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Effect of different culture media, temperature, pH, carbon and nitrogen sources on mycelial growth and sporulation of *Alternaria carthami* causing Alternaria blight of safflower

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ABSTRACT

Eight culture media, eight temperature levels, nine pH levels, seven carbon and six nitrogen sources tested exhibited better growth of *Alternaria carthami*. Results revealed that Potato dextrose agar gave significantly highest growth (90.00 mm), followed by Potato malt agar (84.16 mm) with excellent sporulation. Temperature levels indicated that highest mean mycelial growth (85.66 mm) was recorded at 30°C followed by 25°C (83.83 mm) and 20°C (66.33 mm). However, maximum mean mycelial growth (85.83 mm) was recorded at pH 6.5 with excellent sporulation, followed by at pH 6 (82.00 mm) and pH 7 (70.33 mm) with excellent and good sporulation, respectively. The carbon sources exhibited varied radial mycelial growth and sporulation of the test pathogen. However, highest radial mycelial growth (86.00 mm) and excellent sporulation was recorded on glucose, followed by on maltose (82.83 mm) and starch (80.33 mm) with excellent sporulation. Nitrogen sources resulted highest radial mycelial growth (82.55 mm) and excellent sporulation on potassium nitrate, followed by on peptone (75.83 mm) with good sporulation. Least radial mycelial growth (19.00 mm) was recorded on urea with poor sporulation.

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INTRODUCTION

Safflower (*Carthamus tinctorious*) is an important oilseed crop, belonging to the family Asteraceae and believed to be native of Afganistan. In India, it is most commonly known as Kardai in Marathi and Kusum in Hindi.

Safflower, a multipurpose crop has been grown for the orange red dye (*carthamin*) extracted from it which is brilliant coloured flowers and for its quality oil (30%) rich in polyunsaturated fatty acids (linoleic acid, 78%). Safflower is known to have many medicinal properties for curing several chronic diseases and is widely used in Chinese herbal preparations. It is rich in Vitamin-A, iron, phosphorus and calcium. Bundles of young plants are commonly sold as green vegetables in market in India and some neighboring countries (Nimbkar, 2002).

The important safflower growing countries are: India, Mexico, USA, Argentina, Canada, China, Spain, Italy, Turkey, Iraq, Iran, Egypt, Ethiopia and Sudan. Presently, India is the largest producer of safflower in the world followed

by U.S., Mexico and China. In India it is mainly grown in Maharashtra, Karnataka, and part of Andhra Pradesh, Madhya Pradesh, Orissa and Bihar. In Maharashtra, it is mainly grown in Solapur, Pune, Ahmednagar, Latur, Osmanabad, Parbhani, Hingoli and Jalna districts (Das, 1997). In Marathwada, popularly grown safflower varieties/ cultivars are: Bhima, Phule Kusum, NARI-6, NARI-34, A-1, JSI-7, Sharda, PBNS-12, Nira, NARI-NH-1 and Tara.

Like other agronomical crops, safflower is also affected by many biotic and abiotic stresses/agents. Of the biotic agents, fungi cause major diseases, followed by bacteria, viruses and nematodes. Major safflower diseases caused by fungi are: leaf spot/blight (*Alternaria carthami*), wilt (*Fusarium oxysporum f.* sp. *carthami*), root rot (*Rhizoctinia bataticola*), powdery mildew (*Erysiphe* sp.) and anthracnose (*Colletotrichum capsici*), by bacteria; leaf blight/spot (*Pseudomonas syringae*); by viruses: mosaic (Cucumber mosaic virus), necrosis (tobacco streak virus) and root knot (*Meliodogyne hapla*) nematode disease (Bhale *et al.*, 1998).

Among these diseases, under present situation leaf spot/ blight caused by *Alternaria carthami* (Choudhary, 1944) has become one of the major constraints in the production and productivity of safflower in the country in general as well as in the state of Maharashtra particularly. The disease (*A. carthami*) has been reported to cause 25 to 60 per cent yield losses in safflower (Singh and Prasad, 2005). The disease has been also reported to reduce drastically the seed size, seed volume test as well as per cent oil content.

Typical symptoms of the disease (*A.carthami*) appear as irregular necrotic lesions on leaves and stem. Dark necrotic lesions, 2-5 mm in diameter are firstly found on hypocotyls and cotyledons. In mature plant, small brown to dark brown concentric spots of 1-2 mm appear on leaves and brown discoloration appear on the stem, dark brown spot with concentric ring up to 1 cm in diameter appear on leaves which later develop in to large lesions (Mortensen and Bergman, 1993).

MATERIAL AND METHODS

A. carthami was isolated from the infected leaves and its pure culture was maintained on PDA medium for further studies.

Effect of culture media :

To study the effect of different solid culture media on characteristics of *A. carthami*, eight culture media *viz.*, Potato dextrose agar, Corn meal agar, Potato malt agar, Czapek's Dox agar, Yeast extract agar, Yeast manitol agar, Malt extract agar and Ashby's manitol agar were used The media were sterilized in autoclave at 15 lbs/inch^2 pressure for 15 min.

Autoclaved and cooled media were poured (@ 20 ml/ plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the media, Petri plates of each culture medium (five plates/medium/replication) were inoculated by placing in the centre 5 mm mycelial disc of actively growing 7 days old pure culture of *A. carthami*. Each culture medium was replicated thrice. Plates were incubated at room temperature (27 $\pm$ 1°C).

Physiological studies :

Effect of temperature :

For studying the effect on the growth and sporulation, the fungus was grown temperature were maintained 5, 10, 15, 20, 25, 30, 35 and 40^{0}C temperature the adjustability of the instruments.

Experiment was conducted in Petri plates on Potato dextrose agar (PDA). PDA was sterilized and poured in sterile Petri plates. For each treatment of 5 mm inoculums disc were used. Plates were incubated at respective treatment of temperature and observations on colony diameter and sporulation were recorded after seven days of inoculation.

Effect of pH :

Different eight pH levels *i.e.* 4, 4.5, 5, 5.5, 6, 6.6, 7 and 7.5 were evaluated to study the influence of pH on growth and sporulation of pathogen on PDA. The required pH levels were adjusted by adding 0.10 N HCI/NaOH. After adjustment of pH, basal medium PDA was sterilized in autoclave. The cooled medium was poured in Petri plates (@ 20 ml/plate) and allowed to solidify at room temperature. On solidification Petri plates were inoculated with 5 mm disc of actively growing culture organism and incubated at temperature 27$\pm$1°C for seven days. Three replications of each treatment was maintained.

The observations on radial mycelial growth or colony diameter (mm) and sporulation on each pH level were recorded at 24 hrs interval and continued till seven days after inoculation.

Nutritional studies :

Effect of carbon and nitrogen sources :

Different fungi are known to have different requirements of carbon and nitrogen for their optimum growth and sporulation. Therefore, in the present study seven sources of carbon *viz.*, lactose, manitol, starch, glucose, maltose, sucrose and fructose and six sources of nitrogen *viz.*, urea, potassium nitrate, peptone, sodium nitrate, ammonium nitrate and thiourea were evaluated for the growth and sporulation of *A. carthami* using Richard's agar as the basal medium. The quantity of selected carbon and nitrogen sources required was determined on the basis of their molecular weight. For evaluation of carbon sources, instead

of dextrose test carbon were used in the basal medium, and for evaluation of nitrogen sources basal medium Richard's agar was enriched separately with the test nitrogen compounds. The requisite basal media amended individually with the test carbon and nitrogen sources were autoclaved at 15lbs/inch^2 for 15 minutes and after cooling at room temperature and poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.). These plates were inoculated with 5 mm mycelial disc obtained from seven days old actively growing cultures of *A. carthami* and incubated at 27±1°C temperature. Each treatment was replicated thrice.

The observations on radial mycelial growth/colony diameter (mm) and sporulation were recorded at 24 hrs interval and continued till 10 days after inoculation. Observations obtained were averaged and the data were analyzed statistically.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Effect of culture media :

Cultural characteristics *viz*., mycelium, colony growth, colony elevation, colony colour and sporulation of *A. carthami* were studied *in vitro* on eight culture media and results obtained are presented in Table 1.

Mycelial growth :

The results of Table 1 revealed that, all the media tested encouraged better growth and variable sporulation of *A. carthami*. The mean colony growth recorded with all the test media ranged from 41.66 mm (Yeast extract agar) to 90.00 mm (Potato dextrose agar). However, significantly highest mean mycelial growth (90.00 mm) was recorded on Potato dextrose agar. The second and third best media were Potato malt agar (84.16 mm) and Yeast manitol agar (73.33 mm), respectively. In order of superiority were Malt extract agar (69.16 mm), Asbhy's manitol agar (65.33 mm) and Czapak Dox agar (51.33 mm). Corn meal agar (48.83 mm) and Yeast extract agar (41.66 mm) were found least suitable to the growth of the test pathogen.

All the media tested exhibited varied radial mycelial growth and sporulation of the test pathogen. However, excellent sporulation was recorded on Potato dextrose agar, followed by Potato malt agar. Least radial mycelial growth and fair sporulation was recorded on yeast extract agar.

The results of the present study on the effect of various culture media on morphological and cultural characteristics and sporulation in *Alternaria carthami* are in consonance with those reported earlier by several workers (Jash *et al*., 2003; Yadav and Khan, 2008 and Hubballi *et al*., 2010).

Physiological studies :

Effect of temperature regimes :

Temperature is one of the most crucial factors, which affect the pathogen host or host pathogen interaction during pathogenesis. Therefore, present study includes mycelial growth and sporulation at eight different temperature levels ranging from 5°C to 40°C. The data obtained during the study are presented in Table 2.

The results of Table 2 revealed that, all the temperature tested encouraged variable mycelial growth and sporulation of *A. carthami*. The mean colony growth recorded at different temperatures ranged from 5.66 mm at 5°C to 85.66 mm at 30°C. However, significantly highest mean mycelial growth (85.66 mm) was recorded at 30°C. The second and third best temperature regimes were 25°C (83.83 mm) and 20°C (66.33 mm) followed by at 35°C (60.16 mm), 15°C (46.16 mm) and 40°C (25.66 mm). Temperature 5°C and 10°C were found least suitable which recorded minimum mean mycelial growth of 5.66 mm and 21.33 mm, respectively.

The excellent sporulation was recorded at 30°C, followed by 25°C. Least radial mycelial growth was recorded

Table 1 : Effect of different culture media on mycelial growth and sporulation of *A. carthami*

Treatments	Mean colony diameter (mm)*	Characteristics	Sporulation
Potato dextrose agar	90.00	Circular, loose and wooly growth, grayish colour	++++
Corn meal agar	48.83	Circular, loose and wooly growth, grayish black with whitish grey periphery	++
Potato malt agar	84.16	Circular, loose growth, olivaceous grayish colour	++++
Czapek's dox agar	51.333	Circular, loose and wooly growth, grayish black with whitish grey periphery	++
Yeast extract agar	41.66	Circular, loose growth, grayish black	++
Yeast manitol agar	73.33	Circular, loose and wooly growth, grayish colour	+++
Malt extract agar	69.16	Circular, loose and whitish grey coloured growth	+++
Ashby's manitol agar	65.33	Irregular, close cottony growth, olivaceous white colour	+++
S.E. ±	0.43	-	-
C.D. (P = 0.05)	1.30	-	-

* Mean of three replications, + : Poor, ++ : Fair, +++ : Good, ++++ : Excellent

Table 2 : Effect of different temperatures on the mycelial growth and sporulation of *A. carthami*

Temperature (°C)	Mean colony diameter (mm)*	Sporulation
5°C	5.66	-
10°C	21.33	+
15°C	46.16	++
20°C	66.33	+++
25°C	83.83	++++
30°C	85.66	++++
35°C	60.16	++
40°C	25.66	+
S.E. ±	0.40	-
C.D. (P = 0.05)	1.20	-

*Mean of three replications,
+ : Poor, ++ : Fair, +++ : Good, ++++ : Excellent

at 5°C with poor sporulation. The temperatures between 25°C to 30°C was better for growth and sporulation of test pathogen and the temperature below 15°C and above 35°C were unfavourable for growth and sporulation of the test pathogen.

The results of the present study on the effect of different temperatures on the growth and sporulation of *A. carthami* and different *Alternaria* spp. were reported earlier by several workers (Ramegowda and Naik, 2006; Hubballi *et al.*, 2010 and Mishra and Mishira, 2012).

Effect of pH :

Hydrogen ion concentration (pH) has marked effect on the growth of fungus. The pH requirement may differ from fungus to fungus. In the present study included nine different pH levels ranging from 4.0 to 8.0. The results obtained of study are presented in Table 3.

The results of Table 3 revealed that all the pH levels tested encouraged variable growth and sporulation of *A. carthami*.

The mean colony growth recorded with all the pH levels ranged from 30.50 mm at pH 4.0 to 85.83 mm at pH 6.5. However, significantly maximum mean mycelial growth (85.83 mm) was recorded at pH 6.5 followed by an order of superiority pH 6 (82.00 mm), pH 7 (70.33 mm), pH 7.5 (63.16 mm), pH 8.0 (51.00 mm) and pH 5.5 (53.16 mm). The pH 4.0 and 4.5 were found least suitable which recorded minimum mycelial growth of 30.50 mm and 35.83 mm, respectively of the test pathogen.

All the pH levels exhibited varied radial mycelial growth and sporulation of the test fungus. However, excellent sporulation was recorded at pH 6.5 and pH 6.0. Least radial mycelial growth with poor sporulation pH between 6 to 6.5 was better for growth and sporulation of test pathogen. pH below 5.0 and above 8.0 was not only unfavorable for growth and sporulation of the test pathogen.

The results of the present study on the effect of different

Table 3 : Effect of pH on mycelial growth and sporulation of *A. carthami*

Treatments	Mean colony diameter (mm)*	Sporulation
4.0	30.50	+
4.5	35.83	++
5.0	44.50	++
5.5	53.16	+++
6.0	82.00	++++
6.5	85.83	++++
7.0	70.33	+++
7.5	63.16	+++
8.0	51.00	+++
S.E. ±	0.48	-
C.D. (P = 0.05)	1.44	-

*Mean of three replications
+ : Poor, ++ : Fair, +++ : Good, ++++ : Excellent

pH levels on growth and sporulation of *Alternaria carthami* and other *Alternaria* spp. are supported by earlier workers. (Jash *et al*., 2003; Prathibha *et al*., 2008 and Hubballi *et al*., 2010).

Nutritional studies :

Effect of carbon sources :

Cultural characteristics *viz*., mycelial growth and sporulation of *A. carthami* were studied *in vitro* using seven carbon sources. All the sources of carbon tested exhibited better growth of the test fungus (Table 4).

The results of (Table 4) revealed that of the seven carbon sources tested, glucose was found most suitable and encouraged maximum radial mycelial growth (86.00 mm). The second and third best carbon sources found were maltose (82.83 mm) and starch (80.33 mm). The other sources in order of superiority were sucrose (78.33 mm), lactose (76.83

Table 4 : Effect of different carbon sources on mycelial growth and sporulation of *A. carthami*

Treatments	Mean colony diameter (mm)*	Sporulation
Lactose	76.83	++++
Manitol	74.00	+++
Starch	80.33	++++
Glucose	86.00	++++
Maltose	82.83	++++
Sucrose	78.33	+++
Fructose	69.83	+++
Control (Untreated)	66.16	+++
SE ±	0.45	-
C.D. (P = 0.05)	1.36	-

*Mean of three replications
+: Poor, ++ : Fair, +++ : Good, ++++ : Excellent

Table 5 : Effect of different nitrogen sources on mycelial growth and sporulation of *A. carthami*

Treatments	Mean colony diameter (mm)*	Sporulation
Urea	19.00	+
Potassium nitrate	82.55	++++
Peptone	75.83	+++
Sodium nitrate	70.16	+++
Ammonium nitrate	44.83	++
Thiourea	26.16	++
Control (Untreated)	65.33	+++
SE ±	0.43	-
C.D. (P = 0.05)	1.30	-

*Mean of three replications
+: Poor, ++: Moderate, +++: Good, ++++ : Excellent

mm) and manitol (74.00 mm). Comparatively fructose was found less suitable which recorded minimum radial mycelial growth (69.83 mm) over untreated control (66.16 mm) of the test fungus.

Thus, at all the carbon sources tested exhibited varied radial mycelial growth and sporulation of the test pathogen. However, excellent sporulation was recorded on glucose, followed by maltose. Comparatively less radial mycelial growth with good sporulation was recorded on untreated control.

The similar results on the effect of carbon sources on growth and sporulation in *Alternaria carthami* and other *Alternaria* spp. reported by Jash *et al*. (2003) and Kumar *et al*. (2006).

Effect of nitrogen sources :

Mycelial growth and sporulation of *A. carthami* were studied *in vitro* using six nitrogen sources. All the sources of nitrogen tested encouraged better growth of the test pathogen (Table 5).

The results (Table 5) revealed that of the six nitrogen sources tested, potassium nitrate was found most suitable and encouraged maximum radial mycelial growth (82.55 mm). The second best nitrogen source found was peptone (75.83 mm) and this was followed by sodium nitrate (70.16 mm), ammonium nitrate (44.83 mm) and thiourea (26.16 mm). Urea was found least suitable which recorded minimum radial mycelial growth (19.00 mm) over the control (65.33 mm) of the test pathogen.

The nitrogen sources exhibited varied radial mycelial growth and sporulation of the test pathogen. However, highest radial mycelial growth and also excellent sporulation was recorded on potassium nitrate, followed by peptone with good sporulation. Least radial mycelial growth was recorded on urea with poor sporulation.

The present study on the effect of sources of nitrogen *viz*., potassium nitrate, peptone, sodium nitrate and ammonium nitrate to support maximum growth and sporulation in *Alternaria carthami* and several *Alternaria* spp. was reported by Kumari *et al*. (1998).

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