Journal of Applied and Natural Science 5 (1): 171-178 (2013)

JANS

Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler blight of gerbera (*Gerbera jamesonii* H. Bolus ex J.D. Hook)

Dipak T. Nagrale[1]*, Anil P. Gaikwad[2] and Lalan Sharma[1]

Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri-413722, INDIA
[1]National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan-275101, INDIA
[2]Regional Agricultural Research Station, Mahatma Phule Krishi Vidyapeeth, Lonavala- 410401, INDIA
*Corresponding author. E-mail: dip29unique@gmail.com

Received:February 22, 2013; Revised received:April 10, 2013; Accepted:April 25, 2013

Abstract: Fungal blights are among the major concern for limiting the cultivation and production of many ornamental and flowering plants. Gerbera is an important cut flower with great export potential. However, it is infected by many pathogens in the protected cultivation. The severe infection of fungal blight caused by *Alternaria alternata* (Fr.) Keissler was observed in the polyhouse condition. Hence, in this study, critical morphological and cultural studies were done to understand the pathogen behaviour. The fungus *A. alternata* produced profuse mycelium on Potato Dextrose Agar (PDA) with an average width of 4.42 µm in diameter, conidiophores, conidia and intercalary chlamydospores measured as 42.26 x 4.29 µm, 47.16 x 13.49 µm and 7.22 µm in diameter, respectively. The synthetic media *viz.,* Leonions's agar, Glucose-peptone agar and Sabourand's agar and non-synthetic media, Oat meal agar and PDA were excellent for the mycelial growth and conidial production of *A. alternata*.

Keywords: *Alternaria alternata*, Blight, Cultural characters, *Gerbera jamesonii*, Morphology

INTRODUCTION

Gerbera, commonly known as Transvaal Daisy, Barberton Daisy or African Daisy produces very attractive flowers and it belongs to family Asteraceae. It is an important commercial flower grown throughout the world for beds, borders, pots and rock gardens. In India, gerbera is distributed in the temperate Himalayas from Kashmir to Nepal at altitudes of 1300 to 3200 meters. Gerbera is in demand as cut flower in the world markets and has very good export potential. The production of cut flowers of gerbera under the protected cultivation is threatened by many diseases. Under protected cultivation number of fungal pathogens are known to cause diseases in gerbera like *Alternaria alternata* (Fr.) Keissler, the foot rot (*Pythium irregularae, Phytophthora cryptogea* and *Rhizoctonia solani*), Sclerotium rot (*Sclerotium rolfsii*), grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphae cichoracearum* and *Oidium erysiphoides* f.sp. *gerbera*) and downy mildew (*Bremia lactucae*). However, *Alternaria* blight is an important disease of gerbera in protected cultivation (Ghosh, 1998, Mirkova and Konstantinova, 2003; Nagrale, 2007; Farhood and Hadian, 2012). The severe incidence of blight disease was noticed on two to three months old plants of gerbera under polyhouse condition at polyhouses of Mahatma Phule Krishi Vidyapeeth, Rahuri. The symptomatology and detail study of the disease revealed that the disease is caused by *A. alternata* (Fr.) Keissler. The fungal pathogen, *A. alternata* mostly affects the foliar parts causing light brown to dark brown, roundish-oval to irregular spots of 1 to 2 mm in diameter in initial stage, while later expanded, often coalesced and produced 'Shot hole' during severe infection. The disease severity on foliage was more common during humid weather and symptoms were most pronounced on nutrient deficient leaves (Nagrale, 2007 and Nagrale *et al.*, 2012). *Alternaria* is a genus of of ascomycete fungi. *Alternaria* species are known as major plant pathogens. There are 299 species in the genus *Alternaria* (Kirk *et al.*, 2008, Nowicki- Marcin *et al.*, 2012). Therefore it was decided to study in detail the morphological and cultural characters of the fungal pathogen.

MATERIALS AND METHODS

Collection of diseased sample: The samples of gerbera showing typical symptoms of blight were collected from different greenhouses and polyhouses of university campus and western Maharashtra.

Isolation and maintenance of fungal pathogen: The typical diseased samples of gerbera were collected for isolation and processed for isolation of fungal pathogen. The infected plant part were cut into smaller pieces with a sterile scalpel and disinfected with mercuric chloride solution (0.1%) for one minute with three subsequent

ISSN : 0974-9411 (Print), 2231-5209 (Online)

washings in sterilized distilled water. Then cut samples were dried by sterilized blotting paper. The isolation of blight causing fungal pathogen was made by standard agar plate technique (APT) on potato dextrose agar and was incubated at 27 $\pm$ 1^{0}C temperature. The growth of fungi noticed after four days of inoculation was sub-cultured on Potato Dextrose Agar (PDA) slants to obtain pure cultures. One month old healthy gerbera saplings of highly susceptible variety were selected for proving the pathogenicity of the fungal pathogen. The pathogenicity of test fungus was proved in artificial epiphytotics condition with high relative humidity. The pathogenicity of isolated cultures was proved by 'Microdroplet Inoculation Technique' *i.e.* MDIT (Munaut *et al.*, 1997) and by 'Mycelial Bit Inoculation Method' i.e. MBIM (Rocha *et al.*, 1998). The reisolation was carried out from artificially infected leaves and flowers in the same way as described earlier. The isolates of the pathogenic fungi thus obtained were transferred on PDA slants for comparison with original culture. The pure fungal pathogen thus obtained was maintained on PDA slants in refrigerator at 4^{0}C for further studies.

Morphology of the fungal pathogen: Morphological characters of the fungal pathogen infecting gerbera, were studied from the culture growth on PDA for 5 to 10 days at 27 $\pm$ 1^{0}C. As suggested by Chowdhry and Varshney (2000), observations regarding morphological characters of different structures *viz.*, mycelium (young and matured), conidiophores, conidia and chlamydospores were noted by adopting slide culture technique. The microscopic measurements were taken with the help of filar micrometer. Averages based on 50 observations for each structure, recorded from 5 different slides of 10 randomly selected individuals from each slide. The measurements for young and old mycelium were recorded from five and ten day's old cultures, respectively.

Growth and cultural characters of fungal blight pathogen: The fungal pathogen was grown on different media by using agar plate technique (APT) in order to study its growth and cultural characters on different media. The sterilized petriplates of uniform size were poured in with twenty different synthetic (Table 2) and non-synthetic media (Table 3) separately for each medium. After solidification of media the plates were inoculated at the centre with uniform sized bits (5 mm) of seven days old culture of the pathogen. A set of quadruplicate plates was maintained for each medium. The inoculated plates were then incubated at 27 $\pm$ 1^{0}C temperature in BOD incubator in inverted position. The observations on mean colony diameter and degree of sporulation were recorded at 48 hrs interval, while spore count and other growth characters were noted eight days after inoculation. Colour of the fungal colony was judged by using standard of Methuen Handbook of colour (Kornerup and Wanscher, 1967). The spore count was measured with the help of haemocytometer as per the standard methodology. The growth rate of the fungus on each medium was calculated as follows.

$$GR = \frac{S_{x+1} - S_x}{T_{x+1} - T_x}$$

Where, G R = Growth rate (mm hr^{-1}), S = Colony diameter (mm), T = Time (hrs.)

RESULTS

Isolation and characterization of pathogenic fungi: The isolated fungi from the blighted gerbera leaves were compared with reisolated fungal pathogen. The symptomatology, pathogenicity and morphology confirmed the identity of pathogenic fungi as *A. alternata*. The pathogenicity studies by MDIT and MBIM showed that the fungus infects the gerbera foliage causing typical symptoms of disease. The symptoms on the leaves by both the methods of inoculation were appearance of small, circular to irregular spots of 2 to 4 mm in size. Further, light brown to dark brown patches with characteristic concentric zonations inside the spots were conspicuous and in severe cases, the spots enlarged in size with complete drying and blightening of leaves occurred.

Morphological characters of the fungus *A. alternata*: Morphological observations of the fungus were recorded by adopting slide culture technique. The measurements of different morphological structures of *A. alternata* are presented in Table 1. The fungus produced profuse mycelial growth on PDA. Initially, the mycelium was hyaline that turned to grey- brownish, multicelled, septate and irregularly branched. In early growing stage, hyphae were thin (2.84 µm in diameter), narrow, hyaline but became slightly thick (4.42 µm in diameter) as they grew old. Conidiophores arised singly or in clusters, usually 2-6 and were long or short. They were pale olivaceous to olivaceous- brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. The conidiophores measured 42.26 µm (27.30-112 µm) in length and 4.29 µm (3.12-8.43 µm) in width. The length : width ratio was 9.85 (8.75 - 13.29). Conidia were born in chains upto 10 or more on conidiophores. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. The muriform conidia inclusive of beak measured 47.16 µm (21.82 - 96.40 mm) x 13.49 mm (8.26 - 16.52 µm). The length of the condium was 3-5 times (2.64 to 5.84 times) more than its width. The beak measured 27.12 µm (22.62 - 58.69 µm) in length. The chlamydospores were formed in the old culture of *A. alternata*. They were intercallary, thick

Table 1. Measurement of different morphological structures of *A. alternata* causing blight of gerbera.

Sr. No.	Morphological structures	Measurement parameters (µm)		
		Length	Width	L : W ratio
1.	Mycelium (young)	-	2.84 (1.52 - 4.66)	-
2.	Mycelium (Old)	-	4.42 (4.28 - 6.69)	-
3.	Conidia	47.16 (21.82 – 96.40)	13.49 (8.26 – 16.52)	3.50 (2.64 – 5.84)
4.	Conidiophores	42.26 (27.30 – 112)	4.29 (3.12 – 8.43)	9.85 (8.75 – 13.29)
5.	Chlamydospores	-	7.22 (4.92 – 9.89)	-

walled, roundish to oval in shape, dark brown in colour and measuring 7.22 µm (4.92 - 9.89 µm) in diameter.

Growth and cultural characters of *A. alternata* on synthetic media: The growth and colony characters of fungus on different synthetic media are presented in Table 2. The treatment differences in respect of colony diameter and growth rate at every 48 hrs interval as well as sporulation time and spore count were statistically significant. The synthetic culture media evaluated for growth characters exhibited varying degree of growth rates, mean colony diameter, sporulation time and spore count.

Growth: The maximum growth rate of 0.39 mm hr^{-1} was recorded on Leonion's agar with colony diameter of 7.50 cm. The media *viz.*, Tap water agar, Glucose-peptone agar, Sabourand's agar and Ashby's agar recorded the colony diameter of 6.42, 6.30, 6.13 and 6.03 cm and growth rates of 0.34, 0.33, 0.32 and 0.31 mm hr^{-1}, respectively. The media *viz.*, Jenson's agar, M_2 agar and Czapek's Dox agar recorded the moderate colony diameter of 5.47, 5.48 and 5.35 cm and growth rates of 0.29, 0.29 and 0.28 mm hr^{-1}, respectively. On the contrary, the media *viz.*, Asthana-Hawker's and Richard's agar produced the least colony diameter of 4.06 and 2.75 cm and growth rates of 0.21 and 0.14 mm hr^{-1}, respectively.

Sporulation: The sporulation data recorded eight days after inoculation revealed that significantly highest spore count (0.48 x 10^4 cm^{-2}) was observed in Leonion's agar. It was followed by Sabourand's agar, Glucose-peptone agar and M_2 agar, which sporulated 0.40, 0.32 and 0.29 x 10^4 conidia cm^{-2}, respectively. Further, moderate sporulation was noted on Richard's, Czapek's, Jenson's and Ashby's agars, which sporulated 0.12, 0.09, 0.08 and 0.07 x 10^4 conidia cm^{-2}, respectively. While, very scanty sporulation was noticed on Tap water agar that sporulated just 0.02 x 10^4 conidia cm^{-2}.

Sporulation time: The significantly minimum time of just 48 hours was required for sporulation of *A. alternata* on Jenson's agar. After this, the pathogen on Czapek's, Sabourand's, M_2 and Leonion's agars took moderate time of 96 hours for sporulation. Then, Glucose-peptone agar supported sporulation at 144 hours while Richard's, Ashby's, Asthana-Hawker's and Tap water agars significantly sporulated very late (i.e. at 192 hours). Thus, the synthetic media *viz.*, Leonions's agar, Glucose-peptone agar and Sabourand's agar were the best for growth and sporulation of *A. alternata*.

Growth and cultural characters of *A. alternata* on non-synthetic media: The data in Table 3 revealed that the differences in respect of colony diameter, growth rate, sporulation time and spore count were statistically significant.

Growth: The non-synthetic media exhibited varying degree of growth characters as indicated in Table 3. The growth attained by the pathogen on different non-synthetic media is shown in Fig. 22. The non-synthetic medium, Prune agar showed maximum colony diameter (9.07 cm) and mean growth rate (0.47 mm hr^{-1}) of the pathogen. However, it was at par with Carrot agar and Wheat meal agar, which recorded 8.5 and 8.44 cm colony diameter and growth rate of 0.44 and 0.44 mm hr^{-1}, respectively. Thereafter, the media *viz.*, PDA, Oat meal, Bean meal, Starch meal, Corn meal and Tap water agars showed the colony diameter of 8.01, 7.95, 7.77, 7.29, 7.20 and 6.42 cm and growth rate of 0.42, 0.41, 0.38, 0.37 and 0.34 mm hr^{-1}, respectively.

The significantly maximum sporulation (0.61 x 10^4 spores cm^{-2}) was noticed in Potato dextrose agar. It was followed by Wheat meal (0.48 x 10^4 spores cm^{-2}), Oat meal (0.51 x 10^4 spores cm^{-2}) and Bean meal (0.47 x 10^4 spores cm^{-2}) agars. The moderate sporulation was noticed in Carrot and Prune agars (0.29 x 10^4 spores cm^{-2}), Corn meal agar (0.28 x 10^4 spores cm^{-2}) as well as Starch agar (0.23 x 10^4 spores cm^{-2}). In contrast, Host decoction with 2 per cent dextrose (0.08 x 10^4 spores cm^{-2}) and without dextrose (0.06 x 10^4 spores cm^{-2}) and also Tap water agar (0.02 x 10^4 spores cm^{-2}) yielded significantly least spores of the pathogen.

Sporulation time: The pathogen *A. alternata* sporulated within significantly minimum (48 hrs.) time on Potato

Table 2. Colony diameter, growth rate, sporulation and growth characters of *A. alternata* from Gerbera on different synthetic media

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm hr^{-1}) after hours of inoculation					Growth (degree)	Sporulation			Colony characters			
		48	96	144	192	Mean		Time (hr)	Count (x 10^4 cm^{-2})	Degree	Colour	Margin	Shape	Nature
1.	Richard's	0.90 (0.19)	1.58 (0.14)	2.07 (0.10)	2.75 (0.14)	(0.14)	+	192	0.12	+	Dull white to green	Serrated	Roughly circular	Sub-aerial
2.	Czapeck's	1.05 (0.22)	2.75 (0.35)	4.22 (0.31)	5.35 (0.24)	(0.28)	++	96	0.09	+	Dull white to indigo	Serrated	Roughly circular	Sub-aerial, moderately raised,tree like branching of mycelium
3.	Jenson's	1.23 (0.26)	2.78 (0.32)	3.88 (0.23)	5.47 (0.33)	(0.29)	++	48	0.08	+	Dark brown to black	Entire	Circular	Sub-marged, slightly raised mycelium
4.	Glucose peptone	1.91 (0.40)	3.61 (0.35)	4.95 (0.28)	6.30 (0.28)	(0.33)	+++	144	0.32	+++	Light to ashy white	Entire	Circular	Sub-merged to slightly raised mycelium
5.	Sabourand's	2.25 (0.47)	3.95 (0.35)	5.03 (0.23)	6.13 (0.23)	(0.32)	+++	96	0.40	+++	Green to dark greenish	Wavy	Roughly circular	Sub-aerial, moderately raised mycelium with concentric rings
6.	M_2	1.32 (0.28)	3.13 (0.38)	4.50 (0.29)	5.48 (0.20)	(0.29)	+++	96	0.29	++	Light to dull white	Entire	Circular	Sub-aerial, slightly raised mycelia with light concentric rings
7.	Leonions	2.22 (0.46)	4.27 (0.43)	5.88 (0.34)	7.50 (0.34)	(0.39)	++++	96	0.48	++++	Light to dull white	Entire	Circular	Sub-aerial, slightly raised mycelia with light concentric rings
8.	Ashby's	1.03 (0.21)	2.15 (0.23)	3.75 (0.33)	6.03 (0.48)	(0.31)	+++	192	0.07	+	White to light ashy	Entire	Circular	Aerial, raised mycelium without concentric rings
9.	Asthana-Hawker's	1.09 (0.23)	2.01 (0.19)	2.52 (0.11)	4.06 (0.32)	(0.21)	++	192	0.14	+	Cottony white	Wavy	Sub-circular	Aerial, raised mycelium without concentric rings
10.	Tap water	1.98 (0.41)	3.30 (0.28)	4.72 (0.30)	6.42 (0.35)	(0.34)	+	192	0.02	+	Dull white	Entire	Circular	Submerged, very sparse mycelium, growth with one concentric ring
	S.E. ±	0.10	0.25	0.22	0.29	0.01		2.19	0.01					
	CD at 5 %	0.29	0.73	0.65	0.84	0.03		6.44	0.04					
	C.V. (%)	6.65	8.46	5.29	5.17	4.03		1.63	7.27					

+ = Scanty,++ = Moderate, +++ = Good, ++++ = Abundant,- = Nil

Note: Figures in the parentheses are of growth rates

Table 3. Colony diameter, growth rate, sporulation and growth characters of *A. alternata* from Gerbera on different non synthetic media

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm hr^{-1}) after hours of inoculation					Growth degree	Sporulation			Colony characters			
		48	96	144	192	Mean		Time (hr)	Count (x 10^4 cm^{-2})	Degree	Colour	Margin	Shape	Nature
1.	Oat meal	2.13 (0.44)	4.07 (0.40)	5.87 (0.38)	7.95 (0.43)	(0.41)	++++	48	0.51	++++	Ashy white to light green	Entire	Circular	Aerial, profuse mycelium with concentric rings
2.	Bean meal	2.28 (0.48)	4.43 (0.45)	6.10 (0.35)	7.77 (0.35)	(0.41)	++++	48	0.47	++++	Light ashy to dark blackish	Entire	Circular	Aerial, profuse mycelium with broad concentric rings
3.	Corn meal	1.98 (0.41)	3.68 (0.35)	5.27 (0.33)	7.20 (0.40)	(0.37)	++++	48	0.28	++	Indigo to dark brown	Entire	Circular	Sub merged mycelium with small concentric rings
4.	Host decoction i. 2 % dextrose	0.85 (0.18)	2.07 (0.25)	3.15 (0.23)	4.48 (0.28)	(0.24)	++	96	0.08	+	Dark green to dark blackish	Entire	Circular	Aerial mycelium with white concentric rings
	ii. Without dextrose	0.60 (0.13)	1.23 (0.13)	2.18 (0.20)	3.40 (0.25)	(0.18)	+	144	0.06	+	White to light brown	Entire	Circular	Sub-aerial mycelium with concentric rings
5.	Carrot	2.50 (0.52)	4.80 (0.48)	6.58 (0.37)	8.50 (0.40)	(0.44)	++++	48	0.29	++	Creamy to dark blackish	Entire	Circular	Aerial, highly profuse mycelium with broad concentric rings
6.	PDA	2.15 (0.45)	4.39 (0.47)	6.11 (0.36)	8.01 (0.40)	(0.42)	++++	48	0.61	++++	Creamy to ashy white	Entire	Circular	Sub merged mycelium with small concentric rings
7.	Prune	3.07 (0.64)	6.18 (0.65)	7.88 (0.35)	9.07 (0.25)	(0.47)	++++	48	0.29	++	White, creamy to ashy	Entire	Circular	Aerial, highly profuse with island of white mycelial growth
8.	Starch	2.19 (0.46)	4.22 (0.42)	5.65 (0.30)	7.29 (0.34)	(0.38)	+++	96	0.23	++	Creamy grayish white	Entire	Circular	Sub-aerial mycelium with concentric rings
9.	Wheat meal	2.38 (0.50)	3.70 (0.28)	6.10 (0.50)	8.44 (0.49)	(0.44)	++++	48	0.48	++++	Ashy to dark green	Entire	Circular	Aerial, raised mycelium with concentric rings
10.	Tap water	1.98 (0.41)	3.30 (0.28)	4.72 (0.30)	6.42 (0.35)	(0.34)	++	192	0.02	+	Dull white	Entire	Circular	Submerged very sparse mycelium growth with one concentric ring
	S.E. ±	0.11	0.12	0.12	0.32	0.01		2.28	0.02					
	CD at 5 %	0.33	0.35	0.35	0.93	0.03		6.66	0.05					
	C.V. (%)	5.64	3.12	2.18	4.46	2.56		2.90	6.19					

+ = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant, - = Nil

Note : Figures in the parentheses are of growth rates

dextrose, Wheat meal, Oat meal, Bean meal, Corn meal, Carrot and Prune agars. Further, host decoction agar with 2 per cent dextrose and Starch agar sporulated at 96 hrs. after inoculation. Whereas, Host decoction agar without dextrose (144 hrs.) and Tap water agar (192 hrs.) took significantly maximum time to initiate sporulation. Therefore, these results clearly indicated that PDA, Oat meal agar, Wheat meal agar and Bean meal agar were the excellent non-synthetic media for growth and sporulation of *A. alternata*, while Prune agar and Carrot agar noticed to be good for mycelial growth only, which supported moderate sporulation.

DISCUSSION

Fungal blight is among the major diseases of many cultivated and ornamental plants under protected cultivation. The occurrence of the fungal blight on gerbera caused by *A. alternata* was observed severely in many polyhouses of the western Maharashtra. Recently, Ghosh (1998),Mirkova and Konstantinova (2003),Nagrale (2007) and Farhood and Hadian (2012) have also reported species of *Alternaria* to cause the disease in gerbera. The Koch's postulate as pathogenicity test in the present investigation for fungal pathogen *A. alternata* is in congruence with the results of Mirkova and Konstantinova (2003), Nagrale (2007) and Farhood and Hadian (2012). The symptoms on leaves were appeared as small, circular to irregular spots of 2 to 4 mm in size. Further, light brown to dark brown patches with characteristic concentric zonnations inside the spots were conspicuous and in severe cases, the spots enlarged in size with complete drying and blightening of leaves occurred. Similar results were reported by Wellman (1949), Ghosh (1998), Mirkova and Konstantinova (2003), Nagrale (2007), Farhood and Hadian (2012).

The various morphological structures *viz.*, mycelium, conidiophores, conidia and chlamydospores of *A. alternata* were studied. The fungus produced profuse mycelial growth on PDA. Initially, the mycelium was hyaline that turned to gray-brownish, multicelled, septate and irregularly branched, measuring 4.42 µm in diameter as they grew old. The finding about mycelium colour is in conformity with results of Sonawane (1983), Narain *et al.* (1985) and Deshmukh (1998), who noticed mycelium of *Alternaria* spp., as grayish to green, brown and deep olive grey in colour those infected pomegranate, watermelon and chilli, respectively. The profuse mycelial growth of *Alternaria* on PDA was noticed by Shinde (1995), Deshmukh (1998) and Shinde (2003) that was isolated from soybean, chilli and aster, respectively, which is also tallying with present investigations. The characters like irregular branching of mycelium and septation are in conformity with results of Rao (1965), Sonawane (1983), Narain *et al.* (1985), Shinde (1995) and Deshmukh (1998) who observed similar kind of mycelium of *Alternaria* spp. infecting gerbera, pomegranate, watermelon, soybean and chilli, respectively. The mycelium width measurement of 4.42 µm from present study tallies with the results of Shinde (1995), Deshmukh (1998) and Shinde (2003) who noticed it as 4.53, 4.10 and 4.53 µm in diameter, respectively.

Conidiophores aroused singly or in clusters, usually 2-6 and were long or short. The conidiophore characters are in agreements with the results of Rao (1965), Sonawane (1983), Narain *et al.* (1985) and exactly matching with the report of Ghosh (1998) who also noted similar kind of conidiophores. The conidiophores were pale olivaceous to olivaceous-brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. The present findings are in conformity with the result of earlier workers (Rao, 1965; Sonawane, 1983; Narain *et al.*, 1985, Shinde, 1995; Deshmukh, 1998; Shinde, 2003 and Ghosh, 1998) and Ramjegathesh and Ebenezar (2012) wherein, they observed more or less same types of conidiophores. The conidiophores measured 42.26 mm in length and 4.29 mm in width. These measurements varied slightly from the previous reports of Rao (1965) and Narain *et al.* (1985), but are very close to results of Shinde (1995), Deshmukh (1998), Ghosh (1998) and Shinde (2003) and Farhood and Hadian (2012) in either one of or both the components, *i.e.* length and breadth of conidiophores.

Conidia were born in chains upto 10 or more on conidiophores. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2 to 10 transverse septa. The present finding about conidial chains and colour are in agreements with results of Narain *et al.* (1985), Shinde (1995), Deshmukh (1998), Ghosh (1998), Shinde (2003) and Akhtar *et al.* (2004). Similarly, Rao (1965) also observed the conidial chains and Sonawane (1983) noticed the same colour of conidia indicating similarity in results in respective conidial parameters. Further, the present findings about shape and septation of conidia are in conformity with the reports of Rao (1965), Sonawane (1983), Narain *et al.* (1985), Shinde (1995), Deshmukh (1998), Ghosh (1998) and Shinde (2003). The muriform conidia inclusive of beak measured 47.16 µm (21.82 – 96.40 µm) x 13.49 µm (8.26 – 16.52 µm). The conidial measurement nearly tally with results of Rao (1965), Shinde (1995) and Shinde (2003). However, the size of conidia as recorded by Deshmukh (1998) and Ghosh (1998) differed from the above mentioned results. The chlamydospores were intercalary, thick, roundish to oval in shape, dark brown in colour and measuring 7.22 µm in diameter. The observations regarding chlamydospores are not reported by earlier workers.

The cultural characters of fungus on different synthetic media under study revealed that the media *viz.,* Leonions agar, Glucose-peptone agar and Sabourand's agar were the best for growth and sporulation of fungus. The results in respect of Leonion's agar are in conformity with the finding of Shinde (1995) who also noticed good growth and sporulation of the fungus *A. alternata* on this medium. The effectiveness of Glucose-peptone agar medium as a good medium for growth and sporulation of *A. alternata* was not reported earlier by any scientist. Similarly, the observations in respect of Sabourand's agar as best medium for *A. alternata* are in conformity with the findings of Gaikwad (1987) and Pingale (1996) wherein they reported the effectiveness of this medium for growth as well as sporulation of the same fungus. However, the present findings do not tally with the work carried out by Kalane (1979), Sonawane (1983), Ghosh (1998) and Bhorde (2006) who noticed excellent mycelial growth and sporulation of fungus *A. alternata* on Richard's agar as synthetic media. This medium was observed to be very poor in the present studies.

The cultural characters of the pathogen on different non-synthetic media under study indicated that PDA, Oat meal agar, Wheat meal agar and Bean meal agar were the excellent media for growth and sporulation of *A. alternata* from gerbera. These results are exactly matching with results of Kapoor and Hingorani (1958) who also noticed excellent growth and sporulation of *A. alternata* on PDA and Oat meal agar. Similarly, the findings are nearer to report of Lonnaidis and Main (1973) who obtained optimum sporulation of *A. alternata* on PDA and Oat meal agar. The findings in respect of PDA as an excellent non-synthetic media are also in conformity with Kalane (1979), Sonawane (1983), Gaikwad (1987), Pingale (1996), Ghosh (1998) and Ramjegathesh and Ebenezar (2012), while the effectiveness of Wheat meal and Bean meal agars were not studied by the earlier workers.

Hence, the results in this study revealed that the fungus *A. alternata* produced profuse mycelium on PDA with average width of 4.42 µm in diameter. However, conidiophores were short or long, pale olivaceous to olivaceous-brown, geniculate and measured 42.26 x 4.29 µm. Conidia were in chains, light olivaceous to dark brown, septate, muriform and measured 47.16 x 13.49 µm. Chlamydospores were intercalary, thick, roundish to oval, dark brown in colour and measured 7.22 µm in diameter. The synthetic media *viz.,* Leonions's agar, Glucose-peptone agar and Sabourand's agar; and non-synthetic media, Oat meal agar and PDA were the excellent for mycelial growth and conidial production of *A. alternata*.

REFERENCES

Akhtar, K.P., Saleem, M.Y., M. Asghar and M.A. Haq (2004). New report of *Alternaria alternata* causing leaf blight of tomato in Pakistan, *New Disease Reports*, 9: 43·

Bhorde, B.M. (2006). Studies on pathogen (*A. alternata*) associated with fruit rot of Aonla (*Emblica officinalis* Gaertn.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Chowdhry, P.N. and Varshney, A. (2000). Identification of different *Colletotrichum gloeosporioides* species. In manual on identification of plant pathogenic and biocontrol fungi of Agricultural importance (pp 73-78).P.N. Chowdhry (Ed), Center of Advance studies in plant pathology, Division of Plant Pathology, IARI, New Delhi.

Deshmukh, A.N. (1998). Studies on leaf spot and fruit rot of chilli caused by *Alternaria alternata* (Fr.) Keissler. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Farhood,S. and Hadian,S. (2012). First report of *Alternaria* leaf spot on Gerbera (*Gerbera Jamesonii* L.) in North of Iran. *Adv. Environ. Biol.*, 6(2): 621-624.

Gaikwad, B.S. (1987). Studies on fruit rot and leaf spot of ber (*Zizyphus mauritiana* L.) caused by *Alternaria alternata* (Fr.) Keissler. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Ghosh, C.L. (1998). Studies on *Alternaria alternata* (Fr.) Keissler of Gerbera (*Gerbera jamesonii* Hook.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Kalane, D.G. (1979). Studies on leaf blight of (*Chrysanthemum indicum* L.) caused by *Alternaria alternata* (Fr.) Keissler. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri (M.S.), India.

Kapoor, J.N. and Hingorani, M.K. (1958). *Alternaria* leaf spot and fruit rot of brinjal. Indian *J. Agric. Sci.*, 28: 109-114.

Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J.A. (2008). *Dictionary of the Fungi. 10th ed*. Wallingford: CABI.

Kornerup, A. and Wanscher, J.H. (1967). Methuen Handbook of colour. Publ. By Methuen and Co. Ltd., 11 Newsletter lane, London, E.C. 4.

Lonnaidis, N. M. and Main, C. E. (1973). Effect of culture medium on production and pathogenicity of *Alternaria alternata* conidia. *Pl. Dis. Reptr.*, 57 (1): 39-42.

Mirkova, E. and Konstantinova, P., (2003). First report of *Alternaria* leaf spot on Gerbera (*Gerbera Jamesonii* H. Bolux ex J.D.Hook) in Bulgaria. *Journal of Phyttopathology*, 151(6): 323-328.

Munaut, F., Hamide, N. and Marate, H. (1997). A standardized microdroplet inoculation technique for analysis of the interaction between *Colletotrichum gloeospoiroides* and *Stylosanthes guianensis*. *Parasitica*, 53 (4): 117-125.

Nagrale D.T. (2007). Studies on blight of gerbera (*Gerbera jamesonii* Hook.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Nagrale, D.T., Gaikwad, A.P., Goswami, S. and Sharma, L. (2012). Fungicidal management of *Alternaria alternata* (Fr.) Keissler causing blight of gerbera (*Gerbera jamesonii* H. Bolus ex J.D. Hook). *J. Appl. & Nat. Sci.*, 4 (2): 220-227.

Narian, Avahesh, Swain, N.C., Sahoo, K.C., Dash, S.K. and Shukla, V.D. (1985). A new leaf blight and fruit rot of watermelon, *Indian Phytopath.*, 38(1): 149-151.

Nowicki, M., Nowakowska,M., Niezgoda, A. and Kozik, E.(2012). *Alternaria* black spot of crucifers: Sympotoms, importance of disease and perspectives of resistance breeding, *Veg. Crops Res. Bull.*, 76(1):5-19.

Pingale, A.P. (1996). Studies on *Alternaria* leaf spot of Feba bean (*Vicia feba* L.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Ramjegathesh, R. and Ebenezar, E.G. (2012). Morphological and physiological characters of *Alternaria alternata* causing leaf blight disease of onion. *Int. J. Pl. Pathol.*, 3(2):34-44.

Rao, V.G. (1965). *Alternaria tenuis* auct. in Bombay-Maharashtra. Ibid. *Mycopath. Mycol. Appl.* 27: 257-260.

Rocha, J. de. R-de.S., Oliveira, N.T. de and Menezes, M. de. (1998). Comparison of inoculation methods efficiency for evaluation of *Colletotrichum gloeospoiroides* isolates pathogenicity on passion fruits (*Passiflora edulis*). *Brazilian-Archives of Biol. and Technol.* 41(1): 145-153.

Shinde, B.M. (1995). Investigation on leaf spot disease of soybean (*Glycine max* L. Merill) caused by *Alternaria* and *Dreschslera*. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Shinde, M.A. (2003). Studies on leaf spot of Aster (*Callistephus chinensis* L. Nees). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Sonawane, C.S. (1983). Part-I Studies into Post harvest diseases of pomegranate (*Punica granatum* L.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.

Wellman, F.L. (1949). Successful spray control of *Alternaria* blight of petunias grown for seed in Costa Rica. *Pl. Dis. Reptr.*, 33(2): 69-72.