
Saboraud's agar	82.00	++
Peptone	63.70	+++
Malt	49.30	++
Carrot agar	52.30	+++
Host seed	53.30	+++
Water agar	16.30	++
S. Em ±	0.21	_
CD @ 1 %	0.860	_

media followed by host leaf extract + sucrose (72.70 mm).Among synthetic media maximum radial growth (85.00 mm) of *A. helianthi* was in Richard's agar followed by Saboraud's agar (82.00 mm), whereas least growth was recorded on water agar medium (16.33 mm).

Though the radial growth of the pathogen was highest on Richard's agar, profuse sporulation was observed in host leaf extract + sucrose and host leaf extract, more than 20 conidia per microscopic field followed by PDA+$CaCO_3$(Table 1). Similar types of results were obtained by Singh and Prasad (1973) who recorded the maximum growth of *A.cyamapsis* on Potato Dextrose Agar followed by Richard's and Sabourd's agar.

Besides variation in growth rate, the pathogen exhibited variation in respect of colony colour, texture and surface topography also and the details are furnished in Table 2.

Effect of different carbon sources on the growth of Alternaria helianthi

The growth and sporulation of *A. helianthi* varied significantly in the different carbon sources tested. The radial growth of *A. helianthi* was maximum (89.30 mm) in media supplemented with maltose as the carbon source, which was significantly superior over all other carbon sources followed by glucose (88.20 mm) and dextrose (82.33 mm). The radial growth of the pathogen was minimum (52.00 mm) on media supplemented with cellulose as carbon source and rest of the carbon sources supported radial growth of *A. helianthi* ranging from 62.00 mm to 72.23 mm. Control plates recorded 51.33 mm radial growth of the pathogen (Table 3). The results of the study are in conformity with the reports of Ramjegathesh and Ebenezar (2012)who suggested that the maltose encouraged significantly highest mean mycelial growth of *A. helianthi*.

Effect of different nitrogen sources on the growth of Alternaria helianthi

Growth of *A. helianthi* on seven media having different sources of nitrogen varied significantly. The maximum mycelial growth of 72.33 mm was noticed in media supplemented with potassium nitrate as nitrogen source, followed by sodium nitrate (65.70 mm) and ammonium nitrate (59.33 mm). The least mycelial growth was recorded in media supplemented with ammonium sulphate. Poor mycelial growth of 19.00 mm to 19.70 mm was observed on media supplemented with Ammonium phosphate and Ammonium chloride respectively. Sporulation on the media supplemented with sodium nitrate was good while, potassium nitrate and ammonium nitrate supported moderate sporulation (Table 4).These findings are in agreement with the findings of Chavan *at el*. (2015) who studied the effect of different nitrogen sources on mycelial growth of *A. brassicae* and reported that potassium nitrate was found best with significantly highest mean mycelial growth (87.67 mm), followed by sodium nitrate (72.87 mm).

Effect of different temperature levels on the growth of *Alternaria helianthi*

Temperature is most important physical environmental nor there can a universal substrates or artificial medium on factor for regulating the growth

Table 2: Cultural characteristics of *Alternaria helianthi* on different media

Medium	Color	Colony texture	Surface topography	Margin	Luster
Oat meal agar	Light grey	Raised	Smooth colony, irregular margin	Irregular	Dull
PDA+ $CaCo_3$	Creamish	Raised	Smooth colony with regular margin	Regular	Shiny
PDA	Light grey	Raised	Fluffy, irregular towards margin	Irregular	Dull
Host leaf extract agar	Light brown	Flat	Smooth, buff white	Irregular	Dull
Rose Bengal agar	Light brown	Flat	Buff white, scanty growth	Regular	Shiny
Richard's agar	Dark grey	Raised	Irregular, raised colony	Irregular	Dull
Host extract + sucrose	Light brown	Flat	Light brown colony	Irregular	Dull
Saboraud's agar	Light grey	Raised	Light grey at centre	Wavy	Dull
Peptone	Light grey	Flat	Fluffy regular margin	Regular	Shiny
Malt	Dark grey	Raised	Raised, sectoring margin	Irregular	Dull
Carrot agar	Light grey	Flat	Smooth colony with wavy margin	Irregular	Shiny
Host seed	Light grey	Flat	Wavy margin	Irregular	Dull
Water agar	Creamish	Flat	Buff white, scanty growth	Irregular	Shiny

Table 3 : Effect of different carbon sources on the growth of *Alternaria helianthi*

Carbon sources	Radial Colony Growth(mm)	Sporulation
Glucose	88.20	+++++
Mannitol	71.00	+++
Maltose	89.30	+++++
Dextrose	82.30	+++
Sucrose	72.30	++++
Fructose	70.00	+++
Cellulose	52.00	+++
Pectin	62.00	++
Starch	66.00	++
Control	51.30	++
S. Em ±	0.21	-
CD @ 1 %	0.87	-

Table 4: Effect of different nitrogen sources on the growth of *Alternaria helianthi*

Nitrogen sources	Radial Colony Growth (mm)	Sporulation
Potassium nitrate	72.30	++++
Sodium nitrate	65.70	++++
Ammonium nitrate	59.30	+++
Ammonium sulphate	16.30	++
Ammonium chloride	19.70	++
Ammonium phosphate	19.00	++
Ammonium oxalate	20.30	+++
Control	19.00	++
S. Em ±	0.127	-
CD @ 1 %	0.52	-

Table 5: Effect of different temperatures on dry mycelial weight of *Alternaria helianthi*

Temperature(^{0}C)	Dry mycelial weight (mg)
15	181.6
20	212.00
25	221.2
30	216.67
35	197.0
S. Em ±	4.62
CD @ 1 %	14.75

Table 6: Effect of hydrogen ion (pH) concentration on dry mycelial weight of *A. helianthi*

pH	Dry mycelial weight (mg)
5	60.00
6	170.20
7	155.00
8	55.00
9	32.00
10	21.33
S. Em ±	0.89
CD @ 1 %	3.53

Table 7: Effect of light conditions on the dry mycelial weight of *Alternaria helianthi*

Light Source	Dry mycelial weight (mg)
Light +Dark	207.40
Light	189.20
Dark	178.80
S. Em ±	1.24
CD @ 1 %	3.72

and reproduction of which all fungigrow well. So different media including fungi Maximum dry mycelial weight of 218.33 mg was recorded when temperature was maintained at 25^{0}C which was followed by 216.67 mg when temperature was maintained at 30^{0}C. Dry mycelial weight of the pathogen was minimum at 15^{0}C (181.2 mg) (Table 5). The study agreed well with results of Neelakanth *et al* (2012) and Thejakumar and Devappa (2016) who reported the optimum temperature for pathogen *A. helianthi* range between 25°C to 30°C.

Effect of hydrogen ion (pH) concentration on the growth of Alternaria helianthi

Fungi generally utilize substrates the form of solution only if the reaction of solution conducive to fungal growth and metabolism. This brings importance of hydrogen ion concentration forbetter fungal growth. Of all the six pH levels, pH 6.0 was found to be ideal and produced the maximum mean dry mycelial weight of 170.2mg followed by pH 7.0 (155 mg) and pH 5.0 (55 mg). The mean mycelial growth was lowest at pH 10.0 which recorded 21.33 mg .The pH below six and above seven was noticed to be inhibitory to the growth (Table 6). The results of experiment indicated that *A. Helianthi* prefers pH range of 6.00- 7.00. This showed that the fungus prefers slightly acidic pH for the growth.The results obtained by Neelakanth *et al* (2012)) indicated that pH of 6.0 favored better growth (395.00 mg) of the pathogen *A. Helianthi*

Effect of different light sources on the growth of Alternaria helianthi

Growth of the pathogen in terms of dry mycelial weight differ significantly with exposure to various light conditions *viz*., continuous light, continuous dark and alternate cycles (12 h each).The dry mycelial weight of *A. helianthi* was maximum (207.4mg) when exposed to alternate cycles (12 h each) of light and dark as compared to continuous light (189mg) and continuous dark (178.8 mg) (Table 7).Manjunath *et al* (2010) also observed that the exposure of the fungus *A. alternata* to alternate cycles of 12 hour light and 12 hours of darkness resulted in the maximum mycelial growth as compared to continuous light and dark.

ACKNOWLEDGEMENT

The author are thankful to AICRP on Sunflower, ZARS, GKVK, UAS, Bengaluru-560065, Karnataka

REFERENCES

Allen, S. J., Brown, J. F. and Kochman, J. K. 1983. Effect of temperature, dew period and light on the growth and development of *Alternaria helianthi*. *Phytopathol*. **73** : 893-896

Archana, B. C. 2012. *Studies on Leaf spot and Fruit rot of Pomegranate caused by Alternaria alternata(Fr.) Keissler*. M.Sc (Agri) Thesis, Univ. Agric. Sci., Dharwad, 57 pp.

Balasubramanyam, N. and Kolte, S. J. 1980. Effect of *Alternaria* blight on yield components, oil content and seed quality of Sunflower. *Indian. J. Agric. Sci.* ***50****: 701-706.*

Chavan, P. G. Apet, K.T. Wagh S. S. and Hingole, D.G., 2015. Nutritional and physiological requirement of *Alternaria brassicae*, causing *Alternaria* Leaf spot of Cauliflower. *Trends in Biosciences.* **8**: 1914-1919.

Gulya, J. J. and Masirevie. 1991. Common names for the plant diseases of Sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosa* L). *Plant Disease*, **75**: 30.

Hiremath, P. C., Kulakarni, M. S. and Lokesh, M. S. 1990. An epiphytotic of *Alternaria* blight of Sunflower in Karnataka. *Karnataka J. Agric. Sci*. **3**: 277-278.

Manjunath, H., Sevugapperumal, N., Thiruvengadam, R., Theerthagiri, A. and Ramaswamy, S. 2010. Effect Of environmental conditions on growth of *Alternaria alternata* causing leaf blight Onion. *World J. Agril.Sci*. **6:** 171-177.

Neelakanth, S., Hiremani., Shivananda, J. and Mantur, S. G. 2012. Cultural studies on *Alternaria ricini* causing Leaf spot of Castor . *Inter. J. Plant Prot*., **5** : 116-119.

Prathibha, V. H. 2005. *Studies on leaf spot of sunflower caused by Alternaria helianthi (Hansf.) Tubaki and Nishihara*. M.Sc. (Agri.) Thesis, Univ. Agric. Sci., Bengaluru, 118 pp.

Ramjegathesh, R. And Ebenezar, E. G. 2012. Morphological and physiological characters of *Alternaria alternata* causing Leaf blight disease of Onion. *Inter. J. Plant Pathol*.**3**: 34-44.

Singh, D. B. and Prasad, R. 1973. Studies on physiology and control of *Alternaria cyamapsis*, the incitant of Blight disease of Guar. *Indian J. Mycol. Plant Pathol*. **3**: 33-39.

Thejakumar, M. B. and Devappa, V. 2016. Efficacy of different fungicides against *Alternaria alternata* and *Cercospora capsici* under *in vitro* conditions. *Int. J. Adv. Res. Biol. Sci*. **3**: 126-129.