# Impact of various temperature conditions and culture media on the development and sporulation of *A. brassicae* responsible for Alternaria blight in mustard

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***Abstract*— Rapeseed-mustard (*Brassica juncea L.*) ranks as the second most significant oilseed commodity in India. Alternaria blight (*A. brassicae*) is a highly destructive fungal pathogen that leads to substantial yield losses in this crop. The current study was conducted to examine the impact of different temperature conditions and culture media on the mycelial growth and sporulation of *A. brassicae*. The results clearly indicate that all the temperature conditions and culture media tested exhibited variations in colony diameter and other cultural characteristics. A temperature of 25°C notably promoted mycelial growth (85.00 mm) and demonstrated excellent sporulation of *A. brassicae* when compared to 15 and 35°C. Regarding culture media, Potato Dextrose Agar proved to be significantly more effective than the other media, achieving maximum mycelial growth (82.00 mm) along with excellent sporulation. Conversely, Oat Meal Agar resulted in the least mycelial growth (45.00 mm) with only fair sporulation. Additionally, variations in cultural characteristics such as colony color, growth, appearance, and shape were also noted across the different culture media.**

***Index Terms*— Rapeseed-mustard, Temperature, Culture media, , *A. brassicae*, Alternaria blight.**

## I. INTRODUCTION

Rapeseed-mustard is a globally significant and economically valuable oilseed crop. In India, it is cultivated across various agro-climatic regions and is recognized as the third most crucial oilseed crop, following soybean and palm (Kumar, 2014), contributing 25 percent to the overall oilseed production (Yadav et al., 2019). This crop serves as an essential source of edible oil and is also utilized as vegetables, condiments, livestock feed, and for soil enhancement. Additionally, mustard oil is in demand in various industries for the production of items such as soap, paints, hair oil, and numerous other products. It is rich in erucic acid, linoleic acid, and linolenic acid, which are not found in many other edible oils (Singh et al., 2011). India ranks second in terms of area, covering 6.23 million hectares, with an annual production of 9.43 million tons and an average yield of 1499 kg per hectare (Agricultural Statistics at a Glance, 2021). The primary mustard-producing states include Rajasthan (46.06%), Haryana (12.60%), and Madhya Pradesh (11.38%), collectively accounting for approximately 70% of the total production in India (Agricultural Statistics at a Glance, 2021).

The quantity and quality of agricultural crops are affected by a variety of biotic and abiotic factors. In India, crops are susceptible to more than 30 different diseases, which not only diminish seed quality but also result in a notable reduction in oil content (Saharan et al., 2005). Among the numerous fungal diseases, Alternaria blight, caused by *Alternaria brassicae*, *A. brassicicola*, *A. raphani*, *and A. alternata*, is recognized as one of the most destructive diseases worldwide. Of these four species, *Alternaria brassicae* and *A. brassicicola* are the main leaf pathogens that commonly infect crops. The severe attacks by these pathogens negatively affect seed quality and lead to a significant decrease in oil content across various oil-producing Brassica species (Kolte, 1985; Meena et al., 2010). This disease causes substantial losses in mustard crops and is particularly prevalent in Uttar Pradesh (Meena et al., 2010). Under ideal conditions, the losses can soar to as much as 47 percent (Saharan et al., 2016).

The disease affects all above-ground parts of the plants, including the stem, leaves, fruits, and siliquae. Initially, the symptoms appear as small black spots on the leaves, stem, and siliquae. These spots gradually enlarge and evolve into distinct round shapes with concentric rings, resembling a target board. In severe cases of infection, many small spots can merge to form larger ones, resulting

in blighting and the loss of leaves. In some Brassica species, the lesions are characterized by prominent concentric rings and a yellow halo surrounding them (Saharan and Mehta, 2002). The pathogen can be found both in seeds and in the soil, where it survives as saprophytes on decomposing plant material.

*A. brassicae* is classified as a pseudo-fungus due to its absence of a distinct sexual reproduction phase, and it is known to have a global distribution. The conidia produced by this organism are notably large and obclavate in shape, exhibiting a color spectrum that ranges from olive grey to dark. These conidia are characterized by the presence of both longitudinal and transverse septa, as noted by Aneja et al. in 2014. To gain a deeper insight into the host-pathogen dynamics, it is essential to conduct a thorough analysis of dietary habits and the environmental factors influencing the growth of this pathogen, particularly temperature and pH levels. Consequently, this study is designed to be conducted in vitro, focusing on how varying temperature conditions and different types of culture media affect the mycelial growth and sporulation of *A. brassicae*.

## II. MATERIAL AND METHODS

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

Potato dextrose agar medium served as a fundamental medium to evaluate the differences in radial growth and sporulation of A. brassicae across five distinct temperature settings, with intervals of 5°C, specifically at 15, 20, 25, 30, and 35°C. Inoculation involved a 5 mm mycelial segment, extracted from a 7-day-old culture of A. brassicae, which was then placed in 90 mm diameter Petri plates containing sterilized solidified PDA. These Petri plates were subsequently separated for incubation in various BOD incubators set to temperatures of 15, 20, 25, 30, and 35°C. The Petri plates were regularly monitored after 48 hours to observe the initiation of mycelial growth of the fungus. Each treatment was replicated three times, and mycelial growth along with sporulation was documented after 7 days of inoculation in each Petri plate maintained at different temperatures. The measurements of radial growth were conducted by drawing two perpendicular lines intersecting at the center of the lower surface of the Petri plate, and the average of the three replicates was calculated.

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

The current experiment was designed to investigate the impact of eight distinct nutrient media, namely 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 using a standardized method and autoclaved at 121.6°C, 15 lbs p.s.i. for a duration of 15 minutes. Equal volumes (20 ml) of each culture medium were dispensed into Petri plates with a diameter of 90 mm. Subsequently, each Petri plate was inoculated with a 5 mm disc from a 4-5 day old culture of A. brassicae using a sterilized cork borer. Each treatment was replicated three times, and the Petri plates were incubated at a temperature of 25±2°C for 7 days. These Petri plates were regularly monitored for the radial growth of the fungus. The cultural characteristics, including colony color, growth, margin, zonation, appearance, shape, and sporulation, on all media were also documented.

## III. RESULT AND DISCUSSION

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

The results presented in Table 1 clearly indicate that the pathogen exhibited robust growth across all temperature ranges from 15 to 35°C. The most significant mycelial growth, measuring 85.00 mm, along with excellent (++++) sporulation, was recorded at 25°C. This was succeeded by 30°C with a growth of 68.66 mm, 20°C with 47.33 mm, and 35°C with 38.00 mm, all demonstrating good (+++) to fair (++) sporulation, respectively. Conversely, the lowest mycelial growth was noted at 15°C, which showed poor (+) sporulation.

Table 1. Impact of various temperature conditions on the growth and sporulation of *A. brassicae* on PDA

| Temperature ($^0$C) | Mycelial growth (mm)*7DAI | Sporulation |
|---|---|---|
| 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 | - |

The results indicate that the optimal temperature for the mycelial growth and sporulation of A. brassicae is 25°C. These findings align with those of other researchers who have also observed the impact of different temperature conditions on the mycelial growth and sporulation of A. brassicae (Taware et al., 2014; Yadav et al., 2016). In a separate study, Panchal (2008) reported remarkable mycelial growth and sporulation of A. alternata, which infects tomato, at 25°C. Additionally, Kurhade et al. (2021) noted that A. tagetica, responsible for leaf blight in marigold, exhibited the highest mycelial growth and excellent sporulation at 25°C.

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 | - | - | - | - | - | - | - |

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

This study was conducted to observe the variation in the response of A. brassicae concerning their cultural characteristics across eight distinct nutrient media. The pathogen exhibited significantly the highest 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), as shown in Table 2. Czapek's Dox Agar was the next significantly superior nutrient medium, recording a growth of 59.00 mm. The least growth (45.00 mm) was observed on Oat Meal Agar, followed by Rose Bengal Agar (56.66 mm). *A. brassicae* exhibited variations in cultural characteristics across different nutrient media. A grey-colored colony was observed on Oat Meal Agar and Czapek's Yeast Extract Agar, whereas a dark green colony was noted on Potato Dextrose Agar. In contrast, the remaining nutrient media tested resulted in colony colors ranging from greyish white to blackish white (Table 2). .

Fluffy to compressed growth patterns were noted across all tested nutrient media. The fungus demonstrated rapid growth on Potato Dextrose Agar, Richard's Synthetic Agar, Nutrient Agar, and Czapek's Yeast Extract Agar. Medium growth was recorded on Czapek's Dox Agar, Rose Bengal Agar, and Corn Meal Agar. Conversely, slow growth was observed on Oat Meal Agar (Table 2).

The margins, zonation, and shape vary according to the nutrient media used. Circular margins were observed, while shapes ranged from regular to irregular across all tested media. Zonation within the colony was not present in any of the tested media,

with the exception of Nutrient Agar, Czapek's Dox Agar, and Rose Bengal Agar (refer to Table 2).

Each of the nutrient media tested displayed a broad spectrum of sporulation. Notably, Potato Dextrose Agar and Richard's Synthetic Agar demonstrated exceptional (++++) levels of sporulation. The remaining nutrient media showed good (+++) sporulation, with the exception of Oat Meal Agar, which displayed fair (++) sporulation (see Table 2). The current research aligns with the findings of Charith et al. (2020), who investigated the growth of the fungus across various media and demonstrated that the Potato Dextrose Agar medium significantly facilitated the highest radial growth (68.40 mm) of A. brassicae, followed by the Oat Meal Agar medium, while the least growth was recorded in the Czapex Dox Agar and Corn Meal Agar media. Additionally, other studies conducted 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) corroborated these results.

## IV. CONCLUSION

Recent studies have shown that the ideal temperature for both growth and spore production of the fungus is 25°C. In stark contrast, at a lower temperature of 15°C, the fungus displayed significantly reduced growth, which in turn resulted in insufficient sporulation. Additionally, among the eight different culture media evaluated, Potato Dextrose Agar emerged as the most effective, promoting the highest levels of mycelial growth and optimal sporulation of the pathogen. On the other hand, Oat Meal Agar was found to be the least effective medium, yielding only minimal mycelial growth and producing sporulation that was merely adequate.

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