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A CASE STUDY

Effect of different culture media, temperature and pH on growth and sporulation of *Alternaria carthami*

■ **V.M. GHOLVE, M.R. TAWARE AND S.S. WAGH**

SUMMARY

All eight culture media tested encouraged better growth of *Alternaria carthami*. However, Potato dextrose agar gave significantly highest growth (90.00 mm). The second and third best culture media found were Potato malt agar (84.16 mm) and Yeast manitol agar (73.33 mm). Rest of the culture media recorded good amount of mycelial growth in the range of 41.66 mm (Yeast extract agar) to 69.16 mm (Malt extract agar). Colony growth was circular, cottony, grayish-black or olivaceous-black coloured with fair to excellent sporulation. The mean colony growth recorded with all the different temperature ranged from 5.66 mm at 5^0C to 85.66 mm at 30^0C. However, significantly highest mean mycelial growth (85.66 mm) was recorded at 30^0C with excellent (++++) sporulation. The second and third best temperature found were 25^0C (83.83 mm) and 20^0C (66.33 mm). The mean colony growth recorded with all the pH values 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 with excellent (++++) sporulation. The second and third best pH values found were pH 6 (82.00 mm) and pH 7 (70.33 mm) with excellent and good sporulation, respectively.

Key Words : Culture media, Temperature, pH, *A. carthami*, Growth, Sporulation

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Safflower is known to suffer from many fungal diseases at different stage of crop growth are listed as leaf spot/blight (*Alternaria carthami*), wilt (*Fusarium oxysporum* f. sp. *carthami*), root rot (*Rhizoctonia batatiocla*), powdery mildew (*Erysiphe cichoracearum* DC), anthracnose (*Colletotrichum capsici*) (Bhale *et al*., 1998).

Out of several diseases reported on safflower, *Alternaria* blight caused by *Alternaria carthami* is one of the most important disease. This diseases was first reported by Chowdhury (1944) at Pune in India. In India, disease plays an important role in safflower cultivation and responsible to cause 25 to 60 per cent yield losses every year. In general, the season was suitable for the development of foliar diseases particularly *Alternaria* leaf spot/ blight development as there were rains after

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
V.M. GHOLVE, Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA
Email: vikramgholve@rediffmail.com

Address of the Co-authors:
M.R. TAWARE AND S.S. WAGH, Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

sowing in Maharashtra and a disease severity from 10 to 95 per cent was observed. In Marathwada region of Maharashtra, *Alternaria* leaf spot intensity was 30 to 40 per cent, while in Karnataka and Andhra Pradesh maximum intensity of *Alternaria* leaf spot upto 25 per cent and 20 per cent, respectively was observed (Anonymous, 2012). With this view, the present investigation was undertaken to evaluate the efficacy of different culture media and physiological studies on *Alternaria* leaf spot of safflower.

MATERIAL AND METHODS

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. The experiment was conducted by using CRD design with eight treatments and three replications.

Autoclaved and cooled media were poured (@ 20 ml/plate) in sterilized glass Petriplates (90 mm dia) and allowed to solidify at room temperature. On solidification of the media, Petriplates 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 ± 1^{0}C).

Physiological studies :

Effect of temperature :

For studying the temperature effects different levels of temperature were maintained either in refrigerator, incubator, or in hot air oven at 5, 10, 15, 20, 25, 30, 35 and 40^{0}C as per the adjustability of the instruments. The experiment was conducted by using Complete Randomized Design with eight treatments and three replications.

Experiment was conducted in Petriplates on Potato dextrose agar (PDA) medium. PDA was sterilized and poured in sterile Petriplates. 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 :

Hydrogen ion concentration or pH plays a key role in the maintenance of metabolic rate of the pathogen *Alternaria carthami*. The experiment was conducted by using CRD design with nine treatments and three replications. For this experiment PDA was used as basal medium. The pH values of PDA were adjusted by using 0.1 N HCl or 0.1 N NaOH. After adjustment of pH, basal medium PDA was sterilized in autoclave. The cooled medium was poured in Petriplates @ 20 ml/plate and allowed to solidify at room temperature. On solidification of the media Petriplates were inoculated with 5 mm disc of actively growing culture organism and incubated at temperature 27 ± 1^{o}C for seven days. Three replications of each treatment were maintained.

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

RESULTS AND DISCUSSION

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 (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 with Potato dextrose agar. The second and third best media found were Potato malt agar (84.16 mm) and Yeast manitol agar (73.33 mm), respectively. This was followed by 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.

Characteristics :

All the culture media tested exhibited a wide range of colony morphology, growth and colour. The mycelial growth produced on all the culture media was mostly circular to irregular profuse, wooly and loose cottony colonies developed were circular to irregular. Colour of

colonies produced varied from greenish grey to black, olivaceous black with whitish grey periphery, circular periphery of the colonies were whitish grey coloured.

Sporulation :

All the eight culture media tested exhibited a wide range of sporulation from fair (++) to excellent (++++). However, Potato dextrose agar and Potato malt agar recorded excellent (++++) sporulation. Good (+++) sporulation was recorded on Yeast manitol agar, Malt extract agar and Ashby's manitol agar. Whereas, fair (++) sporulation was recorded on Corn meanl agar, Czpack dox agar and Yeast extract agar.

Thus, at all the media tested exhibited varied of radial mycelial growth and sporulation of the test pathogen. However, highest radial mycelial growth (90.00 mm) and excellent sporulation of (++++) was recorded on Potato dextrose agar, followed by Potato malt agar (growth: 84.16 mm and sporulation: excellent). Least radial mycelial growth was recorded on Yeast extract agar (41.66 mm) with fair (++) sporulation.

Thus, 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 (Awadhiya, 1991; Jash *et al*., 2003; Khan *et al*., 2007; Yadhav and Khan, 2008; Hubballi *et al*., 2010).

Physiological studies :

Effect of temperature regimes :

Temperature is one of the most crucial factors, which affects 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.

Mycelial growth :

The results (Table 2) revealed that, all the temperature tested encouraged variable mycelial growth and sporulation of *A. carthami*. The mean colony growth recorded with all the different temperature ranged from 5.66 mm at 5°C to 85.66 mm at 30°C. However,

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

Treatments	Mean colony dia. (mm)*	Characteristics	Sporulation
T_1 : Potato dextrose agar	90.00	Circular, loose and wooly growth, grayish coloured	++++
T_2 : Corn meal agar	48.83	Circular, loose and wooly growth, grayish black with whitish grey periphery	++
T_3: Potato malt agar	84.16	Circular, loose growth, olivaceous grayish coloured	++++
T_4 : Czapek's dox agar	51.33	Circular, loose and wooly growth, grayish black with whitish grey periphery	++
T_5 : Yeast extract agar	41.66	Circular, loose growth, grayish black	++
T_6 : Yeast manitol agar	73.33	Circular, loose and wooly growth, grayish coloured	+++
T_7 : Malt extract agar	69.16	Circular, loose and whitish grey coloured growth	+++
T_8 : Ashby's manitol agar	65.33	Irregular, close cottony growth, olivaceous white coloured	+++
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*

Treatments	Mean colony diameter (mm)*	Sporulation
T_1 : 5°C	5.66	-
T_2 : 10°C	21.33	+
T_3: 15°C	46.16	++
T_4 : 20°C	66.33	+++
T_5 : 25°C	83.83	++++
T_6 : 30°C	85.66	++++
T_7 : 35°C	60.16	++
T_8 : 40°C	25.66	+
S.E. ±	0.40	-
C.D. (P=0.05)	1.20	-

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

significantly highest mean mycelial growth (85.66 mm) was recorded at 30^{0}C. The second and third best temperature regimes were found 25^{0}C (83.83 mm) and 20^{0}C (66.33 mm). This was followed by at 35^{0}C (60.16 mm), 15^{0}C (46.16 mm) and 40^{0}C (25.66 mm) temperature. Temperatures 5^{0}C and 10^{0}C were found least suitable and recorded minimum mean mycelial growth 5.66 mm and 21.33 mm, of the test pathogen, respectively.

Sporulation :

All the eight temperature regimes tested exhibited a wide range of sporulation from poor (+) to excellent (++++). However, 25^{0}C and 30^{0}C recorded excellent (++++) sporulation. Good (+++) sporulation was recorded at 20^{0}C. At 15^{0}C and 35^{0}C temperature fair (++) sporulation was recorded. Whereas, poor (+) sporulation was recorded at 10^{0}C and 40^{0}C temperature. At temperature 5^{0}C sporulation was not observed.

Thus, at all the temperature regimes tested exhibited varied of radial mycelial growth and sporulation of the test pathogen. However, highest radial mycelial growth (85.66 mm) and excellent sporulation (++++) was recorded at 30^{0}C, followed by 25^{0}C (growth: 83.83 mm and excellent sporulation). Least radial mycelial growth (5.66 mm) was recorded at 5^{0}C with poor sporulation (+). The temperatures between 25^{0}C to 30^{0}C were better for growth and sporulation of test pathogen and the temperature below 15^{0}C and above 35^{0}C were unfavorable for growth and sporulation of the test pathogen.

Thus, the similar results of the present study on the effect of different temperature *viz*., 20^{0}C, 25^{0}C, 30^{0}C and 35^{0}C to supported maximum growth and sporulation in *A. carthami* and different *Alternaria* spp. were reported earlier by several workers (Raranciuc, 2002; Singh *et al*., 2005; Ramegowda and Naik, 2006; Hubballi *et al*., 2010 and Mishra and Mishra, 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 therefore; present study included nine different pH values ranging from 4.0 to 8.0. The results obtained during the study are presented in Table 3.

Mycelial growth :

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

The mean colony growth recorded with all the pH values was 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. The second and third best pH values found were pH 6 (82.00 mm) and pH 7 (70.33 mm). This was followed by pH 7.5 (63.16 mm), pH 8.0 (51.00 mm) and pH 5.5 (53.16 mm). The pH values 4.0 and 4.5 were found least suitable and which recorded minimum mycelial growth 30.50 mm and 35.83 mm, respectively of the test pathogen.

Sporulation :

All the nine pH values tested exhibited a wide range of sporulation from poor (+) to excellent (++++). However, at pH 6.0 and 6.5 recorded excellent (++++) sporulation. Good (+++) sporulation was recorded at

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

Treatments	Mean colony diameter (mm)*	Sporulation
T_1 : 4.0	30.50	+
T_2 : 4.5	35.83	++
T_3: 5.0	44.50	++
T_4 : 5.5	53.16	+++
T_5 : 6.0	82.00	++++
T_6 : 6.5	85.83	++++
T_7 : 7.0	70.33	+++
T_8 : 7.5	63.16	+++
T_9 : 8.0	51.00	+++
S.E. ±	0.48	-
C.D. (P=0.05)	1.44	-

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

pH 5.5, 7.0 7.5 and 8.0. At pH 4.5 and 5.0 fair (++) sporulation was recorded. Whereas, poor (+) sporulation was recorded at pH 4.0.

Thus, all the pH values exhibited varied radial mycelial growth and sporulation of the test pathogen. However, highest radial mycelial growth (85.83 mm) and excellent sporulation (++++) was recorded at pH 6.5. This was followed by pH 6.0 (growth 82.00 mm and sporulation: excellent). Least radial mycelial growth (30.50 mm) was recorded at pH 4.0 with poor sporulation (+). The pH between 6 to 6.5 was better for growth and sporulation of test pathogen. The pH below 5.0 and above 8.0 was unfavorable for growth and sporulation of the test pathogen.

Thus, the similar results of the present study on the effect of different pH *viz*., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 to support maximum growth and sporulation in *Alternaria carthami* and different *Alternaria* spp. were reported earlier by several workers (Jash *et al*., 2003; Singh *et al*., 2005; Prathibha *et al*., 2008; Hubballi *et al*., 2010; Taware *et al*. (2014) and Ramjegathesh and Ebenezar, 2012).

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