

Effect of different nutrient media on radial growth of *Alternaria brassicae* (Berk.) Sacc. infecting different *Brassica* host crops

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<p>Original Research Article Received on October 20, 2018 Revised on October 27, 2018 Accepted on November 12, 2018 Published on November 20, 2018</p> <p>Article Authors Vaibhav Pratap Singh, R. U. Khan Corresponding Author Email vaibhavpratapsingh10392@gmail.com</p>	<p><i>Alternaria brassicae</i> (Berk.) Sacc. is the most common and destructive pathogen of a number of <i>Brassica</i> crops belong to family Brassicaceae. It has been reported from all the continent of the world and causes considerable losses in terms of quality and quantity of crop produce. The present investigation was undertaken <i>in vitro</i>, to know the effect of different nutrient media <i>viz.</i>, Potato Dextrose Agar (PDA), V-8 Juice Agar (V8JA), Richard's Agar (RA), Czapeck's Dox Agar (CDA) and Corn Meal Agar (CMA) on the growth of <i>A. brassicae</i> isolates collected from the different <i>Brassica</i> host crops <i>i.e.</i> Mustard, Cauliflower, Cabbage and Radish. These isolates were designated on the basis of their hosts as Acae M, Acae Cf, Acae Ca and Acae R, respectively. Radial growth of all isolates was observed after seven days of incubation. The result showed a marked variation in radial growth of <i>A. brassicae</i> isolates. However, maximum growths of all isolates were recorded in V-8 Juice Agar (V8JA) followed by Potato Dextrose Agar (PDA) while the minimum growth of pathogen was observed in Corn Meal Agar (CMA).</p>
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Brassica crops play an important role for its uses as vegetable as well as oil yielding seed. Important *Brassica* crops include vegetables like *B. oleracea* (vegetables), *B. rapa* (vegetables, oilseeds, and forages) and oilseeds like *B. juncea* (vegetables and seed mustard), *B. campestris* (rapeseed) and *B. napus* (oilseeds) (Westman *et al.*, 1999). The Indian mustard (*B. juncea*) is the main source of cooking oil in Asia. India is one of the leading oilseeds producing country in the world accounting for 11.12 per cent of the world's rapeseed-mustard production, and ranks third in the world next to China and Canada. In India, oilseed *Brassica* are grown over an area of about 6.3 million hectares with an annual production of 7.4 million tonnes and an average

yield of 1176 kg/ha (www.drmr.res.in, Kumar, 2014). India is also the second largest vegetable producer in the world, next only to China with an annual production of 81 million tonnes from 5.1 million hectares of land (Karanth, 2002) and it accounts for about 15% of the world production of vegetables. In general, the Brassicaceous crops have low average productivity due to the prevalence of various biotic and abiotic stresses. Among the major biotic stresses, *Alternaria* blight disease caused by *Alternaria brassicae* (Berk.) Sacc. is the most important and destructive disease which causes heavy losses in all over the world attacking all *Brassica* species (Kolte, 1985, Meena *et al.*, 2010).

Alternaria blight (Black spot) of oil seed rape, cabbage, cauliflower and mustard crops have been reported from many countries, viz., India (Kadian and Saharan, 1983), Italy (Tosi and Zizzerini, 1985), USA, UK and several other European countries (Gladders, 1987), Canada (Berkeramp and Kirkham, 1989, Conn and Tewari, 1990), Iran (Nourani *et al.*, 2008) etc. Yield losses may vary from 10 to 70% depending on the type of crop species grown and prevailing environmental conditions; maximum (>70%) being in yellow sarson and low to moderate high (35-40%) in mustard (Chattopadhyay, 2008). Symptoms of this disease include presence of irregular, often circular brown to dark brown colour leaf spots on the leaves with concentric lines inside the spots. Often the circular spots coalesce to form large patches resulting in the leaf blight.

In several cases, small dark coloured spots are also formed on pods and tender twigs (Valkonen and Koponen, 1990). *Alternaria* blight severity on oilseed *Brassicaceae* differ season to season, region to region and also individual crop to crop in India (Chattopadhyay *et al.*, 2005). This might be due to the existence of variability among geographically similar isolates of *A. brassicae*. Several researchers have reported existence of variability based on morphology, sporulation and cultural characteristics (Gupta *et al.*, 1972, Saharan and Kadian, 1983, Awasthi and Kolte, 1989, Vishwanath and Kolte, 1997). Therefore, the present study was conducted to know the variation in *A. brassicae* isolates collected from different *Brassica* host crops on different nutrient medium.

Materials and Methods

The present study was carried out under laboratory condition during *rabi* season 2014-2015 at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh. During this study, the infected plant parts were collected on the basis of symptoms appeared on the leaves and stem. Such infected plant parts were collected in a polythene bags and brought to the laboratory for the isolation of *A. brassicae*. The pathogen was isolated and purified by single spore technique as described by Tousan and Nelson (1976) and maintained on PDA slants for its further use in experiments. These petriplates were incubated in

BOD incubator at $25\pm 1^{\circ}\text{C}$ for to 4-5 days. Various isolates of *A. brassicae* yielded from the samples of different crops of *Brassicaceae* family i.e. Mustard, Cauliflower, Cabbage, Radish. These isolates were designated on the basis of their hosts as Acae M, Acae Cf, Acae Ca and Acae R, respectively. The study was conducted to ensure the existence of the variability in *A. brassicae* isolates in relation to their response on different nutrient media (Potato Dextrose Agar, V-8 Juice Agar, Richard Agar, Czapek Dox Agar and Corn Meal Agar). According to the composition, the nutrient media were prepared and sterilized in an autoclave at 15 lbs p.s.i. for 15 minutes. The glass wares used in this study were thoroughly cleaned and also sterilized in a hot air oven at $140-160^{\circ}\text{C}$ for 8-10 hours. After sterilization, a 30 ml sterilized medium poured separately into each 90 mm sterilized petriplates. Thereafter, these petriplates were inoculated with a 5 mm disc of the pathogen obtained with the help of sterilized cork borer and cut from the advancing growth region of a week old culture of *A. brassicae*.

These discs were used as an inocula of the pathogen. Each treatment was replicated three times and the petriplates were kept in an incubator at $25 \pm 1^{\circ}\text{C}$ for incubation. After one week, these petriplates were observed for the measurement of radial growth of the pathogen by drawing two perpendicular lines passing through the centre of the lower surface of the bottom of petriplate. The measurement of radial growth was done. This procedure was followed in all culture media and the observations were recorded accordingly and the average of three replicates was calculated.

Results and Discussion

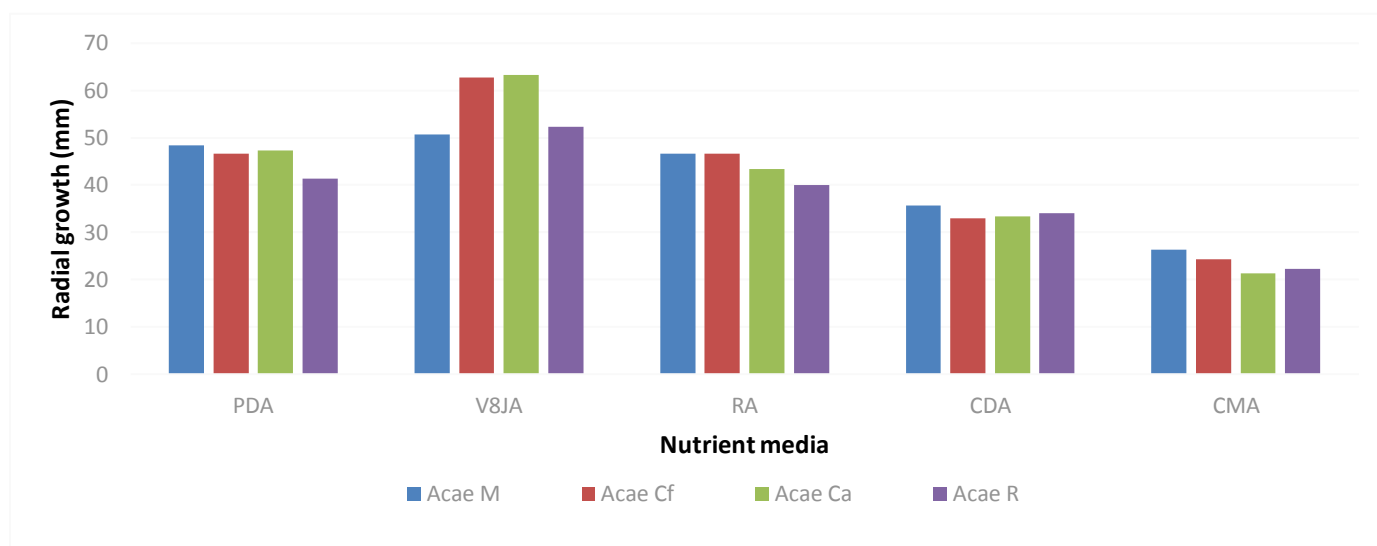
The result showed that the significant variation in radial growth of *A. brassicae* isolates was found on different nutrient media when compared to one another. Maximum radial growth of all isolates were observed in V-8 Juice Agar medium i.e. Acae M (50.66 mm), Acae Cf (60.00 mm), Acae Ca (62.00 mm) and Acae R (52.66 mm). While minimum radial growth of all *A. brassicae* isolates were recorded on Corn Meal Agar medium i.e. Acae M (26.33 mm), Acae Cf (21.33 mm), Acae Ca (21.33 mm) and Acae R (22.33 mm). Corn Meal Agar (CMA) did not facilitate the growth of fungus as compared to other (Table 1).

Table 1. Effect of different nutrient media on radial growth of *A. brassicae* isolates

Isolates	Nutrient media (mm)					
	PDA	V8JA	RA	CDA	CMA	PDA
Acae M	48.33 (44.02)	50.66 (45.36)	46.66 (43.06)	34.00 (35.65)	26.33 (30.85)	48.33 (44.02)
Acae Cf	46.66 (43.06)	60.00 (50.75)	46.66 (43.07)	34.33 (35.85)	21.33 (27.48)	46.66 (43.06)
Acae C	47.33 (43.45)	62.00 (51.92)	43.33 (43.33)	36.33 (37.05)	21.33 (27.48)	47.33 (43.45)
Acae R	41.33 (39.99)	52.66 (46.52)	40.00 (39.21)	31.00 (33.81)	22.33 (28.17)	41.33 (39.99)
CD at 5%	2.82	4.50	2.20	1.48	2.20	2.82
S.E (m)	0.85	1.36	0.66	0.44	0.66	0.85
S.E (d)	1.20	1.92	0.94	0.63	0.94	1.20
C.V.	3.46	4.84	2.77	2.18	4.04	3.46

Figures in parentheses are the arcsin $\sqrt{\text{percent transformed values}}$. *Each value is an average of 3 replicates.

PDA= Potato Dextrose Agar, **V8JA**= V-8 Juice Agar, **RA**= Richard's Agar medium, **CDA**= Czapek's Dox Agar, **CMA**= Corn meal agar

**Fig 1. Effect of nutrient media on radial growth of *A. brassicae* isolates**

Potato Dextrose Agar (PDA) and Richard's Agar (RA) recorded more or less similar growth of fungus against all isolates (table 1). On Potato Dextrose Agar (PDA), Acae M isolates recorded maximum growth of pathogen *i.e.* 48.33 mm while Acae Cf, Acae Ca and Acae R isolates recorded 46.66 mm, 47.33 mm and 41.33 mm, respectively. Both Acae M and Acae Cf isolates registered maximum radial growth of 46.66 mm on Richard's Agar (RA) while Acae Ca and Acae R recorded 43.33 mm and 40.00 mm growth of fungus respectively. The pathogen did not produced good growth on Czapek's Dox Agar (CDA) and Corn Meal Agar (CMA) as compare to V-8 Juice Agar (V8JA), Potato Dextrose Agar (PDA) and Richard's Agar (RA).

It is clear from the results that all *A. brassicae* isolates preferred V-8 Juice Agar (V8JA) for its maximum radial growth in comparison to other medium. However, the growth of on Potato Dextrose Agar (PDA) was slightly good as in comparison to Richard's agar (RA). But Corn Meal Agar (CMA) and Czapek's Dox Agar (CDA) were not proved good nutrient substrate for growth of fungus in this study. However (Sharma *et al.*, 2013) tested seven types of media *viz.* Potato Dextrose Agar (PDA), Cauliflower Agar Media (CAM), Carrot Potato Agar (CPA), Oat Meal Agar (OMA), Czapek's Dox Agar (CDA), V-8 Juice Agar (V8JA) and Corn Meal Agar (CMA) and they found that Potato Dextrose Agar, Cauliflower (Host) Agar medium and Carrot Potato Agar were good for the growth and sporulation of *A. brassicae*.

Selvamani *et al.* (2013) also reported that all the 40 isolates of *A. brassicae* collected from different host *viz.* cauliflower, cabbage and mustard from various locations of India, showed high level of variability *in vitro* in respect to mycelia growth, growth pattern and sporulation. All the isolates depicted high growth rate and high number of spore production on Cauliflower Leaf Extract Agar followed by Potato Dextrose Agar and Czapek Dox Agar media. In this study, *A. brassicae* isolates showed significant variation in the growth of pathogen, it may be due to variation in medium concentration and location/site of different field.

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