INTRODUCTION

The fungus Webber (Phylum-; Sub Phylum-; Class-; Sub Class-; Order-; Family-) was first used for controlling whiteflies in citrus groves in Florida in the early 1900s. A combination of predators, parasitoids with has been in use for managing the citrus whiteflies for decades (Berger, 1921).

Biological control based on the use of entomogenous fungi is not simply dependent upon interaction between the host and the pathogen, but also on the environment to which they are exposed. Range of factors, including, temperature, relative humidity (RH), light, air, nutrient availability and host physiological status influences fungal pathogenicity (Padmini and Padmaja, 2010). Of the diverse factors, temperature, humidity and light are reported to be the foremost essential environmental factors affecting the survival and effectiveness of entomogenous fungi for their use as biocontrol agents (Bugeme et al., 2008).

Stimulating effect of temperature, relative humidity and light on the growth of mycelium and sporulation is well known in case of some of the entomogenous genera including Nomuraea, Beauveria, Metarhizium, and Paecilomyces (Alves et al., 1984; Sharma et al., 1998; Gopalkrishnan and Mohan, 2002; Miętkiewski et al., 1994). However, little information is available on the influence of environmental factors on entomopathogenic fungus Aschersonia aleyrodis.

The purpose of the present study was to find out the range of environmental factors which influence the growth and sporulation of this fungus.

MATERIALS AND METHODS

Isolation and Morphological Identification: The present study was conducted at the Department of Plant Pathology and AICRP on Fruits, Dr. PDKV, Akola-444104 Maharashtra, India. A combination of predators, parasitoids with A. aleyrodis has been in use for managing the citrus whiteflies for decades (Berger, 1921).

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dextrose agar media for 10 days and its cultural characters with respect to growth, colony morphology, colony colour, mycelial characters, sporulation, conidia formation, conidial morphology and their size were recorded.

**Effect of temperature on growth and sporulation of *A. aleyrodis* isolate:** Effect of temperature regimes on mycelial growth and sporulation of *A. aleyrodis* was determined using PDA medium. A 6 mm disc (contains 0.5 mg of mycelial load) of young sporulating culture of *A. aleyrodis* cut with the help of sterilized cork borer placed at the centre on the agar surface was incubated at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C in the BOD incubator. Four replications of each temperature were maintained. Following inoculation, radial growth of the mycelium was measured after 10 days using two cardinal diameters drawn at the bottom of each plate and recorded. Conidia were harvested from mycelial discs into 10 mL triton water in a universal bottle, which was vortexed for 3 min so as to obtain a homogenous solution. The conidial density was measured from this solution under a light microscope (40X) using a Neubauer hemocytometer.

**Effect of relative humidity (RH):** Effect of different levels of relative humidity was assessed by using desiccators of equal size containing different concentrations of sulphuric acid + water as per the method suggested by Solomon (1951). The experiment was conducted in five replications and inoculated Petri plates were kept in desiccators’ maintaining the levels of humidity at 80, 85, 90, 95 and 100 per cent. The lid was fixed with grease in order to make the desiccators air tight maintaining definite percentage of relative humidity. The experiment was run at room temperature (22-25 ± 10°C). The colony diameter was recorded after ten days interval while sporulation was recorded after fifteen days interval.

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Figs. 2  (a-d) a: Stromata covering nymphs of citrus black fly; b: Colony of *A. aleyrodis* on PDA medium; c: Mycelium of *A. aleyrodis*; d: Microscopic view of fusoid conidia of *A. aleyrodis* (40 X magnification)
Effect of light: For ascertaining the effect of light, the inoculated Petri plates in six replications were exposed to (A) continuous light provided by 40 W white fluorescent tubes arranged 41 cm above Petri plates, (B) alternate cycle of 12 h light and 12 h darkness and (C) complete darkness provided by wrapping black paper from inner and outer side of bell jar. Radial growth of colonies was measured after 10 days and sporulation count was recorded on 15th day.

Statistical analysis: The data collected from the experiments were subjected to analysis of variance for different treatments. Fisher’s protected critical difference (CD) test was used to indicate the difference between the treatments at the probability level of p < 0.01 following the procedure described by Gomez and Gomez (1984).

RESULTS

Morphology and colony characteristics: During the present investigations the young nymphs of black fly with natural mycosis due to infection by the fungus which formed stroma on the dorsal side of the body of insect nymph were examined. To look at, the stroma was flattened pulvinate, and the conidiomata produced reddish orange conidal masses. The coloration of the infected portion of the dead black fly nymphs mainly on the ventral side of citrus leaf varied from white to pale yellow and finally orange. The Stromata (Fig. 2a) exhibited a shape similar to a fried egg with the conidal mass on the top giving yellowish orange to deep orange appearance. Portion of A. aleyrodis when cultured on PDA medium produced many small mycelial extensions which afterward became tightly packed and formed a hard and compressed colony on the culture medium.

As for colony characters are concerned, the colony was white to light orange or even yellow, effuse, fluffy, and convex having spherical surface with smooth edge. The colony which was observed growing relatively slowly (40 mm in 7 days) primarily composed of aggregates of short mycelia that were woven together to form ‘stroma’ (Fig. 2b). Stromata (1.5-5 mm in diameter, 0.3-1.0 mm in height) appeared white to yellowish white with circular to variable shape. Abundant confluent white or light yellow conidial masses were present with creamy yellow to orange conidial surface. Mycelia hyphae were smooth, septate, branched, 3-5 µm wide (Fig. 2c). Yellowish to light yellowish conidial droplets prominently formed near the top and middle of the stroma.

Under the microscope, a small ostiole was found on the stroma which represented the exit point of the conidia. Pycnidia embedded in stroma appeared, ovoid to flattened, globose to even circular, 120-340 x 140-300 µm. Conidiophores arising monomonoetically and laterally from thick walled hyphae with whorls of 2-5 conidigenous cells. Conidia hyaline, fusoid, occasionally slightly curved, apiculate with acute ends, aseptate; smooth measuring 10-12 x 1.5-2.5 µm in size, (Fig. 2d).

Effect of temperature on growth and sporulation of A. aleyrodis: The optimum temperature for growth and sporulation of the fungus was evaluated at 25°C (Table 1) which supported the maximum colony diameter and spore count (8.0 cm and 2.04 x 10⁹ spore/mL, respectively). The next best temperature for growth and sporulation was found to be 30°C (7.3 cm and 1.45 x 10⁹ spore/mL, respectively) followed by 20°C (4.5 cm growth and spore count 1.43 x 10⁹ spore/mL, respectively). There was relatively less growth and sporulation at 15°C and 35°C (2.6 cm and 1.17 x 10⁹ spore/mL and 3.2 cm 1.29 x 10⁹ spore/mL, respectively). At 40°C temperature there was hardly any growth (colony diameter 1.4 cm) and no sporulation at all.

Effect of different humidity levels on radial growth and sporulation of A. aleyrodis: The test fungus gave appreciable growth at all humidity levels (Table 2). At 100% and 85% relative humidity levels significantly higher colony growth and spore count was achieved (9.0 and 8.9 cm; 2.14 x 10⁹ and 2.09 x 10⁹ spore/mL, respectively). Similarly at 90% and 95% relative humidity levels also equally significant colony diameter was there (8.6 cm) and sporulation (2.0 x 10⁹ spore/mL and 2.02 x 10⁹ spore/mL, respectively).

Effect of light conditions on the radial growth and sporulation of A. aleyrodis: The test fungus gave appreciable growth at all humidity levels (Table 2). At 100% and 85% relative humidity levels significantly higher colony growth and spore count was observed (9.0 and 8.9 cm; 2.14 x 10⁹ and 2.09 x 10⁹ spore/mL, respectively). There was relatively less growth and sporulation at 15°C and 35°C (2.6 cm and 1.17 x 10⁹ spore/mL and 3.2 cm 1.29 x 10⁹ spore/mL, respectively). At 40°C temperature there was hardly any growth (colony diameter 1.4 cm) and no sporulation at all.

Table 1: Effect of temperature on growth and sporulation of A. aleyrodis

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Temperature °C</th>
<th>Growth (cm)* 10 DAI</th>
<th>Spore count 15 DAI (&lt; 10⁶ spore/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>2.6</td>
<td>1.17</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>4.5</td>
<td>1.43</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>2.0</td>
<td>2.04</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>7.3</td>
<td>1.45</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3.2</td>
<td>1.29</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>1.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Average of four replications, DAI days after inoculation

Table 2: Effect of Relative humidity on growth and sporulation of A. aleyrodis

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>RH (%)</th>
<th>Growth (cm)* 10 DAI</th>
<th>Spore count 15 DAI (&lt; 10⁶ spore/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>8.4</td>
<td>1.92</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>8.9</td>
<td>2.09</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>8.6</td>
<td>2.03</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>8.6</td>
<td>2.02</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>9.0</td>
<td>2.14</td>
</tr>
</tbody>
</table>

*Average of five replications, DAI days after inoculation
Effect of RH on growth and sporulation of *Aschersonia aleyrodis*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Mean colony (cm)*10 DAI</th>
<th>Spore count15 DAI (*10³ spore/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Continuous light</td>
<td>9.0</td>
<td>1.61</td>
</tr>
<tr>
<td>2</td>
<td>Alternate cycle of 12 hr light and 12 hr darkness</td>
<td>7.3</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>Continuous darkness</td>
<td>5.2</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>SE(m)</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>CD(P=0.01)</td>
<td>0.16</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Average of six replications DAI days after inoculation

**DISCUSSION**

Entomopathogenic fungus *A. aleyrodis* can be a potent candidate for use as bio-control agent in view of its ability to cause notable epizootic disease in whiteflies (*Aleyrodidae*) and scale insects (*Coccidae*) in the tropical and subtropical regions (Evans and Hywel Jones, 1990). In India the natural occurrence of *A. aleyrodis* was recorded by Prasad et al. (2004) in forest of Western Ghat in Goa State on the Homopteran insects. Debnath (2016) in North Eastern region reported its infection on scale insect in citrus orchards. So far no systematic studies have been conducted on the mycological aspects and the environmental factors favouring the growth of this species from Vidarbha region of Maharashtra.

The morphological features of the associated fungus were in conformity with the taxonomic details provided in the standard literature on the basis of which it was identified as *A. aleyrodis* (Samson et al., 1988; Liu et al., 2006; Homrahud et al. 2016).

Temperature is one of the crucial abiotic factors affecting development and sporulation of entomogenous fungi. During the present investigations temperature ranging between 25°C to 30°C was found to be favourable for the vegetative growth and sporulation of *A. aleyrodis* (Fig. 3). Similar observations have been documented by Lima et al. (2017) and Homrahud et al. (2016) who reported 25°C temperature as the optimum for *in vitro* growth and sporulation of *A. aleyrodis*. As has been observed presently, most entomopathogenic fungi are reported to show maximum germination within the temperature range of 20°C to 30°C (Roberts and Campbell, 1977). Similarly Fransen (1987) reported over 90% of the spore germination in case of *A. aleyrodis* when grown on water agar in the temperature range of 15°C to 28°C. Mycelia growth and conidial germination of this fungus ranged from 15°C to 35°C temperature, with 20°C to 30°C being the optimum. These studies clearly indicate the ability of this entomopathogenic fungas to acclimatize fairly over a wide range of temperature provided other environmental conditions are also favourable.

It is a well known fact that epizootics of mycopathogen are facilitated by number of environmental factors and among them humidity play a significant role in its growth and development. In the current study, there was statistically significant difference observed among the humidity levels (Fig. 4). Relative humidity ranging between 90% and 95% was found to be most favourable for the growth and sporulation of this fungus.

![Fig. 4: Effect of RH on growth and sporulation of *A. aleyrodis*](image)

![Fig. 3: Effect of temperature on growth and sporulation of *A. aleyrodis*](image)
reported a photoperiod of 16 hours among four varied light regimes to be most suitable for growth and sporulation of \( M. \) anisopliae. While working with \( N. \) rileyi Glare (1987) reported that in vitro sporulation of \( N. \) rileyi is severely inhibited by complete darkness and light is reported to be essential to produce conidia on the diseased cadavers of \( H. \) spp. This observation is exactly what we have observed while working with \( A. \) aleyrodis using different light and dark regimes. In the present study, the change of mycelial pattern was quite evident on the exposure of light in comparison to when grown under complete darkness. Also the colony was a bit creamy white with circular rings under continuous light exposure (Fig. 5) whereas in complete darkness the colony appeared whitish without any concentric ring. This variation clearly denotes that light plays a significant role in the growth and development of \( A. \) aleyrodis.

CONCLUSION

Ecological factors affect the mycelial growth and sporulation of \( A. \) aleyrodis in a different way. Optimum temperature of 25°C and exposure to more than 85% relative humidity and continuous light favours maximum growth and sporulation of this entomopathogenic fungus. This in vitro data can form baseline information for in vivo application of \( A. \) aleyrodis as a bio-control agent.

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REFERENCES


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