Int.J.Curr.Microbiol.App.Sci (2019) *8*(4): 1900-1910

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 04 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.804.223

Isolation, Pathogenicity and Effect of Different Culture Media on Growth and Sporulation of *Alternaria brassicae* (berk.) Sacc. causing *Alternaria* Leaf Spot Disease in Cauliflower

H.T. Valvi, J.J. Kadam and V.R. Bangar*

Department of Plant Pathology, College of Agriculture, Dapoli, India
Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist.,
Ratnagiri- 415 712(M.S.), India

**Corresponding author*

Keywords

Cauliflower, Isolation, Pathogenicity, *Alternaria brassicae* (Berk.) Sacc., Culture media, Sporulation and mycelial growth

Article Info

Accepted:
15 March 2019
Available Online:
10 April 2019

A B S T R A C T

The leaf spot disease of Cauliflower (*Brassica oleracae* L. var. *Botrytis*) caused by *A. brassicae* (Berk.) Sacc. was noticed in moderate to severe form on farm of College of Agriculture Engineering and Technology, Dapoli during 2014-2016. The pathogenic fungus was isolated on potato dextrose agar medium from infected leaves of cauliflower. The pathogenicity of the isolated fungus was proved by inoculating healthy seedlings of cauliflower. On the basis of typical symptoms on foliage, microscopic observations and cultural characteristics of the fungus, it was identified as *Alternaria* spp. The Chief Mycologist, Agharkar Research Institute, Pune identified the pathogenic fungus as *Alternaria brassicae* (Berk.) Sacc. Eight culture media were tested among that, the potato dextrose agar medium was found most suitable and encouraged maximum radial mycelial growth (90.00 mm) of *A. brassicae*. The second best culture medium found was host leaf extract agar medium (87.00 mm). This was followed by Richard's agar medium (75.33 mm) and oat meal agar medium (71.66 mm). Carrot potato agar medium (55.00 mm), Asthana and Hawker's agar medium (51.66 mm) and Czapek's Dox agar medium (39.00 mm) were moderate in mycelial growth of *A. brassicae*. Poor mycelial growth was recorded in V8 juice agar medium (32.66 mm).

Introduction

Cauliflower (*Brassica oleracae* L. var. *Botrytis*) belongs to cruciferae/brassicae family. It is originated from wild cabbage known as 'Cole warts', through mutation, human selection and adoption. The name cauliflower consists of two Latin words, 'caulis' meaning cabbage and 'floris', meaning flower.

Cauliflower is one of the most important winter vegetables of India. India produces 8573.3 MT of cauliflower in the year 2013-14 from 433.9 ha. area with an average productivity of about 19.8mt/ha. In

Maharashtra, the area under cauliflower is 36.0 ha with total production of 813.2mt and average productivity of 22.6 MT/ha in the year 2013-14. The major cauliflower producing states are Bihar, Uttar Pradesh, Orissa, West Bengal, Assam, Haryana and Maharashtra (Anonymous, 2013).

Several factors are responsible for low production of cauliflower crop, among which diseases also play an important role. The important diseases of Cauliflower crop are leaf spot, Downy mildew, Damping off, Club root, Powdery mildew, White rust, Black rot, Bacterial soft rot and Cauliflower mosaic. Among these diseases *Alternaria* leaf spot is a serious disease of cauliflower.

The disease appears as minute specks on the leaves, which enlarge over a time and result in substantial lesions with concentric rings where spores are produced.

Defoliation of the outer leaves may occur on severely infected plants and extensive trimming may be required to remove infected leaves from the cabbage head at harvest. In susceptible varieties, apart from yield, significant reduction in quality may occur. Considering importance of the crop and disease, present study was planned and conducted with isolation, pathogenicity and cultural characteristics of pathogen to know its survival, association with host plant in Konkan region and *in vitro* characteristics and further management studies.

Materials and Methods

Examination of diseased samples

Visual observations

Visual observations of disease symptoms were recorded in the field to know the development of the disease in a plant population under natural conditions.

Disease sample

The cauliflower leaves showing typical symptoms of leaf spot were collected in the paper bags from the College of Agricultural Engineering and Technology, Dapoli and brought to the laboratory for further studies.

Microscopic examination

These samples were then washed under tap water to remove extraneous material. Temporary mounts were prepared from the diseased specimens in lacto-phenol cotton blue and examined under compound microscope for presence of microorganism if any.

Isolation of causal organism

Small bits of desired size of infected samples were cut by taking care that each bit contained half infected and half healthy portion. Such bits were then disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride.

These bits were then placed on sterilized blotters for drying. Properly dried bits were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium.

The plates were incubated in BOD incubator at 24 ± 1°C till the fungal mycelium fully covered the surface of the medium. The fungal growth obtained was then transferred to PDA slants and maintained as stock culture for further studies.

Pathogenicity test of the isolated organism

Inoculation

Three Seeds of cauliflower (variety-super fast crop) was sown in the earthen pots containing

desired potting mixture. Potting mixture comprising FYM and soil (1:2) was autoclaved for three successive days in order to kill the micro flora present if any.

One healthy growing cauliflower seedling per pot was maintained and watered regularly. Spore-cum mycelial suspension of the test pathogen was prepared by pouring the distilled sterile water in 7-8 days old culture plates.

The resultant spore cum-mycelial suspension was filtered through muslin cloth and filtrate obtained was suitably diluted with distilled sterile water to get inoculum concentration of 10^5 spores/ml (Pattanamahakul and Strange, 1999).

Forty five days old seedlings of cauliflower already grown in earthen pots were artificially inoculated by spraying the spore-cum-mycelial suspension (10^5 spores/ml) of the test fungus with an automizer. Seedlings grown in earthen pots and sprayed only with sterile water (without inoculum) were maintained as control.

Development of symptom

Pots (both inoculated and non-inoculated) were incubated in the moist chamber prepared with a wooden frame covered with a muslin cloth. Proper humidity (85-90%) was maintained in the chamber by frequently spraying sufficient clean water on the muslin cloth. Seedlings were watered as and when required till the development of typical disease symptoms.

The causal organism was re-isolated from the artificially inoculated leaves showing typical symptoms of the disease.

The fungal growth obtained on PDA medium on re-isolation was compared with the original culture obtained from naturally infected leaves under field conditions.

Identification of the causal organism

The re-isolated, pure fungal culture was identified in Department of plant pathology, college of agriculture, Dapoli, Ratnagiri comparing its morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification.

Further, culture was sent to chief Mycologist, Agharkar Research Institute, Pune for identification of the fungus up to species level.

Effect of culture media on growth and sporulation

Five synthetic and three non-synthetic media were evaluated in the present study. Media were prepared with given composition. The initial pH of each medium was adjusted to 6.5 prior to autoclaving. The medium was prepared with given composition and dispended in conical flask.

The flasks were plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs. psi for 20 minutes. Petri plates were sterilized in hot air oven at 160^0C for 1 hour.

Such sterilized Petri plates were poured with 20 ml of molten medium and allowed to solidify. Five millimetre diameter disc of the test fungus was cut with the help of incinerated cork borer and inoculated at the centre of Petri plates.

The inoculated plates were then incubated at room temperature (27 ± 2^0C) for 7 days. The compositions of all the media used were obtained from Ainsworth and Bisby's Dictionary of the fungi as mentioned below.

Media used and their composition

1)	**Czapek's Dox agar medium**		
	i) Sucrose ($C_{12}H_{22}O_{11}$)	:	**30.00 g**
	ii) Sodium nitrate ($NaNO_3$)	:	**2.00 g**
	iii) Potassium dihydrogen phosphate (KH_2PO_4)	:	**1.00 g**
	iv) Magnesium sulphate ($MgSO_4.2H_2O$)	:	**0.50 g**
	v) Potassium chloride (KCl)	:	**0.50 g**
	vi) Ferric chloride ($FeCl_3$)	:	**0.01 g**
	vii) Agar-agar	:	**20.00 g**
	viii) Distilled water	:	**1000 ml**
2)	**Asthana & Hawker's agar medium**		
	i) Glucose ($C_6H_{12}O_6$)	:	**5.00 g**
	ii) Potassium Nitrate (KNO_3)	:	**3.50 g**
	iii) Potassium dihydrogen phosphate (KH_2PO_4)	:	**1.75 g**
	iv) Magnesium sulphate ($MgSO_4.2H_2O$)	:	**6.75 g**
	v) Agar-agar	:	**20.00 g**
	vi) Distilled water	:	**1000 ml**
3)	**Richard's agar medium**		
	i) Sucrose	:	**50.00 g**
	ii) Potassium dihydrogen phosphate (KH_2PO_4)	:	**5.00 g**
	iii) Potassium nitrate (KNO_3)	:	**10.00 g**
	iv) Magnesium sulphate ($MgSO_4.2H_2O$)	:	**2.50 g**
	v) Ferric chloride ($FeCl_3$)	:	**0.02 g**
	vi) Agar-agar	:	**20.00 g**
	vii) Distilled water	:	**1000 ml**
A)	**B) Non-synthetic media:**		
1)	**Oat meal agar medium**		
	i) Oat meal	:	**60.00 g**
	ii) Agar-agar	:	**20.00 g**
	iii) Distilled water	:	**1000 ml**
2)	**Potato dextrose agar (PDA) medium**		
	i) Peeled potato	:	**200.00 g**
	ii) Dextrose	:	**20.00 g**
	iii) Agar-agar	:	**20.00 g**
	iv) Distilled water	:	**1000 ml**
3)	**Vegetable 8 (V8) juice agar medium**		
	i) V8-agar	:	**44.3 g**
	ii) Distilled water 1000 ml	:	**1000 ml**
4)	**Potato carrot agar**		
	i) Potato extract (as made above)	:	**250.0 ml**
	ii) Carrot extract (as made above)	:	**250.0 ml**
	iii) Agar	:	**15.0 g**
	iv) Distilled water	:	**500.0 ml**
5)	**Host leaf extract agar medium**		
	i) **Cauliflower leaves extract (10 %)**	**:**	**100 ml**
	ii) **Agar-agar**	**:**	**20.00 g**
	iii) **Distilled water**	**:**	**900 ml**

For preparing host leaf extract medium, cauliflower leaves were chopped and ground with the help of mixer. The extract was strained through muslin cloth and volume made to 1 lit.

Twenty millilitre of each medium as listed above was poured into sterilized Petri plate, separately. After solidification, 5 mm culture discs of the test fungus from actively growing 7 days old fungal culture were cut using sterilized cork borer and a single disc was placed at the centre of each Petri plate and incubated at 27 ± 2ºC. Each treatment was replicated thrice. The measurement of the colony diameter was taken when the maximum (90mm) growth was achieved in any one of the media tested. The cultural characters such as colony diameter, colony colour and degree of sporulation were recorded.

Statistical analysis

The data obtained were statistically analysed by the methods suggested by Gomez and Gomez (1986). The standard error and critical difference were worked out and the results obtained were compared statistically.

Results and Discussion

Examination of diseased samples

Visual observation

The leaf spot disease of cauliflower (*Brassica oleracae* L. var. *Botrytis*) caused by *Alternaria brassicae* (Berk.) Sacc. was noticed in moderate to severe at the farm of College of Agriculture Engineering and Technology, Dapoli during November, 2015. The disease appeared initially as small, circular, dark, yellow spots on the lower leaves. Later on these spots enlarged into circular areas with concentric rings and possibly surrounded by yellow halo. Later on these spot enlarged into gray to black lesions of 0.5 to 1 cm diameter. As the disease progressed, the lesions attended target board pattern due to formation of many concentric rings with wavy margin. Centres were coated with sooty black spore masses and later it drop out, producing shot holes. (PLATE-I)

Microscopic examination

Temporary mounts were prepared from the diseased samples in lacto-phenol cotton blue. Microscopic examination revealed the presence of fungal structures such as mycelium and conidia. The conidia were obclavate, muriform with a long beak with both transverse (10 to 11) and longitudinal (2 to 3) septa. The conidia were slightly constricted at transverse septa. Conidia were light brown to gray coloured and measured 104.0-142.0 × 11.62-16.95 µm. Length of beak was 43.35-70.57 µm. (PLATE-II)

Isolation and proving pathogenicity

The pathogen was isolated successfully on potato dextrose agar medium from the diseased tissue showing well developed lesions along with healthy portion which were brought to the laboratory from naturally infected cauliflower plants. The inoculated plates were incubated in BOD incubator at 25 ± 2^0C for 5 to 7 days. The culture of the fungus obtained by isolation from diseased tissue was transferred to PDA in Petri plates and multiplied in the laboratory.

Purification of fungal culture

The test fungus produced greenish grey to black coloured, fluffy, lanose to loose cottony growth on potato dextrose agar medium after seven days of incubation. The slants of the pure culture were sealed with paraffin wax and maintained in the laboratory in refrigerator for further use.

Inoculation of fungal culture

Healthy growing, 25 days old seedlings of cauliflower (Variety-supper fast crop) were used for pathogenicity test. Seedlings after making injuries on leaves by pinning were inoculated by spraying with the spore suspension. After 8 - 10 days of incubation, typical symptoms of blight on foliage of artificially inoculated plant were observed (PLATE-III). The lesions on the artificially inoculated plant also exhibited conidia formation. However, the plant kept as control, which was sprayed only with sterilized water did not produced any kind of symptoms.

The fungus was reisolated on PDA from artificially inoculated plants showing typical blight symptoms and was found to be identical to original isolate. Thus, the pathogenicity of the isolated fungus was proved on cauliflower. In present study, symptoms developed on artificial inoculated cauliflower plants were similar to those observed in field. Gaikwad (2013) also proved pathogenicity of *A. brassicae* by inoculating one month old seedlings of cabbage with the spore-cum-mycelial suspension (2×10^5). Similarly Sharma *et al.*, (2013) also proved pathogenicity of *A. brassicae* on detached leaves of cauliflower and mustard. The findings are also in agreement with Giri *et al.*, (2013) and Deep *et al.*, (2014)

Identification of causal organism

Based on the typical symptoms on foliage, microscopic observations and cultural characteristics of the fungus, it was tentatively identified as *Alternaria* spp. This proved that the pathogen responsible for causing leaf spot disease of cauliflower was *Alternaria* spp. Further, the Chief Mycologist, Agharkar Research Institute, Pune, confirm the identification of the pathogenic fungus as *Alternaria brassicae* (Berk.) Sacc. Thus the study revealed that leaf spot of cauliflower under present study was caused by *Alternaria brassicae* (Berk.) Sacc. The pathogen was easily isolated on potato dextrose agar medium. On PDA, the fungus produced greenish grey to black fluffy, lanose to loose cottony growth which resembled to the colony of *Alternaria brassicae.* The *A. brassicae* was already reported to be isolated from diseased tissue of cauliflower leaves by Deep and Sharma (2012), Reshu *et al.*, (2012), Gaikwad 2013, Sharma *et al.*, (2013), Chand and Chandra (2014), Deep *et al.*, (2014), Taware *et al.*, (2014) and Koley and Mahapatra (2015).

Effect of culture media on growth and sporulation of *Alternaria brassicae* (Berk.) Sacc.

Growth and sporulation of *A. brassicae* were studied *in vitro* using eight synthetic and non-synthetic culture media.

The data from Table 1, PLATE-IV and Figure 1 revealed that of the eight culture media tested, potato dextrose agar medium was found most suitable and encouraged maximum radial mycelial growth (90.00mm) of *A. brassicae*. The second best culture medium found was host leaf extract agar medium (87.00 mm). This was followed by Richard's agar medium (75.33 mm) and oat meal agar medium (71.66 mm). Carrot potato agar medium (55.00 mm), Asthana and Hawker's agar medium (51.66 mm) and Czapek's Dox agar medium (39.00 mm) were moderate in mycelial growth of *A. brassicae*. Poor mycelial growth was recorded in V8 juice agar medium (32.66 mm).

Excellent sporulation of *A. brassicae* was observed in potato dextrose agar medium and host leaf extract agar medium. Good

sporulation was observed in Richards's agar medium and oat meal agar medium. Fair sporulation was observed in carrot potato agar medium and Asthana and Hawker's agar medium and it was poor in Czapek's Dox agar medium. The results of present investigation are in close conformity to Singh (1980) who reported that oat meal agar media best for growth and sporulation of *A. brassicae.* Similarly, Sharma *et al.*, (2013) also reported that potato dextrose agar and cauliflower agar medium were optimum for fungal growth of *A. brassicae.* Deep *et al.*, (2014) also reported that cauliflower leaf extract medium and potato dextrose agar were appeared optimum for growth and sporulation of the fungus.

Plate.1 Field symptoms of leaf spot of cauliflower caused by *Alternaria brassicae*

Plate.2 Culture of *Alternaria brassicae* and its spores

Plate.3 Inoculation of *Alternaria brassicae*

Uninoculated plant (Healthy)

Inoculated plant (Infected)

Plate.4 Effect of different culture media on mycelial growth and sporulation

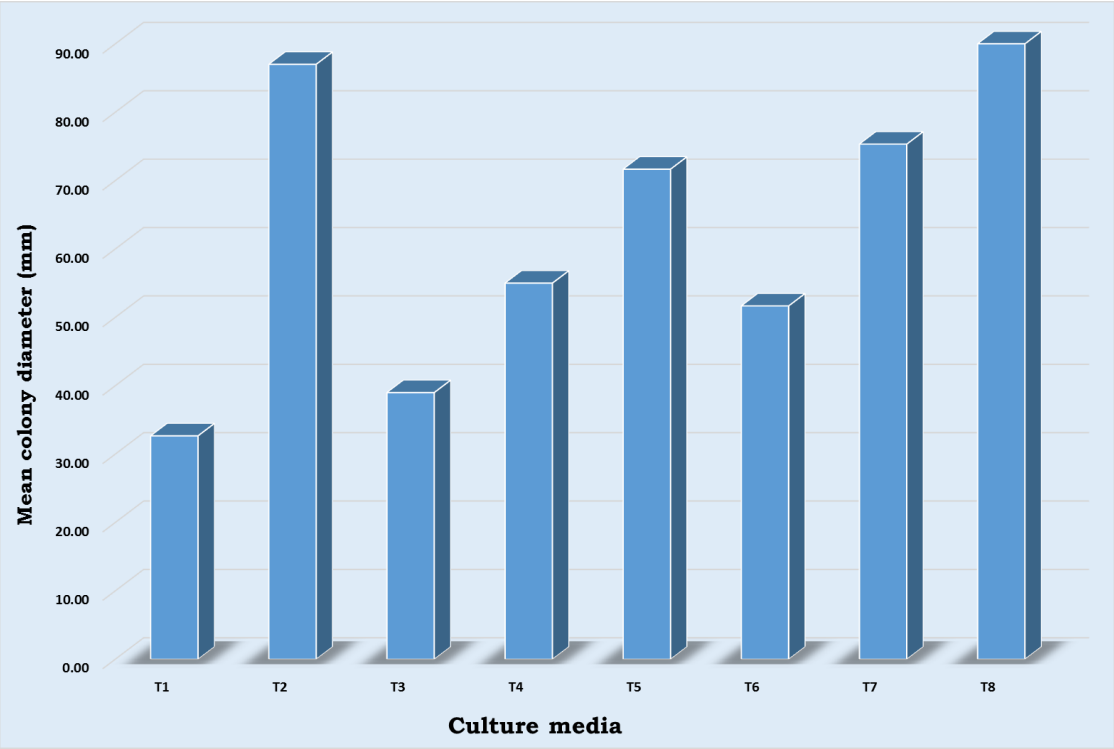
Table.1 Effect of different culture media on mycelial growth and sporulation of *A. brassicae*. (Berk.) Sacc.

Tr. No	Culture media	Average mycelial growth (mm)	Sporulation
T_1	V8 juice agar medium	32.66	+
T_2	Host leaf extract agar medium	87.00	++++
T_3	Czapek's Dox agar medium	39.00	+
T_4	Carrot potato agar medium	55.00	++
T_5	Oat meal agar medium	71.66	+++
T_6	Asthana and Hawker's agar medium	51.66	++
T_7	Richards agar medium	75.33	+++
T_8	Potato dextrose agar medium	90.00	++++
S.Em.±		**0.70**	
C.D. at 1%		**2.94**	

Sporulation

-	=	**No sporulation,**	+++	=	**Good,**
+	=	Poor,	++++	=	Excellent.
++	=	Fair,			

Fig.1 Effect of different culture media on mycelial growth of *A. brassicae*



In conclusion, on the basis of the results of present study it can be concluded that *Alternaria* leaf spot of cauliflower caused by *Alternaria brassicae* (Berk.) Sacc. is an important disease of cauliflower in Konkan region. Among the various biotic factors responsible for low production and productivity of cauliflower, *Alternaria* leaf spot caused by *Alternaria brassicae* (Berk.) Sacc. is one of the constraints.

References

Anonymous, 2013. Area, Production and productivity of cauliflower in India. *Indian Horticulture Database* Pp. 141-151.

Chand, G. and Chandra, K. K. 2014. symptomological, cultural and molecular variability of *Alternaria brassicicola* leaf spot in broccoli (*Brassica oleracea* var. Ltalica L.). *International Journal of Pharma and Bio Sciences*., 5 (2): 680 – 688.

Deep S., Sharma P., Behera N. and Chowdappa P. 2014. Diversity in Indian Isolates of *Alternaria brassicicola* (Schwein) Wiltshire Causing Black Leaf Spot Disease in Cauliflower. *Plant Pathology Journal*., pp.1-14

Deep Swati and Sharma Pratibha. 2012. Host age as predisposing factor for incidence of black leaf spot of cauliflower caused by *Alternaria brassicae* and *Alternaria brassicicola*. *Indian phytopath*., 65(1): 71-75.

Gaikawad, P.A. 2013. Studies on Leaf Spot of Cabbage Caused by *Alternaria brassicae* (Berk.) Sacc. M.Sc. (Ag) Thesis, submitted to V.N.M.K.V., Parbhani, Maharashtra.

Giri, P., Taj, G., Meena, P.D. and Kumar, A. 2013. Microscopic study of *Alternaria brassicae* infection processes in *Brassica juncea* cultivars by drop plus agarose method. *Afr. J. Microbial. Res.,* 7: 4284-4290.

Gomez, K. A. and Gomez, A.A. 1986. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons, London, 680.

Koley, S. and Mahapatra, S. S. 2015. Evaluation of Culture Media for Growth Characteristics of *Alternaria solani*, Causing Early Blight of Tomato. *Plant Pathol Microbiol*., 5(5): 312-314.

Pattanamahakul, P. and Strange, R. N. 1999. Identification and toxicity of *Alternaria brassicicola*, the causal agent of dark leaf spot disease of *Brassica* species grown in Thailand. *Plant Pathology*, 48(6):749-755.

Peruch L.A.M., Michereff S., Araujo I. B. 2006. Survey of intensity of *Alternaria* black spot and black rot on brassica species under organic farming systems in Pernambuco and anta catarina states, *Brazil. Horti.Bras.*, 24(4).

Reshu and Khan, M. M. 2012. Role of different Microbial-origin bioactive antifungal compounds against *Alternaria* spp. causing leaf blight of Mustard. *Plant Pathol. J*., 11(1): 1-9.

Sharma, P., Deep, S., Sharma, M. and Bhati, D.S. 2013. Genetic variation of *Alternaria brassicae* (Berk) Sacc. causing dark leaf spot of Cauliflower and Mustard in India. *J. Gen. Plant Pathology*. 79: 41-45.

Singh, D.B 1980. Effect of culture media, pH and temperature on growth behavior of *Alternaria brassicae* and *Drechslera graminae. Proc.Indian natn.Sci.Acad.*, 46(3): 393-396.

Sreedhar, K.N., Ashokkumar, C.T., Padamabha, K. and Sudhirkumar, A.S. (2013). Compatibility of insecticides and fungicides mixtures against cabbage leaf spot, *Alternaria brassicae* (Sacc.) Berk. *The Asian Journal of Horticulture*., 8:659-666.

Surviliene, E. and Dambrauskiene, E. (2006).

Effect of Different active Ingredients of Fungicides on *Alternaria spp*. growth *in vitro*. Agronomy Research, pp: 403–406.

Thaware, D. S., Fugro, P. A., Jadhav, Y. T., Magar, S. V. and Karande, R. A. 2010. *In vitro* evaluation of different fungicides, plant extracts and bio-agents against *Alternaria alternata* (Fr.) Keissler causing leaf blight of cowpea. *International Journal of Plant*., 3(2): 356-360.

How to cite this article:

Valvi, H.T, J.J. Kadam and Bangar, V.R. 2019. Isolation, Pathogenicity and Effect of Different Culture Media on Growth and Sporulation of *Alternaria brassicae* (berk.) Sacc. causing *Alternaria* Leaf Spot Disease in Cauliflower. *Int.J.Curr.Microbiol.App.Sci.* 8(04): 1900-1910. doi: https://doi.org/10.20546/ijcmas.2019.804.223