Original Research Article

***In Vitro* Growth of *Alternaria alternata* Isolated from Strawberry Under Constant Light or Constant Darkness Conditions**

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ABSTRACT

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| The first step in scientific studies on *Alternaria alternata* is the development of the pathogen *in vitro*. For this purpose, in addition to many media, environmental factors such as light, temperature, and chemicals should be considered and evaluated. On the other hand, studies on these issues can help us reveal the pathogen's habitat and the ways to manage it. A correct optimization will ensure that subsequent studies are carried out healthily. In this study, the development of the phytopathogen, *Alternaria* *alternata,* isolated from strawberries, was investigated under constant light and constant darkness conditions. Colony diameter, colony development rate, conidiospore amount, and colony color were evaluated by observation and measurements. The results showed that on the 8th day, the colony diameter was 58 mm in light and 61,7 mm in the dark, and the colony development rate was 72.7% in the light and 77% in the dark. The numbers of conidiospores were about 2×107 conidiospores/mL in light and dark. Colony color was evaluated from both the front and back in terms of the Red/Green/Blue color ranges method (RGB), and no statistically significant difference was found in either color or any other measured parameter. The results showed that *A. alternata* isolated from strawberries used in this study can be grown in continuous light or continuous dark conditions without any developmental differences. |

*Keywords: Alternaria alternata*; colony growth; conidia; dark; light; pathogen; small-spored; strawberry.

1. INTRODUCTION

The genus *Alternaria,* belongs to the Fungi kingdom, which includes the family Pleosporaceae, order Pleosporales, subclass Pleosporomycetidae, class Dothideomycetes, subphylum Pezizomycotina, and phylum Ascomycota (EPPO, 2025; NCBI, 2025). Within this genus, *Alternaria alternata* (Fr.) Keissl., is the type species (Lawrence *et al*., 2013; INDEX FUNGORUM, 2025). Diseases caused by *Alternaria* species are one of the most economically significant caused by phytopathogen worldwide, with an annual or perennial host range found in fruits, vegetables, field crops, ornamentals, and forest flora. Symptoms of the *Alternaria* spp. diseases vary from plant to plant, but are generally seen as leaf spots and blights. In the seedlings, they can cause trunk-girdling lesions and damping-off, and in mature plants, they can cause stem, tuber, and fruit lesions, necrosis, rot, and cankers (Agrios, 2005). One of the hosts of the pathogen is the strawberry. Strawberry (*Fragaria* × *ananassa*) is a very famous plant worldwide and has a special commercial importance. Aside from being considered a processed food, the fruit is suitable for fresh consumption, necessitating local consumption and production. This raises the possibility that diseases may be regional and that pathogenic species may have adapted to specific regions. It is important to conduct studies on the effect of environmental factors on the pathogen to both identify the species and control it. Determining the response of plants to light can guide the cultural control of this pathogen, which can be grown both in the greenhouses and in the open fields. One of the many fungi encountered on strawberries is *Alternaria* spp.. *Alternaria* is a leaf disease of strawberries, and some studies have been conducted on it. Some of the studies were first reported in some geographical regions of the strawberry. *A. alternata* was reported from Pakistan (Mehmood *et al*., 2018), *A. tenuissima* from Taiwan as fruit rot (Ko *et al*., 2008), *A. tenuissima* and *A. alternata* from Beijing, China (Fu *et al*., 2020), and *A. tenuissima* from Iran (Bagherabadi *et al*., 2015). Besides the initial reports, the toxins of *A. alternata* were studied to elucidate the disease mechanism of the pathogen in strawberries (Maekawa *et al*., 1984). Biological control of fruit rot of strawberry caused by *A. alternata* was also studied (Al-Rahbi *et al*., 2021). The fungicide resistance of *Alternaria* spp. in strawberries is also studied (Li *et al*., 2025). All the studies indicated the importance of *A. alternata* in strawberries. For this purpose, in addition to many media, environmental factors such as light, temperature, and chemicals should be considered and evaluated. On the other hand, the studies on these issues can help us reveal the pathogen's habitat and the ways to manage it. A correct optimization will ensure that subsequent studies are carried out healthily. The aim of the study is to investigate the development of the *A. alternata* pathogen isolated from the strawberries under the constant light and the constant dark conditions, which is one of the most important environmental factors.

2. material and methods

Isolation of the pathogen, *A. alternata*, was done from commercially grown strawberry (*Fragaria* x *ananassa*) leaves with diseased areas, which were irregular spots, in shape, 2 to 5 mm in diameter, and blackish brown. The symptomatic tissue in 4×4 mm was excised from the margins of the necrotic tissue, surface disinfected with 2%NaOCl for 1 minute, rinsed three times in sterile distilled water, and dried. The leaf tissue was then placed on ready-to-use Potato dextrose Agar (PDA) medium (39 g/L distilled water) poured into a Petri-plate with a 9 cm diameter, and five slices of leaves were placed at 25±1 °C in the dark. For each sample, three plates were prepared. From the mixture of the fungal cultures, morphologically Alternaria-like fungi were re-isolated and maintained in the same medium at 25±1°C. A molecular analysis was conducted on a colony derived from single spore isolation, using ITS1and ITS4 primers. The results, confirmed by a previous study, identified the sample as *Alternaria alternata* with 99.77% similarity in the NCBI database, as determined by a professional commercial laboratory. In the experiment, the single spore culture was maintained for eight days under the continuous dark and light (1000 lux illumination with a distance of 50 cm) to find out the difference in the lightness conditions. The colony diameters were measured on two perpendicular axes for each plate, using a digital calliper every two days. After eight days, the colony diameters, colony growth rate, colony color were measured, and conidiospores were counted using the methodology by flooding three different plates each with 10 ml of sterile distilled water and dislodging the spores using a spatula. For spore counting, the standart manual hemocytometer (Thoma lam) count method was used. For its macro and microscopic features, a light microscope (40-x magnification) was used. To evaluate the color of the colonies, the images were used in the IrfanView digital image processing program, which is a freeware program ([http://www.irfanview.com](http://www.irfanview.com/)). Three pieces of each 1 cm2 per Petri plates were taken from the front and back views without patches in saltations. In the part of the study, Red/Green/Blue color ranges (RGB) were evaluated, which define the intensity of the color with a value between 0 and 255. All data were analyzed in IBM SPSS Statistics 22. All data represent the mean of three sets of data from three repeats. In the tables, the means and the standard error of the means (S.E.M.) are shown.

3. results and discussion

The results indicated that both illumination (the continuous light or dark) showed no stimulatory effect on the colony diameter (Table 1) (Fig. 1), the colony growth rate (Table 2), conidiospore quantity (Table 3), and colony color (Table 4) (Fig. 2) of *A. alternata* that is isolated from the strawberry. The colony diameters were 58 mm and 61,7 mm under the light and dark conditions, respectively, on the 8th day. In the study of Li *et al*. (2025), forty-nine isolates were isolated from the symptomatic strawberry leaves and treated with a fungicide active ingredient to determine sensitivity. In the study, the colony diameters were changed between 59,28 mm and 71,98 mm in five sensitive and five resistant isolates after six days of the culture. The data is slightly different from this study, which changed to be 44 mm under the light and 44,3 mm under the dark on the 6th day of culture. Variations among the isolates could explain these differences.

In another study, the effect of constant light, constant darkness, light/darkness alternating, and constant UV on *A. dauci* and *A. alternata* isolates (Perviz & Trkulja, 2023). In the study, the colony diameters were measured on the 3rd, 5th, 7th, and 10th day of culture, and no light treatments affected colony diameter in the isolates of the two species. Besides constant UV, no lighting regime affected average daily increment. The constant light and dark conditions were statistically not different, reaching 85 mm in all treated *A. alternata* isolates on the 10th day. In the same study, sporulation was slightly more under constant light than constant dark. In addition, *A. alternata* sporulated in all light conditions at varying rates depending on the isolates. This study also showed more sporulation, but this is also not statistically different.

The microscopic observations of *A. alternata* conidiospores under both the constant light and darkness are shown in Fig. 3, and Fig. 4. The morphology of the spores was consistent across both conditions, exhibiting typical elliptical and septate conidia characteristic of the species. No significant morphological alterations were observed between treatments, indicating that light did not influence spore structure under *in vitro* conditions.

Yusef & Allam (1967) have found that no stimulatory response to light on the growth of *Myrothecium verrucaria*, *Pestalotia gracilis*, and *Pleurotus ostreatus*. A study was carried out by Cotty & Misaghi, (1985) on *A. tagetica* revealed that the fungus growth is inhibited by both the continuous and alternating light. In the study, the pathogen sporulated only in the one of the tested media (modified V-8 media) under alternating light. In addition, more lesions were observed in the dark in the *in vivo* study. Still, in this study, the alternating light and *in vivo* studies were not applied, and sporulation occurred in both (light and dark) conditions under PDA. This difference may be related to the pathogens tested.

**Table 1. The colony diameter of *A. alternata* from strawberry at the light or the dark conditions**

|  |  |
| --- | --- |
| **Light / Dark** | **Colony Diameters** **(mm)** |
| **2nd day** | **4th day** | **6th day** | **8th day** |
| **Light** | 14,7±0,6 | 31,3±0,6 | 44,0±1,0 | 58,0±1,0 |
| **Dark** | 16,0±1,0 | 32,3±1,5 | 44,3±1,5 | 61,7±3,1 |
| *P* value | N.S.\* | N.S. | N.S. | N.S. |
| Mean | 15,3±1,0 | 31,8±1,2 | 44,2±2,0 | 59,8±2,9 |

*\*N. S.; not significant (P < 0,05) difference between the light and the dark conditions (values expressed as mean ± S.E.M.) according to the ANOVA test*

**Table 2. Daily colony growth rate of *A. alternata* from strawberry at the light or the dark conditions**

|  |  |
| --- | --- |
| **Light / Dark** | **Colony Growth Rates** **(%)** |
| **Between****0-2nd day** | **Between****2nd-4th day** | **Between****4th-6th day** | **Between****6th-8th day** | **Beween****0-8th day** |
| **Light** | 73,3±2,89 | 107±4,00 | 70,3±2,31 | 65,7±2,31 | 72,7±1,53 |
| **Dark** | 80,0±5,00 | 101±1,73 | 68,7±0,58 | 69,7±5,13 | 77,0±3,61 |
| *P* value | N.S.\* | N.S. | N.S. | N.S. | N.S. |
| Mean | 76,7±5,16 | 104,0±4,29 | 69,5±1,76 | 67,7±4,18 | 74,8±3,43 |

*\*N. S.; not significant (P < 0,05) difference between the light and the dark conditions (values expressed as mean ± S.E.M.) according to the ANOVA test*

**Table 3. Conidiospore count of *A. alternata* from strawberry at the light or the dark conditions**

|  |  |
| --- | --- |
| **Light / Dark** | **Conidiospore Count** **(Conidiospores/mL)** |
| **Light** | 20 088 888 (2×107) ±2 155 440 |
| **Dark** | 20 000 000 (2×107) ±1 597 998 |
| *P* value | N.S.\* |
| Mean | 20 044 444 (2×107) ±1 697 698 |

*\* N. S.; not significant (P < 0,05) difference between the light and the dark conditions (values expressed as mean ± S.E.M.) according to the ANOVA test*

**Table 4. Colony color of *A. alternata* from strawberry at the light or the dark conditions in the 8th day**

|  |  |
| --- | --- |
| **Light / Dark** | **Colony Color in the 8th Day** |
| **Front** | **Back** |
| **Red** | **Green** | **Blue** | **Red** | **Green** | **Blue** |
| **Light** | 82,4±8,1 | 72,0±7,6 | 66,2±7,9 | 121,6±1,2 | 91,1±1,0 | 70,3±3,2 |
| **Dark** | 92,4±5,5 | 78,7±5,5 | 71,8±7,6 | 101,8±12,8 | 84,8±13,8 | 70,1±15,1 |
| *P* value | N.S.\* | N.S. | N.S. | N.S. | N.S. | N.S. |
| Mean | 87,4±8,3 | 75,4±7,0 | 69,0±7,6 | 111,7±13,6 | 87,9±9,4 | 70,2±9,7 |

*\* N. S.; not significant (P < 0,05) difference between the light and the dark conditions (values expressed as mean ± S.E.M.) according to the ANOVA test*



b

a



d

c

**Fig. 1. Colony growth of *A. alternata* from strawberry at the light (at the above three Petri plates) or the dark (at the below three Petri plates) conditions; a) in the 2nd day, b) in the 4th day, c) in the 6th day, and d) in the 8th day**



b

a



d

c

**Fig. 2. Colony color of *A. alternata* from strawberry at different conditions in the 8th day; a) at the light front view, b) at the light back view, c) at the dark front view, and d) at the dark back view**

 

a

b

**Fig. 3. Conidiospore count view at hemocytometer of *A. alternata* from strawberry at different conditions in the 8th day; a) at the light, b) at the dark**

 

b

a

**Fig. 4. Conidiospore view under microscope of *A. alternata* from strawberry at different conditions in the 8th day; a) at the light, b) at the dark (bars show 100 µm under 40× Magnification)**

4. Conclusion

Strawberries are extremely popular fruits and have some fungal phytopathogens in both the growth season and the post-harvest period. One of the pathogens is *Alternaria alternata*, which is mostly harmful to leaves, but at the same time, it shows symptoms on fruits. The study showed that the isolates of *A. alternata* were not affected by continuous light or dark conditions in *in vitro* studies at PDA medium under 25±1 °C. Therefore, from the perspective of further research, the authors of this study will deal with the influence of other environmental factors on the *A. alternata* of strawberries to find out biological and morphological differences for its control.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

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