Research Article

Effect of Physical Parameters on the Growth and Sporulation of the *Alternaria Alternate* in Coriander

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Abstract
Coriander is edible spices in India and also used for medicine purpose Out of five different solid media tested, Potato Dextrose Agar (PDA) proved to be the best for mycelial growth (88.35 mm) of the fungus, followed by Corn meal medium (63.17 mm) and Minimum mycelial growth was observed on Martin's medium. Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. Ultra low or high temperature adversely affects the germination and growth. Maximum mycelial growth of *A. alternata* was recorded at 25^0C (89.00 mm). Good mycelial growth of *Alternaria alternata* was observed at 100 per cent RH followed by 90 per cent.

Keywords: Alternaria, blight, coriander, mycelial, temperature

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Introduction

Coriander [*Coriandrum sativum* L.] is a major spice crop of the family *umbelliferae* (or *Apiaceae*) has been cultivated since ancient times. Coriander is mainly used as spice and condiments for seasoning foods and also used for medicinal purpose. On the other hand, in the whole or powdered form, it is considered as an important spice commodity of international trade. In India, it has commercially cultivated mainly in *Rabi* season with widely grown in Rajasthan, Andhra Pradesh, Tamil Nadu, Gujrat, Madhya Pradesh, Uttar Pradesh and Bihar [1]. Andhra Pradesh is the most leading state in area and production because of its agro-climatic conditions are best suited for growth and yield of the crop. The crop suffers from number of diseases caused by phytopathogenic fungi and other pathogenic organisms. Amongst all the diseases of the coriander, *Alternaria* blight of coriander incited by *Alternaria alternata*, is one of the important disease and a big obstacle in successful cultivation of this crop.

During the present study, the isolation of the pathogen was made from disease infected leaves of coriander. The culture was purified by single spore technique. On the basis of cultural and morphological characters, the fungus was tentatively identified as *Alternaria alternata*. The pathogen was found pathogenic when inoculated through foliar spray with fungal spore cum mycelium suspension artificially to coriander plants under mini plots conditions. The characteristic symptoms of blight appeared after 10 days of inoculation. Symptoms of disease infection in the beginning was typically in the form of appearance minute, light brown lesions on the leaflets, which later enlarge gradually and become dark brown in colour with profuse sporulation of the causative fungus. Lesions are usually most common on foliage but in case of severe infection they also appear as angular necrotic lesions on tender branches. Heavy infestation may cause drying of leaflets and complete defoliation due to large petiole lesions with seedling seldom surviving an attack [2-6]. Every living being requires food for growth and reproduction and fungi are not an exception to it [7]. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compound in the medium which are required for their growth and other life process. Neither all media are equally good for all fungi nor there can a universal substrates or artificial medium on which all fungi grow well. So, five different media were tried for the growth of *Alternaria alternata.*

Materials and Methods

Effect of physical parameters on the growth and sporulation of the pathogen

Mycelial growth on different solid media

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the center of colony by excluding initial diameter (5 mm) of bit was recorded. Five solid media whose compositions given below were taken for *in vitro* studies. Petriplates having sterilized medium were inoculated with 5

mm disc of mycelial growth with the help of sterilized cork borer and incubated at 25 $\pm$ 1^0C in incubator for 7 days. Observations on mycelial growth (radial growth) was taken after 7 days of incubation.

The media have highest mycelial growth and excellent formation of conidia, used for further studies.

(1) Potato Dextrose Agar (PDA) medium

Agar- Agar	20.00 g
Dextrose	20.00 g
Peeled potato	200.00 g
Distilled water	1000 ml

(2) Czapeck's Dox Agar medium

Agar-Agar	15.00 g
Sucrose	30.00 g
Distilled water	1000 ml
Dipotassium phosphate	1.00 g
Magnesium	0.50 g
Potassium chloride	0.50 g
Sodium nitrate	2.00 g
Ferrous sulphate	0.01g

(3) Oat Meal medium

Agar- Agar	20.00 g
Glucose	20.00 g
Oat meal	20.00 g
Distilled water	1000 ml

(4) Corn Meal medium

Agar-Agar	20.00 g
Corn meal	20.00g
Glucose	20.00g
Distilled water	1000ml

(5) Martin's medium

Agar-Agar	15.00g
Dextrose	10.00g
Peptone	5.00g
Distilled water	1000ml
Rose Bengal	1 part in 3000 part of media
Potassium dihydrogen phosphate	1.00g
Magnesium sulphate	0.50g

Effect of temperature on mycelial growth

Effect of temperature on mycelial growth of *Alternaria alternata* was studied *in vitro.* Twenty ml of sterilized PDA medium was poured in each sterilized Petriplates. Inoculation was made with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer and incubated at 5 different levels of temperature *viz*., 15, 20, 25, 30 and 35 ^{0}C for 7 days. Observations on mycelial growth was recorded after 7 days of incubation.

Effect of relative humidity on mycelial growth

To study the effect of relative humidity on mycelial growth of *Alternaria alternata,* five different levels of relative humidity *i.e.* 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportions in glass desiccators by the method suggested by Buxton and Mellanby (1934) [8]. The composition of the acid solution was used as followed.

Relative humidity (%)	Stock solution (ml)*	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	-	Only DW
* 50% v/v solution of concentrate sulphuric acid		

Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer. Inoculated Petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 25 $\pm$ 1^{0}C for 7 days. Observations on mycelial growth was recorded after 7 days of incubation.

Results and Discussion

Physiological studies

Growth of Alternaria alternata on solid media

To find out a suitable medium for the mycelial growth of *Alternaria alternata*, five different synthetic and semi synthetic media were tested *in vitro*. On perusal of data (**Table 1**) revealed that among the five different solid media, the Potato Dextrose Agar was significantly superior in supporting maximum mycelial growth (88.35 mm) at 7 days of incubation followed by Corn meal medium (63.17 mm) and Czapeck's dox medium (58.58 mm). Minimum growth of the fungus was observed on Martin's medium (40.47 mm). The PDA medium was selected for further studies. Results showed that Potato Dextrose Agar was good for mycelium growth followed by Corn meal medium and Czapek's dox medium. *Alternaria alternata* fungus grow well on Potato Dextrose Medium also reported by various workers, Joshi *et al*. (2012) [9], Mishra and Mishra (2012) [10], Bochalya *et al.* (2013) [11], and Apet *et al*. (2014) [12].

Table 1 Effect of different solid media on the mycelial growth of *Alternaria alternata* at 25 $\pm$ 1^{0}C after 7 days of incubation

S. No.	Medium	Mycelial growth (mm)*
1.	Czapeck's dox medium	58.58
2.	Martin's medium	40.47
3.	Oat meal medium	55.32
4.	Corn meal medium	63.17
5.	Potato Dextrose Agar medium	88.35
	SEm$\pm$	1.16
	CD (p=0.05)	3.78
* Average of three replications		

Effect of temperature

The effect of temperature on mycelial growth was recorded by incubating inoculated on PDA as basal medium at different tempertures (*viz.*, 15, 20, 25, 30 and 35 ^{0}C). Results depicted in **Table 2** reveals that maximum mycelial growth (89.00 mm) was observed at 25 ºC. while, the temperature of 20^{0}C, 30^{0}C and 35^{0}C were recorded least mycelial growth (79.15 mm), (71.21 mm) and (68.11 mm) respectively, but differ significantly from the growth at 25^{0}C. The results obtained in the present investigation showed that optimum temperature for growth is 25 ºC. Similar results were recorded by Chohan *et al*. (2015) [13] and Devappa and Thejakumar (2016) [14]. Balai and Ahir (2013) [15] recorded optimum growth of *Alternaria alternata* at 27 ^{0}C.

Effect of relative humidity

The effect of relative humidity on the mycelial growth was stidies at different levels *viz.*, 60, 70, 80, 90 and 100 per cent and incubated at 25$\pm$1 °C for 7 days. It was observed (**Table 3**) that maximum mycelial growth (90.00 mm) was recorded at 100 per cent relative humidity which was at par with 90 per cent (87.00 mm) relative humidity. A significantly decrease in mycelial growth was observed at 80, 70 and 60 per cent humidity. Minimum mycelial growth (59.85 mm) was observed at 60 per cent relative humidity. *In vitro* studies on different levels of relative humidity revealed that 100 per cent relative humidity supported maximum mycelial growth of *Alternaria alternata* while minimum mycelial growth at 50 per cent relative humidity. The results are in close conformity with the

observations of Prasad and Roy (1979) who observed maximum growth of *Alternaria alternata* at 90-100 per cent relative humidity. Balai and Ahir (2013) [15], Kumawat *et al*. (2011) [16] and Prasad and Ahir (2013) [17] also reported that 90 to 100 per cent relative humidity was most suitable for mycelial growth of *Alternaria alternata*.

Table 2 Effect of different levels of temperature on the mycelial growth of *Alternaria alternata* at 7 days of incubation (*in vitro*)

S.No.	Temperature (^{0}C)	Mycelial growth (mm)*
1	15	64.05
2	20	79.15
3	25	89.00
4	30	71.21
5	35	68.11
SEm±		1.09
CD (p=0.05)		3.048

Table 3 Effect of relative humidity on the mycelial growth of *Alternaria alternata* at 7 days of incubation at 25 ± 1^{0}C (*in vitro*)

S.No.	Relative humidity (%)	Mycelial growth (mm)*
1	60	59.85
2	70	72.25
3	80	79.00
4	90	87.00
5	100	90.00
SEm±		1.34
CD (p=0.05)		4.36

Conclusion

Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. Ultra low or high temperature adversely affects the germination and growth. Maximum mycelial growth of *A. alternata* was recorded at 25^{0}C (89.00 mm). Good mycelial growth of *Alternaria alternata* was observed at 100 per cent RH followed by 90 per cent.

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Publication History

Received	09.08.2021
Revised	19.11.2021
Accepted	25.11.2021
Online	31.12.2021