Influence of different temperature regimes and culture media on the growth and sporulation of *A. brassicae* causing Alternaria blight of mustard

Comment [RMSS1]: Enter the full name of the species.

Abstract

Rapeseed-mustard (*Brassica juncea* L.) is the second most important oilseed commodity in India. Alternaria blight (*A. brassicae*) is one of the devastating fungal pathogen, resulting severe yield losses to the crop. The present work was undertaken to study the effect of various temperature regimes and culture media on the mycelial growth and sporulation of *A. brassicae*. It is clearly evident from the results that all the tested temperature regimes and culture media showed variation in the colony diameter and other cultural characters. The temperature 25°C significantly encouraged the mycelial growth (85.00 mm) and exhibited excellent sporulation of *A. brassicae* compared to 15 and 35°C. In case of culture media, Potato Dextrose Agar found to be significantly superior over the other media and registered maximum mycelial growth (82.00 mm) with excellent sporulation. However, Oat Meal Agar supported the minimum mycelial growth (45.00 mm) with fair sporulation. The variation in cultural characteristics (colony color, growth, appearance, and shape etc.) was also observed with various culture media.

Comment [RMSS2]: As it is mentioned for the first time in the abstract, include the full name.

Keywords: Temperature, culture media, rapeseed-mustard, *A. brassicae*, Alternaria blight

Introduction

Rapeseed-mustard is an economically immense and useful oilseed crop globally. In India it is grown over in diverse agro-climatic environments and known as the third most important and oilseed crop after soybean and palm (Kumar, 2014) contributing 25 per cent of the total oilseed production (Yadav *et al*., 2019). The crop is vital source of edible oil and also used as vegetables, condiments, livestock feed and soil amendment. Mustard oil also has demand in industries for manufacturing various products like soap, paints, hair oil and many other products. It contains ample amount of erucic acid, linoleic acid and linolenic acids which are absent in many other edible oils (Singh *et al*., 2011). India occupies second position in terms of area covering 6.23 mha, contributing 9.43 MT annual production and an average productivity of 1499 kg/ha (Agricultural Statistics at a Glance, 2021). The major mustard producing states are Rajasthan (46.06%), Haryana (12.60%) and Madhya Pradesh (11.38%) and collective contribute about 70% of the total production in India (Agricultural Statistics at a Glance, 2021).

The quantity and quality of the crops is affected by various biotic and abiotic constraints. The crop suffers from various diseases, more than 30 diseases reported in India which not only deteriorates the seed quality but also results significant reduction in oil content (Saharan *et al*., 2005). Amongst the fungal diseases, Alternaria blight (*Alternaria brassicae, A. brassicicola*, *A. raphani* and *A. alternata*) is one of the world's most catastrophic disease. Out of the four species, *Alternaria brassicae* and *A. brassicicola* are the major leaf pathogens which are known to infect the crop frequently. The severe attack of these pathogens affects not only the seed quality but also reduces the oil content significantly in different oil-yielding *Brassica* species (Kolte, 1985; Meena *et al.*, 2010). The disease causes significant loss in mustard and much prevalent in Uttar Pradesh (Meena *et al.*, 2010). Under favorable conditions, the losses may be as high as up to 47 per cent (Saharan *et al*., 2016).

The disease affects all aerial parts of the plants such as stem, leaves, fruits and siliquae. The initial symptoms start with the appearance of black spots on leaves, stem and siliquae which later enlarges and convert into conspicuous round spots with concentric rings exhibiting target board like appearance. In severe infection, many small spots conjoin to form large spots, resulting blighting and defoliation of leaves. In few *Brassica* species, prominent concentric rings and yellow halo are formed around the lesions (Saharan and Mehta, 2002). The pathogen is seed as well as soil borne in nature and can survive in soil as saprophytes on the decaying plant debris.

A. brassicae is considered pseudo-fungi with no distinguishable sexual phase and reports as cosmopolitan. Conidia are large obclavate, olive grey to dark color with longitudinal and transverse septa (Aneja *et al.*, 2014). Crucial analysis and ample knowledge of alimentary patterns and factors influencing the growth of the pathogen (like temperature, pH, etc.) are essential for any study to understand the host-pathogen relationship. Hence, the present study is conducted *in-vitro* to know the effect of different temperature regimes and various culture media on the mycelial growth and sporulation of *A. brassicae.*

Material and methods

I. Effect of different temperature regimes on the growth of *A. brassicae*

Potato dextrose agar medium was used as a basal medium to check the disparity in radial growth and sporulation of *A. brassicae* at 5 different temperature regimes with an interval of 5°C, i.e., 15, 20, 25, 30, and 35°C. Inoculation was done with a 5 mm mycelial bit,

cut from the 7 days-old culture of *A. brassicae*, and placed in the Petri plates of 90 mm diameter consisting of sterilized solidified PDA. These Petri plates were then kept apart for incubation in various BOD incubators operating at temperatures 15, 20, 25, 30, and 35°C. Theses Petri plates were routinely checked after 48 h for the mycelial growth initiation of the fungus. Each treatment was replicated three times, and mycelial growth and sporulation were recorded after 7 days of inoculation in each Petri plate kept at varying temperatures. The observations of the radial growth were made by drawing 2 perpendicular lines passing through the centre of the lower surface of the bottom of Petri plate and the average of three replicates was assessed. (methodology reference???)

II. Effect of different culture media on the growth of *A. brassicae*

The present experiment was laid out to know the effect of eight different nutrient media *viz*., Oat Meal Agar, Nutrient Agar, Richard's Synthetic Agar, Czapek's Dox Agar, Czapek's Yeast Extract Agar, Rose Bengal Agar, Corn Meal Agar, and Potato Dextrose Agar on the growth of *A. brassicae*. The media were prepared by the standardized method and autoclaved at 121.6°C, 15 lbs p.s.i. for 15 minutes. Uniform quantities (20 ml) of each culture media were poured into 90 mm diameter Petri plates. Thereafter, each Petri plate was inoculated with a 5 mm disc of 4-5 days old culture of *A. brassicae* with the help of a sterilized cork borer. Each treatment was replicated thrice, and the Petri plates were incubated at 25±2°C for 7 days. These Petri plates were frequently examined for the radial growth of the fungus. The cultural characteristics (colony color, growth, margin, zonation, appearance, shape, sporulation) on all media were also recorded. (methodology reference???)

Result and Discussion

I. Effect of different temperature regimes on growth and sporulation of *A. brassicae*

It is evident from the results (Table 1) that the pathogen grew well at all the temperature regimes varying from 15 to 35°C. Significantly highest mycelial growth (85.00 mm) with excellent (++++) sporulation was observed at 25°C, which was followed by 30°C (68.66 mm), 20°C (47.33 mm) and 35°C (38.00 mm) with good (+++) to fair (++) sporulation, respectively. However, the least mycelial growth was recorded at 15°C having poor (+) sporulation.

Table 1. Effect of different temperature regimes on growth and sporulation of *A. brassicae* on PDA

Temperature (℃)	Mycelial growth (mm)*	Sporulation
	7DAI	
15	29.66[e]	+
20	47.33[c]	++
25	85.00[a]	++++
30	68.66[b]	+++
35	38.00[d]	++
L.S.D. (P≤0.05)	1.93	++
S.E(m)±	0.51	-

It is revealed from the results that found most suitable temperature for the mycelial growth and sporulation of *A. brassicae* was 25°C. The results thus obtained in the current study are in agreement with the finding of other workers, who have also noted the influence of various temperature regimes on mycelial growth and sporulation of *A. brassicae* (Taware *et al.*, 2014; Yadav *et al.*, 2016). In a study, Panchal (2008) recorded excellent mycelial growth and sporulation at 25°C of *A. alternata* infecting tomato. Kurhade *et al*. (2021) also observed the highest mycelial growth and excellent sporulation of *A. tagetica*, causing leaf blight of marigold at 25°C.

II. Effect of different culture media on the growth of *A. brassicae*

This experiment was carried out to note the variation in response of *A. brassicae* with respect to their cultural characteristics on eight different nutrient media. The pathogen measured significantly maximum growth (82.00 mm) on Potato Dextrose Agar followed by Richard's Synthetic Agar (76.33 mm), Czapek's Yeast Extract Agar (69.66 mm), Nutrient Agar (63.66 mm) and Corn Meal Agar (61.33 mm), (Table 2). Czapek's Dox Agar was next significantly superior nutrient medium and registered 59.00 mm growth. Minimum growth (45.00 mm) was measured on Oat Meal Agar followed by Rose Bengal Agar (56.66 mm).

*Each value is an average of three replicates. Values within a column followed by different alphabets are significant and some alphabets are non-significant according to Tukey's Test at P≤0.05. This is in the wrong place. To correct.

Comment [RMSS3]: When citing the table in the text, place it right below so as not to confuse the reader.

A. brassicae also showed variation in cultural characteristics on different nutrient media. The grey color colony appeared on Oat Meal Agar and Czapek's Yeast Extract Agar, while a dark green color colony appeared on Potato Dextrose Agar. However, the rest of the tested nutrient media produced greyish white to blackish white colony color (Table 2). The fluffy to compressed growth was observed in all the tested nutrient media. The growth of the fungus was found to be fast on Potato Dextrose Agar, Richard's Synthetic Agar, Nutrient Agar, and Czapek's Yeast Extract Agar. Medium growth was found on Czapek's Dox Agar, Rose Bengal Agar, and Corn Meal Agar. While as, slow growth was observed on Oat Meal Agar (Table 2).

Margin, zonation, and shape also differ with the nutrient media. Round to circular margins, whereas regular to irregular shape was formed on all the tested media. Zonation in the colony was absent in all the tested media except Nutrient Agar, Czapek's Dox Agar, and Rose Bengal Agar (Table 2). All the tested nutrient media exhibited a wide range of sporulation. However, Potato Dextrose Agar and Richard's Synthetic Agar showed excellent (++++) sporulation. It was good (+++) on the rest of the tested nutrient media except Oat Meal Agar which exhibited fair (++) sporulation (Table 2).

The present study is in conformity with the results of Charith *et al*. (2020), who studied the growth of the fungus in different media and revealed that Potato Dextrose Agar medium significantly supported the maximum radial growth (68.40 mm) of *A. brassicae* followed by Oat Meal Agar medium and the less growth was observed in the Czapex Dox Agar and Corn Meal Agar medium. The other studies done by Nagrale *et al*. (2013), Meena *et al*. (2013), Koley and Mahapatra (2015), Gholve *et al*. (2017), Krishna *et al*. (2018) and Reddy *et al*. (2019) also supported the findings.

The discussion is too brief and too superficial. It is important to further explore the results found through the discussion.

Table 2. ***In-vitro*** **effect of different nutrient media on mycelial growth of** ***A. brassicae***

S. No	Nutrient media	Mycelial growth (mm)	Colony color	Appearance	Growth	Margin	Shape	Zonation	Sporulation
1.	**Oat Meal Agar**	45.00^{h}	Grey	Fluffy	Slow	Rough	Irregular	Absent	++
2.	**Nutrient Agar**	63.66^{d}	Blackish white	Compressed	Fast	Smooth	Regular	Present	+++
3.	**Richard's Synthetic Agar**	76.33^{b}	Blackish White	Fluffy	Fast	Rough	Regular	Absent	++++
4.	**Czapek's Dox Agar**	59.00^{f}	Greyish White	Compressed	Medium	Rough	Irregular	Present	+++
5.	**Czapek's Yeast Extract Agar**	69.66^{c}	Grey	Compressed	Fast	Rough	Irregular	Absent	+++
6.	**Rose Bengal Agar**	56.66^{g}	Greyish White	Compressed	Medium	Smooth	Regular	Present	+++
7.	**Corn Meal Agar**	61.33^{e}	Greyish White	Fluffy	Medium	Smooth	Regular	Absent	+++
8.	**Potato Dextrose Agar**	82.00^{a}	Dark Green	Fluffy	Fast	Smooth	Regular	Absent	++++
L.S.D(P≤0.05)		1.86	-	-	-	-	-	-	-
S.E(m)±		0.62	-	-	-	-	-	-	-

*Each value is an average of three replicates. Values with in a column followed by different alphabets are significant and same alphabets are non-significant according to Tukey's Test at P≤0.05

Conclusion

The present study revealed that most appropriate temperature for the growth and spore production was 25°C. On the contrary, the fungus did not grow well at 15°C, resulting in poor sporulation. In addition, among the eight tested culture media, the highest mycelial growth and excellent sporulation of pathogen was observed with Potato Dextrose Agar. However, Oat Meal Agar was found to be the least effective and supported minimum mycelial growth with fair sporulation.

Is there any future prospects for this work? If so, write here.

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References

Agricultural Statistics at a Glance (2021). Directorate of Economics and Statistics, Department of Agriculture and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India.

Aneja J. K., Agarwal A. and Agnihotri, A. (2014). Inter and intra-specific diversity in *Alternaria* species infecting oilseed *Brassicas* in India. *Journal of Oilseed Brassica* 5(2), 102-117.

Charith, P., Mishra, J. P., Prasad, R., Chandra, J., Sekhar, V. P. R. and Kumar, S. (2020). Favorable cultural conditions for mycelial growth of *Alternaria brassicae*, causing leaf blight of cabbage under *in-vitro* conditions. *Journal of Pharmacognosy and Phytochemistry*, 9(4), 831-834.

Gholve, V. M., Ghuge, S. B., and Pawar, S. V. (2017). Effect of different culture media, temperature and pH on growth and sporulation of *Alternaria carthami. The Indian Society of Oilseeds Research*, 256.

Khan, M. M., Khan, R. U. and Mohiddin, F. A. (2007). Variation in occurrence and morphology of *Alternaria brassicae* (Berk.) Sacc. causing blight in rapeseed and mustard. *Annals of Plant Protection Sciences*, 15(2): 414-417.

Koley, S. and Mahapatra, S. S. (2015). Evaluation of culture media for growth characteristics of *Alternaria solani*, causing early blight of tomato. *Journal of Plant Pathology and Microbiology*, s1. 10.4172/2157-7471.S1-005.

Kolte, S. J. (1985). Diseases of Annual Edible Oilseed Crops, Rapeseed-mustard and Sesame

Diseases. *CRC Press Inc Boca Raton*, 2, 135p.

Taware, M. R., Gholve, V. M., Wagh, S. S., Kuldhar, D. P., Pawar, D. V. and Chavan, A. A. (2014). Effect of different culture media, temperature, pH, carbon and nitrogen sources on mycelial growth and sporulation of *Alternaria carthami* causing Alternaria blight of safflower. *International Journal of Plant Protection*, 7(2): 349-353.

Krishna, V. P., Suryawanshi, A. P., Prajapati, S. and Surekha, S. (2018). Effect of different culture media on growth and sporulation of *Alternaria dauci* causing carrot leaf blight. *Journal of Pharmacognosy and Phytochemistry*, 7(6), 1789-1792.

Kumar, A. (2014). Challenge of edible oils: Can Brassicas deliver. *J Oilseed Brassica*, 5: 83-86.

Kurhade, K. C., Gangawane, H. D., Deokar, C. D. and Raghuwanshi, K. S. (2021). Effect of temeperature and pH on growth of *Alternaria tagetica*, Leaf blight of marigold. *Bhartiya Krishi Anusandhan Patrika*, 36(1), 76-78.

Meena, P. D., Awasthi, R. P., Chattopadhyay, C., Kolte, S. J. and Kumar, A. (2010). Altemaria blight: a chronic disease in Rapeseed-mustard. *J. Oilseed Brassica* 1: 1-11.

Meena, P. D., Gour, R. B., Gupta, J. C., Singh, H. K., Awasthi, R. P., Netam, R. S., Godika, S., Sandhu, P. S., Prasad, R., Rathi, A. S., Rai, D., Thomas, L., Patel, G. A. and Chattopadhyay, C. (2013). Non-chemical agents provide tenable, eco-friendly alternatives for the management of the major diseases devastating Indian mustard (*Brassica juncea*) in India. *Crop Protection*, 53: 169-174.

Nagrale, D. T., Gaikwad, A. P. and Sharma, L. (2013). Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler blight of gerbera (*Gerbera jamesonii* H. Bolus ex J D Hook). *Journal of Applied and Natural Science*, 5(1), 171-178.

Panchal, D. G. (2008). Alternaria fruit rot of tomato (*Lycopersicon esculentum* Mill.) and its management. M. Sc. thesis Submitted to Anand Agricultural University, Anand.

Reddy, V. V., Ghante, P. H. and Kanase, K. M. (2019). Studies on morpho-cultural characters of *Alternaria alternata* infecting groundnut crop by using various culture media. *Journal of Pharmacognosy and Phytochemistry*, 8(2), 85-87.

Saharan, G. S. and Mehta, N. (2002). Fungal Diseases of Rapeseed-Mustard. In: Diseases of Field Crops (Gupta, V. K. and Paul, Y. S. eds.). Indus Publishing Company, New Delhi, pp.193-228.

Saharan, G. S., Mehta, N. and Sangwan, M. S. (2005). Diseases of oilseed crops. Indus Publication Co., New Delhi, 643p.

Saharan, G. S**., Mehta, N., Meena, P. D. and Dayal, P. (2016).** Alternaria diseases of crucifers: biology, ecology and disease management. *Springer*, 17-51.

Singh, L., Bagri, R. K., Thakore, B. B. L., Singh, J and Sharma, P. (2011). Effect of pH source on production of enzyme by *Fusarium oxysporum* f. sp. *pisi. Ann. Pl. Protec. Sci*. 11: 388-390.

Yadav, M. S., Godika, S., Yadava, D. K., Ahmad, N., Mehta, N., Bhatnagar, K., Agrawal, V. K., Kumar, A., Thomas, L. and Chattopadhyay, C. (2019). Prioritizing components of package of integrated pest management in Indian mustard (*Brassica juncea*) in India for better economic benefit. *Crop Protection*, 120: 21-29.

Yadav, S. P., Kumar, S., Prasad, R., Upadhyay, H. and Bansal, M. (2016). Identification of morphological, cultural and pathogenic variability of *Alternaria brassicae* causing Alternaria blight of Indian mustard (*Brassica juncea*). *Indian Phytopathology*, 69(1), 102-104.