

Original Research Article

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Physiological Characterisation of *Alternaria brassicicola* causing Alternaria Leaf Spot in Cabbage

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ABSTRACT

Cabbage is a cool season crop grown in temperate regions and belongs to family Brassicaceae. In Kerala, cabbage is grown in an area of 197 ha and major cultivated regions were Idukki and Wayanad districts. The leaf spot of cabbage is caused by *Alternaria brassicicola* is a widespread disease affecting the yield of cabbage. This disease can be managed by using fungicides or bioagents but the efficacy of these measures depend on host pathogen interaction, which is influenced by environmental factors. The present study was undertaken to study the effect of Temperature, pH levels and Light intensity on the growth of *A. brassicicola* as the growth and development of pathogen was influenced by the environmental factors. Studies on physiological characters revealed that optimum temperature for the growth of *A. brassicicola* was 25°C. The pathogen was completely inhibited at 35°C and moderate growth was recorded at 20°C and 30°C. The ideal pH for the growth of pathogen was 5.5 and 6. The optimum light intensity favoured the growth of pathogen was normal day and night condition (20 lux) followed by dark. The growth and sporulation was affected at higher light intensities.

Keywords

A.brassicicola,
Cabbage, *In vitro*,
Physiological studies

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Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is an economically important vegetable crop which forms a compact head with leaves and grown as biennial for the production of seeds. It is a cool season crop grown in temperate regions and belongs to family Brassicaceae. Cabbage has its centre of origin in Mediterranean regions and now its cultivation is seen all over the world due to its nutritional benefits. It is mostly cultivated under temperate conditions as a rabi season crop and as cool season crop in the tropics.

Cabbage production is affected by many fungal, bacterial and viral diseases at different stages of growth and development. Among fungal diseases, clubroot, *Alternaria* leaf spot and damping off are the most prominent and destructive ones affecting the yield of cabbage. *Alternaria* leaf spot of cabbage caused by *A. brassicicola* is a widespread disease affecting the yield of cabbage worldwide.

Ansari (1988) stated that initial symptoms of *A. brassicicola* were dark spots on the lower leaves of cabbage and caused damping off

during seedling stage. These dark spots on leaves ranged in size from one to four cm in diameter and developed concentric rings within the spots (Dillard *et al.*, 1997). As the disease progressed these spots merged together on leaf and reduced the photosynthetic ability of plant (Kucharek, 2000). Later the infection appeared on the reproductive parts of the plant and caused significant yield losses in cabbage (Doullah *et al.*, 2006).

Dillard *et al.*, (1997) stated that most favourable temperature for growth and sporulation of *A.brassicicola* was 20-30⁰C in cabbage but optimum temperature for infection and conidial germination in cabbage was 25⁰C and 28-31⁰C respectively. Mehta (2014) stated that the congenial temperature for the growth and sporulation of *A.brassicicola* was 25⁰C and also reported that optimum temperature required for the development of disease was 12-25⁰C and relative humidity should be more than 70% in rapeseed-mustard. Singh (1980) reported that for mean mycelial growth and conidial development of *A.brassicicola*, the ideal was at pH 5.5. Hubballi *et al.*, (2010) reported that average growth of mycelium was highest at pH 6-6.5 for *A. alternata*. Alhussaen (2012) and Chohan *et al.*, (2015) stated that best hydrogen ion concentration for the growth of *A.solani* range from pH 6 to 7.

Hubballi *et al.*, (2010) conducted a study and determined the effect of light on the growth of *A. alternata* and observed that the radial growth of the pathogen was highest (8.7 cm) at 12 h of alternate light and dark condition compared to continuous darkness and light intensity. Mehta (2014) studied the effect of light on the sporulation of *A. brassicicola* and reported that growth and sporulation was inhibited at higher light intensity (>1000 lux) and vice versa with darkness and less intensity of light.

Materials and Methods

Isolation

The cabbage leaves infected with *Alternaria* spp. were collected during the survey and isolation was done by cutting small pieces of diseased portion of leaf along with healthy portion and immersed in mercuric chloride solution (0.1%) for 30 seconds.

Then these leaf bits were washed thrice in sterile water and placed aseptically on the sterile petriplate which was poured with the sterilized potato dextrose agar (PDA) medium (Rahimloo and Ghosta, 2015). These plates were incubated at room temperature (27±4⁰C) for proper development of the pathogen. Pure culture of the fungus was obtained by hyphal tip isolation method.

Physiological studies

Effect of temperature on growth of pathogen

The growth of virulent pathogen was tested at five different temperatures viz., 15⁰C, 20⁰C, 25⁰C, 30⁰C and 35⁰C. For this, PDA medium was prepared and sterilized. Then melted and cooled PDA medium was poured in sterilized petriplates. Five mm fungal discs of pathogen were taken from seven days old culture and inoculated at the centre of the petriplate under aseptic conditions. Four replications were maintained for each treatment. Then these plates were incubated at different temperatures and observations on radial growth was recorded when pathogen attained full growth in the petriplate at any one treatment.

Effect of pH on growth of pathogen

In order to study the effect of pH on the growth of pathogen, PDA medium ±were prepared having different pH range 4.0, 4.5,

5.0, 5.5 and 6.0. The pH levels of this PDA media were adjusted by adding acid (HCl) or alkali (NaOH) to get the desired pH. PDA medium of respective pH were poured into the sterilized petriplates separately and four replications were maintained for each pH. Then five mm disc of fungus were taken from seven days old culture pathogen using sterile cork borer and inoculated at the centre of petriplate.

These plates were incubated at room temperature ($27\pm 4^{\circ}\text{C}$). Observation were taken on the radial growth of the pathogen (cm) when the fungi attained full growth at any one of pH levels tested.

Effect of light on growth of pathogen

The virulent pathogen were grown at different light intensities 1000 lux, 2000 lux, 3000 lux, dark condition and normal day and night condition to determine the influence of light on the growth of pathogen. For this, sterilized PDA medium was prepared and poured into sterilized petriplates in laminar airflow chamber. Then five mm mycelial bits of the pathogen were taken from the 7 day old culture and inoculated at the centre of petriplate and they were incubated at respective light intensities. Four replications were maintained for each treatment and observations were noted when the fungus attained full growth in the petriplate at any one of the light intensities. Light intensities were measured by using the lux meter. By using the lux meter the distance from the light source has been determined at which the required light intensity is attained.

Results and Discussion

Isolation

Pure culture of the pathogen obtained from the cabbage leaves exhibiting typical blight

symptoms were identified as *A.brassicicola* based on the morphological characteristics according to Alternaria identification manual (Simmons, 2007).

Physiological studies

Effect of temperature on growth of pathogen

The pathogen was grown at different temperatures i.e., 15, 20, 25, 30 and 35°C on the PDA medium to determine the optimum temperature for the growth of pathogen. The results showed that the treatments differ significantly.

The growth of the pathogen was maximum at 25°C (9 cm) followed by 20°C (6.3 cm). The growth was completely inhibited at 35°C . Optimum temperature for the growth of pathogen was observed as 25°C and growth was inhibited with further increase or decrease in temperature (Table 1, Plate 1).

Humpherson-jones and Phelps, (1989) and Dillard *et al.*, (1997) reported that the growth of the pathogen was maximum at temperature $25-30^{\circ}\text{C}$. In the present study, the growth of *A. brassicicola* was moderate at temperature 20°C and 30°C (Fig. 1) consistent with results of Alhussaen (2012) and Mehta (2014).

Effect of pH on growth of pathogen

The growth of the pathogen was tested under *in vitro* condition at six acidic (4, 4.5, 5, 5.5, 6 and 6.5) and one neutral (7) pH conditions. The results from *in vitro* evaluation showed that the radial growth of the pathogen was maximum at pH -5.5 (9 cm) and 6 (9 cm). This was followed by pH 6.5 (8.6 cm), pH 5 (8.4) and pH (4.5) which was on par with each other. The least growth of the pathogen recorded was 7.6 cm at pH 7 (Table 2, Fig. 2, Plate 2).

Table.1 Effect of temperature on the growth of *A. brassicicola*

Sl. No.	Temperature (°C)	Radial growth* (cm)
1	15	2.9 ± 0.3 ^d
2	20	6.3 ± 0.4 ^b
3	25	9.0 ± 0.0 ^a
4	30	4.1 ± 0.2 ^b
5	35	Nil
	CD (0.05)	0.29
	SE	0.13

*Mean of four replications

Table.2 Effect of pH on the growth of *A. brassicicola*

Sl. No.	Ph	Radial growth* (cm)
1	4.0	7.6 ± 0.3 ^d
2	4.5	8.1 ± 0.3 ^c
3	5.0	8.4 ± 0.1 ^{bc}
4	5.5	9.0 ± 0.0 ^a
5	6.0	9.0 ± 0.0 ^a
6	6.5	8.6 ± 0.1 ^b
7	7	6.8 ± 0.5 ^e
	CD (0.05)	0.30
	SE	0.154

*Mean of four replications

Table.3 Effect of light on the growth of *A. brassicicola*

Sl. No.	Intensity of light	Radial growth* (cm)
1	Dark	8.5 ± 0.2 ^b
2	1000 lux	8.3 ± 0.2 ^c
3	2000 lux	8.1 ± 0.3 ^d
4	3000 lux	7.8 ± 0.4 ^e
5	Normal day and night condition	9.0 ± 0.0 ^a
	CD (0.05 level)	0.4
	SE	0.134

*Mean of four replications

Fig.1 Effect of temperature on growth of *A. brassicicola*

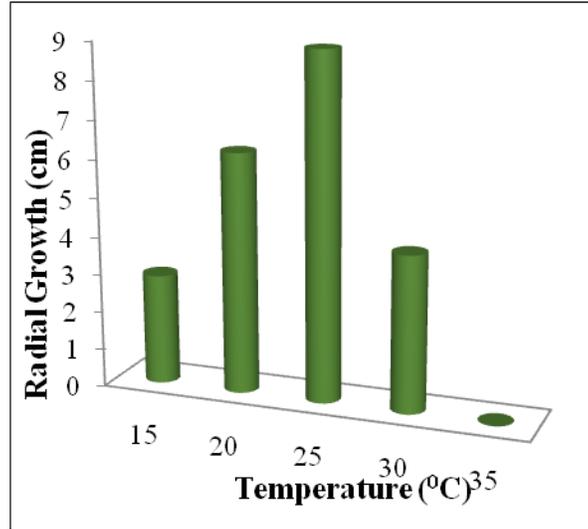


Fig.2 Effect of pH on growth of *A. brassicicola*

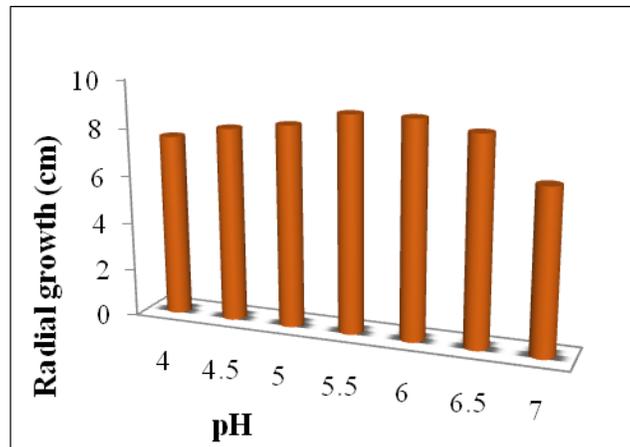


Fig.3 Effect of light intensity on growth of *A. brassicicola*

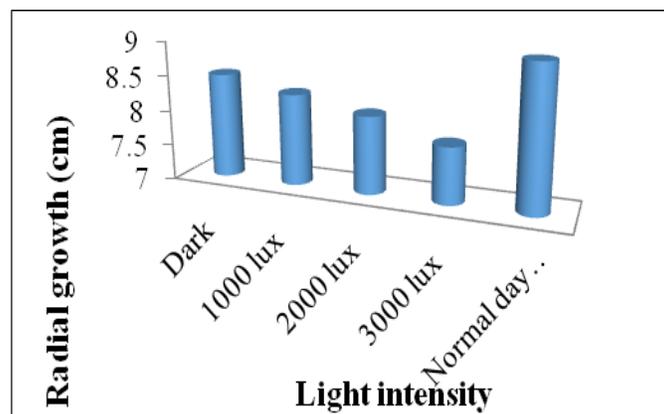


Plate.1 Radial growth of *A. brassicicola* at different temperature

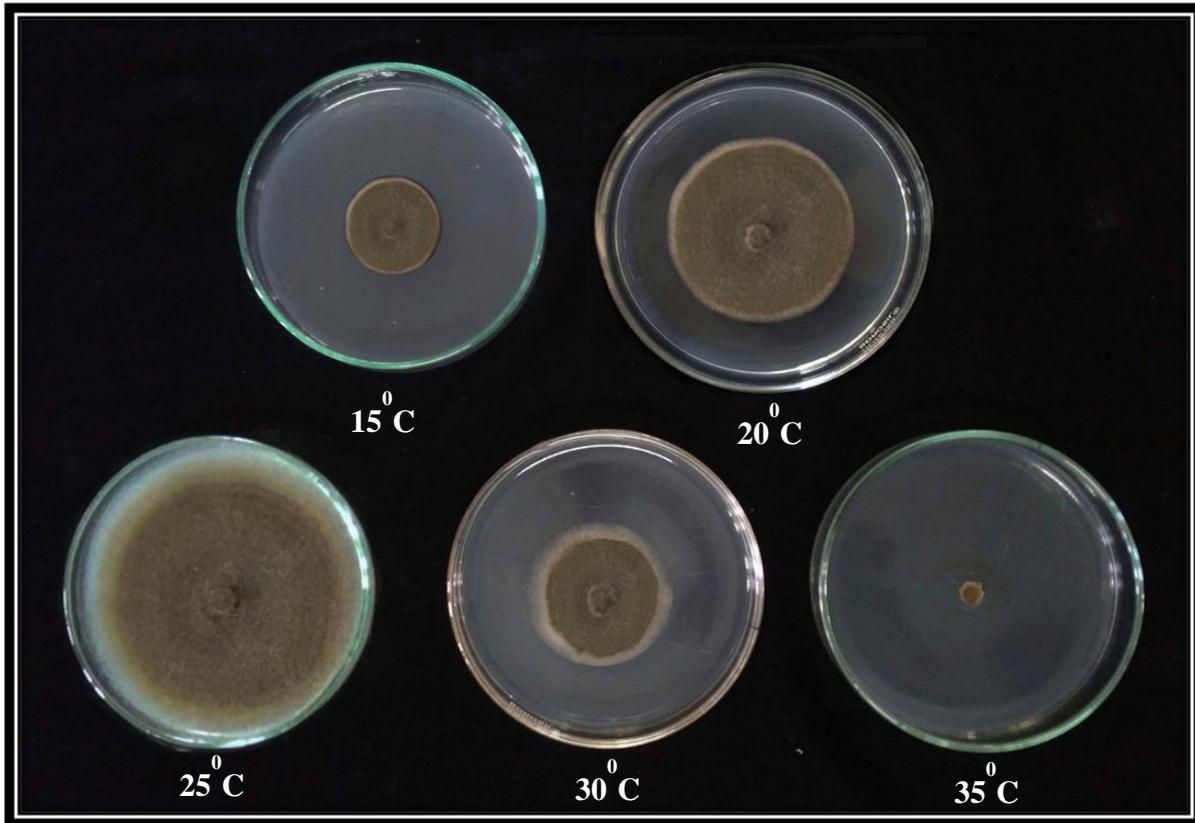


Plate.2 Radial growth of *A. brassicicola* at different pH

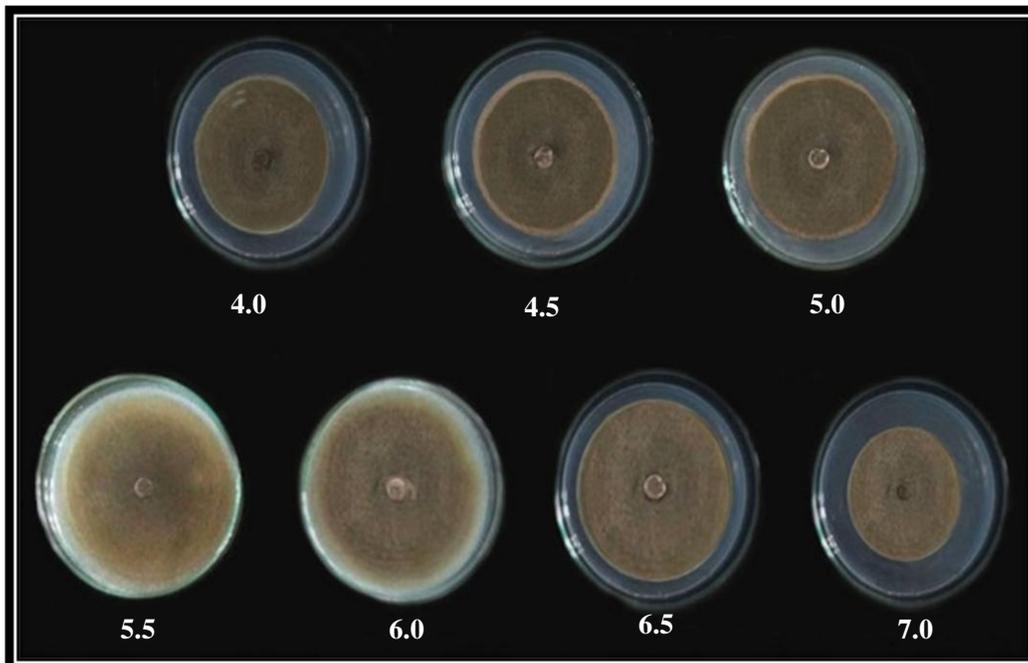
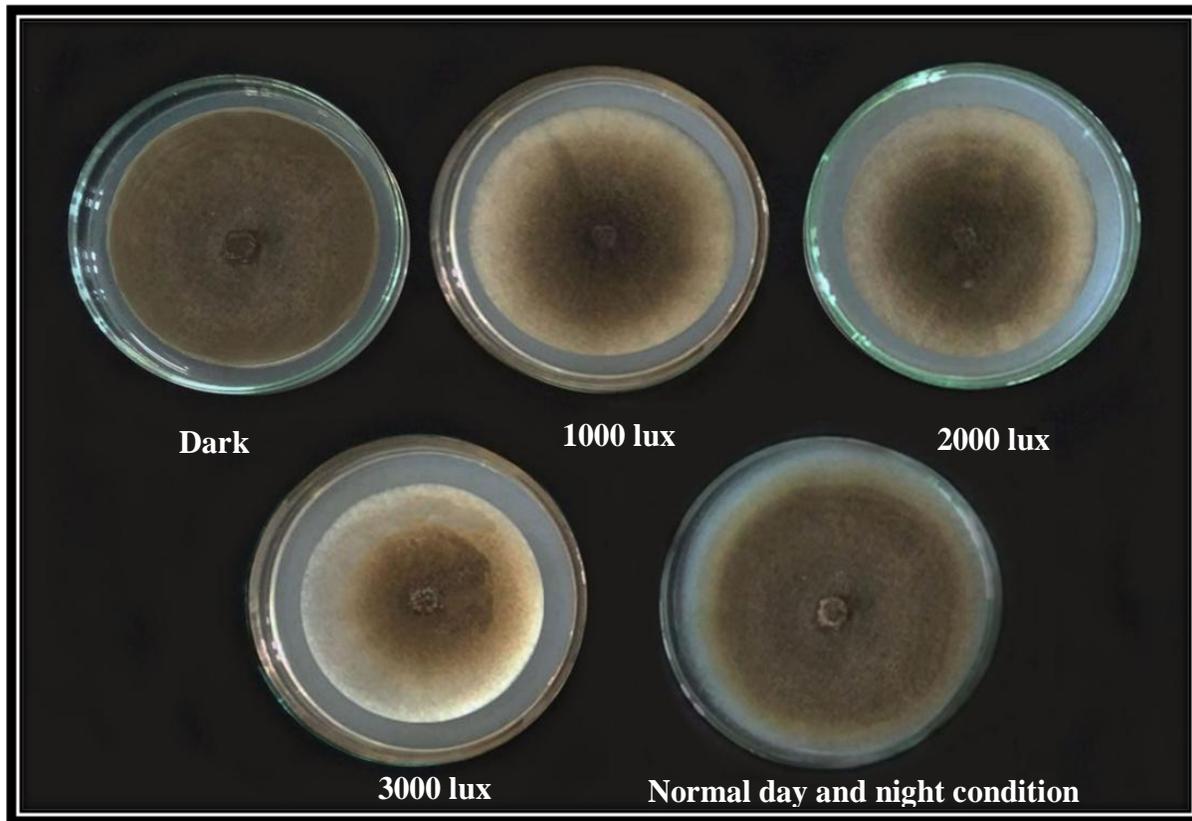


Plate.3 Radial growth of *A. brassicicola* at different light intensity



The results from this *in vitro* study were similar to the results of Kumar (1978), Singh (1980) and Pradnyarani and Kulkarni (2015) but differ with the results of Chohan *et al.*, (2015), Gawai and Mangnalikar (2017) as the ideal hydrogen ion concentration for the growth of pathogen was observed at pH 6.5.

Effect of light on the growth of pathogen

The pathogen was exposed to different light conditions i.e., dark, 1000 lux, 2000 lux, 3000 lux and normal day and night conditions (20 lux) to determine the optimum light intensity for the growth of the pathogen. The results revealed that the radial growth of the pathogen was highest at normal day and night condition (9 cm). This was followed by the dark (8.5 cm), 1000 lux (8.3 cm), 2000 lux (8.1 cm) and 3000 lux (7.8 cm) which were on par with each other (Table 3, Plate 3).

These results showed similarity with the results of Naik (2010) and Hubbali *et al.*, (2010) (Fig. 3). The growth of the pathogen declined with increase in light intensity and these results were in agreement with Lukens (1963) and Humpherson-jones and Phelps (1989).

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