The Pharma Innovation Journal 2023; 12(8): 2661-2664

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 2661-2664

www.thepharmajournal.com
Received: 02-05-2023
Accepted: 06-06-2023

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Cultural variability of *Alternaria alternata* on different media

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Abstract
The *Alternaria* blight is an important disease among the different cultivated and wild hosts. The diseased leaves of different plants species showing typical symptoms of *Alternaria* leaf blight were collected from different cultivated and wild hosts from different locations. Total 12 cultures of *Alternaria* were isolated and proved their pathogenicity. Pure culture of the fungus so obtained was sub cultured on PDA slants and incubated at 27±2 °C. The growth of 12 isolates of *A. alternata* were studied on 6 different media (three Synthetic & three non-synthetic) namely Potato dextrose agar medium, Oat meal agar medium, Richard's medium, Asthana & Howker's medium, Sabourauds agar medium and V8 juice agar medium. Each treatment was replicated thrice. On the basis of linear growth, the best medium for the growth of the fungus was identified. The cultural characteristics, *viz*., topography, colour of colony, growth pattern, type of margin were recorded periodically. The variation was observed in cultural characteristics such as colony colour, growth pattern and zonation.

Keywords: *Alternaria alternata*, isolation, culture, autoclave, sterilization

Introduction
The members of the genus *Alternaria* are anamorphs of the fungus belonging to genus *Lewia* which is a member of the family Pleosporaceae in the order Pleosporales of class Dothideomycetes of Phylum Ascomycota. Most of the *Alternaria* species are destructive pathogens of cultivated crops and wild hosts. A large variation observed in genus *Alternaria.* The genus *Alternaria* was first described by Nees in 1817, with *A. tenuis* as the sole species. The conidial spores of *Alternaria* are airborne and found all over in surrounding, especially in the soil and water. The long chains type structure is formed by club-shaped spores which are single and able to grow on thick colonies. The colonies are fast growing usually green, black to olivaceous black or grey in colour. By and large, *Alternaria* species cause about 20 to 80 per cent losses in field crops, horticultural crops, plantation crops, forest plants and post-harvest storage (Nagrale *et al*., 2013) [11]. The pathogen produces distinctive leaf spots and can also cause stem lesions, ring spots and fruit rot. Initial infection on the leaves is in the form of small, circular dark spots, which gradually enlarge to form larger patches with concentric rings. As the disease progresses, such spots may enlarge to 1 cm or more in diameter and are usually gray, gray-tan, or nearly black in colour. The symptoms vary from host to host.

Materials and Methods
Three Synthetic and three non-synthetic media were evaluated in the study. The initial pH of each medium was adjusted to 6.5 prior to autoclaving. The medium was prepared with given composition and dispended in conical flask. The flasks were plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs pressure for 20 minutes. Petri plates were sterilized in hot air oven at 160 °C for 1 hour. Such sterilized Petri plates were poured with 20 ml of molten medium and allowed to solidify. Five-millimeter diameter disc of the test fungus was cut with the help of incinerated cork borer and inoculated at the center of Petri plates. The inoculated plates were then incubated at room temperature (27±2 °C) for 7days. Each treatment was replicated thrice. On the basis of linear growth, the best medium for the growth of the fungus was identified. The cultural characteristics, *viz*., topography, colour of colony, growth pattern, type of margin were recorded periodically.

Results and Discussion
The twelve isolates were studied for their cultural variability on different media (Table 1) *viz*., Potato dextrose agar (PDA), oat meal agar (OMA), Richard's agar (RA), Asthana and

Hawker's agar (AH), Sabouraud's agar (SA) and V8 Juice agar medium. The isolates grown on different media showed varied colony characters. (All the twelve isolates of *Alternaria alternata* are coded as A1, A2 ……. A12.)

On PDA medium, colonies A1, A8 and A12 were dark olive green in colour. The colonies of A9 was olive green with a blackish tinge. Light olive green colonies with grayish to greyish white surface were formed by A2, A3, A10 and A11. The colony colour of the isolates A4 and A5 was grey with white surface while that of A6 was dark grey with white surface. The isolate A7 formed dark grey colony. The colonies were rough in A1, A2, A3, A4, A5, A6 and A7, fluffy thick growth, smooth in A8, A9, A10, A11 and A12. The isolates (A1, A2, A7, A8, A9, A10, A11 and A12) formed concentric zonate colonies but such zonation was not formed in the colonies of the isolates A3, A4, A5, and A6. The results of present study are in agreement with Jadhav (2003) who reported that *A. alternata* forms circular, profuse, grayish black colonies with whitish growth on the upper surface on PDA. Akbari (2005) [2] observed that, colony of *A. alternata* on PDA was fairly compact, raised, grayish white to olivaceous green. Devappa and Thejakumar (2016) [5] found colonies of *A. alternata* on PDA were grey with raised fluffy growth and irregular margin.

On OMA medium, colonies were dark olive green to black with greyish surface found on A1, greenish white on A2, A6, A7 and A12, greyish white on A3, A10 and A11, dark olive green to black on A5, greenish white on A4 and A8, black with greyish surface on A9. Colonies were rough, fluffy with thick growth on A11, A12. Smooth, fluffy thick growth on A2, A4, A5, A6, A7, A8 and A9. Smooth, fluffy shiny aerial mycelial growth found on A1. Smooth, fluffy raised uniform cottony growth on A3. Smooth, fluffy thick mycelial growth, touching the lid of the Petri plate in A10. The growth patterns of all isolates were regular. The colonies of A2, A12 did not form concentric zonation while remaining all the isolates produced concentric zonation. Akbari (2005) [2] reported that the colony of *A. alternata* on OMA was regular, raised loose cottony growth with whitish gray mycelium. Nagrale *et al*. (2013) [11] found that the colonies of *A. alternata* were ashy white to light green, circular with profuse mycelial growth in the form of concentric rings on OMA medium. The colonies of *A. alternata* on OMA were greyish in colour with irregular colony margin (Devappa and Thejakumar, 2016) [5].

On Richard's agar (RA) medium, colonies of A9 were dark olive green colour while those of A1, A5 and A12 were olive green. The colonies of A8 were also olive green but with greyish surface at the center. Greyish white colonies were formed by the isolates A3, A10 and A11. Dark olive green colonies with greenish white surface at center were formed by A2. While, dark grey with whitish surface were observed in isolates A4, A6 and A7. Flat, smooth colony growth with sectoring at uniform distance was observed in isolates A1. Flat, continuous growth, with white sparse, superficial growth throughout entire diameter of the colony was observed in A2 while similar growth with white sparse, superficial growth in the inner 2-2.5 mm diameter was recorded in A4. The colonies of the isolates A6, A8 and A11 exhibited similar growth. On this medium, the colony of A3 was white with a violet border. The colony of A9 was flat, rough with slightly elevated concentric rings whereas colonies of A10 and A12 were with fluffy, smooth, dense growth. The colonies with sparse, flat and smooth growth were formed by A5 and A7. The growth pattern of isolates A2, A3, A6, A7, A8, A10 and A12 was regular and that of isolates A1, A4, A5, A9 and A11 was irregular. All the isolates formed concentric rings except A2, A4 and A12. The results of the present study are in concurrence with Akbari (2005) [2], who reported that the colony of *A. alternata* on Richard's agar was regular, compact, and grayish to olivaceous mycelium with black center and substrate was pinkish white in colour. Nagrale *et al*. (2013) [11] reported that the colony of *A. alternata* was dull white to green, serrated and roughly circular on Richard's agar medium. Devappa and Thejakumar (2016) [5] reported that *A. alternata* formed cream/ whitish colony on Richards's agar medium with irregular margin. The results of Nagrale *et al*. (2013) [11] and Devappa and Thejakumar (2016) [5] are contradictory to present findings.

On Asthana and Hawker's agar (AH) medium, colonies were dark olive green (A9), olive green (A1, A2, A5 and A12), Greenish grey (A4) and greyish white (A3, A6, A7, A8, A10 and A11). Colonies with sparse, smooth, flat growth were observed in terms of the isolates A1, A2, A3, A4, A5 and A12. Centrally fluffy, smooth, flat marginal growth was formed by the isolates A6, A7, A8 while flat, thick, smooth growth was formed by A9. Fluffy, thick, rough growth was observed in the colonies of A10 and A11. All tweleve isolates formed regular growth pattern. There was no zonation in isolates A3 and A4 but remaining all the isolates produced concentric zonation. Akbari (2005) [2] who reported that the colony of *A. alternata* on Asthana& Hawker's agar was regular and formed scanty growth with whitish gray mycelium. Devappa and Thejakumar (2016) [5] reported that *A. alternata* formed grey colour colony on Asthana and Hawker's medium. Nagrale *et al*. (2013) [11] reported that the colonies of *A. alternata* formed cottony white, wavy, sub-circular, raised mycelium without concentric rings on Asthana and Hawker's.

On Sabouraud's agar (SA) medium, colonies were dark olive green to black (A5), olive green (A1, A2 and A12), grey with white surface at center (A3), black colour (A9), grey colour with whitish surface (A4), dark grey (A6) and greyish white (A7, A8, A10 and A11). Colonies were sparse, smooth, flat growth in isolates A1, A2, A3, A4, A5 and A12, sparse, rough, flat growth in A6, A11, smooth, flat growth in A8, A9, and A10, smooth, fluffy growth (A7). The growth pattern of isolate (A1, A2, A3, A5, A6, A7, A8, A9, A10, A12, while (A11) were slightly irregular and (A4) were formed slightly irregular wavy margin. The isolates (A1, A2, A4, A5, A8, A9, A11 and A12) produced concentric zonation and isolates (A3, A6, A7 and A10) not produced zonation. The results are in conformity with Nagrale *et al*. (2013) [11] and Devappa and Thejakumar (2016) [5]. Nagrale *et al*. (2013) [11] who reported that the colonies of *A. alternata* were green to dark greenish, wavy, roughly circular and moderately raised mycelium with concentric rings on Sabourard's agar medium. Devappa and Thejakumar (2016) [5] reported that the *A. alternata* formed grey colour colony with irregular margin on Sabouraud's agar medium.

On vegetable juice agar (V8) medium colonies were olive green and white (A1, A2), centrally black colour with greyish surface at marginal side (A12), greyish white (A3, A4, A6, A7, A10 and A11), black colour (A8 and A9) and dark olive green to black (A5). Colonies were smooth, flat growth in isolates A1, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12 and centrally raised, flat marginal growth, smooth in isolate A2.

The isolates (A2, A3, A4, A5, A6, A7, A10, and A12) showed violet pigmentation in V8 medium. The growth pattern of isolates on V8 medium showed great variation *viz*; regular, irregular, slightly irregular with white rim, irregular with wavy margin, irregular with thick white mycelial growth at margin and slightly irregular with cream colour rim. The 11 isolates were produced concentric zonation, only A3 isolate not produced zonation on V8 medium. The results of the present study are in agreement with Devappa and Thejakumar (2016) [5] reported that the dark grey colony formed by *A. alternata* with irregular margin on V8 medium.

Table 1: Cultural characteristics of *Alternaria alternata* on different solid media (Synthetic & non-sysnthetic)

M and I	Potato dextrose agar medium	Oat meal agar medium	Richard's medium	Asthana & Hawker's agar medium	Sabouraud's agar medium	V8 juice agar medium
A1	Dark olive green, fluffy thick, rough, regular with zonation	Dark olive green to black with greyish surface, fluffy, smooth, regular with zonation	Olive green, flat, smooth, slightly irregular with zonation	Olive green, sparse, flat, smooth, regular with zonation	Olive green, Sparse, flat, smooth, regular with zonation	Olive green, flat growth, smooth, irregular with zonation
A2	Light olive green with greyish surface, fluffy thick, rough, regular with zonation	Greenish grey, fluffy thick, smooth, regular without zonation	Dark olive green with greenish surface at centre, fluffy thick growth, rough, regular without zonation	Olive green, sparse, flat, smooth, regular with zonation	Olive green, Sparse, flat, smooth, regular with zonation	Olive green, centrally raised, flat margin, smooth, violet pigmentation, irregular wavy, with zonation
A3	Greyish white, fluffy thick, rough, regular without zonation	Greyish white, , cottony, smooth, regular with zonation	Greyish white, flat, violet pigmentation, smooth, regular with zonation	Greyish white, sparse, flat, smooth, regular without zonation	Grey with white surface at Centre, sparse, flat, smooth, regular without zonation	Greyish white, Flat, smooth, slightly irregular wavy margin with white rim, violet pigmentation, no zonation
A4	Grey with white surface, fluffy thick, rough, regular without zonation	Greenish white, fluffy thick, smooth, regular with zonation	Dark grey with whitish surface, fluffy thick, rough, slightly irregular without zonation	Greenish grey, sparse, flat, smooth, regular without zonation	Grey with whitish surface, sparse, flat, smooth, irregular wavy margin, with zonation	Greyish white, flat growth, smooth, violet pigmentation, slightly irregular, creamy rim with zonation
A5	Grey with white surface, fluffy thick, rough, regular without zonation	Dark olive green to black, fluffy thick, smooth, regular with zonation	Olive green, sparse, flat, smooth, slightly irregular with zonation	Olive green, sparse, flat, smooth, regular with zonation	Dark olive green to black, sparse, flat, smooth, regular with zonation	Dark olive green to black, flat growth, smooth, violet pigmentation, slightly irregular, creamy rim with zonation
A6	Dark grey with white surface, fluffy thick, rough, regular without zonation	Greenish grey, fluffy thick, smooth, regular with zonation	Grey with whitish surface, fluffy thick, rough, regular with zonation	Greyish white, centrally fluffy, flat margin, smooth, regular with zonation	Dark grey, Sparse, flat, rough, regular without zonation	Greyish white, flat, smooth, slightly irregular, violet pigmentation, with zonation
A7	Dark grey, fluffy thick, rough, regular with zonation	Greenish grey, fluffy thick, smooth, regular with zonation	Grey with whitish surface, sparse, flat, smooth, regular with zonation	Greyish white, centrally fluffy, flat margin, smooth, regular with zonation	Greyish white, fluffy, smooth, regular without zonation	Greyish white, flat, smooth, slightly irregular, violet pigmentation, with zonation
A8	Dark olive green, fluffy thick, smooth, regular with zonation	Greenish white, fluffy thick, smooth, regular with zonation	Olive green with greyish surface, fluffy thick, rough, regular with zonation	Greyish white, centrally fluffy, flat margin, smooth, regular with zonation	Greyish white, flat, smooth, regular with zonation	Black colour, flat, smooth, irregular with white end margin with zonation
A9	Dark olive green to black, fluffy thick, smooth, regular with zonation	Black colour with greyish surface, fluffy thick, smooth, regular with zonation	Dark olive green, flat, with grooves, rough, slightly irregular with zonation	Dark olive green, flat thick, smooth, regular with zonation	Black colour, flat, smooth, regular with zonation	Black colour, flat, smooth, violet pigmentation, slightly irregular, creamy rim, with zonation
A10	Greyish white, fluffy thick, smooth, regular with zonation	Greyish white, fluffy thick, reached to the upper lid, smooth, regular with zonation	Greyish white, flat thick, smooth, regular with zonation	Greyish white, fluffy thick, rough, regular with zonation	Greyish white, flat, smooth, regular without zonation	Greyish white, flat, smooth, violet pigmentation, irregular, with zonation
A11	Greyish white, fluffy thick, smooth, regular with zonation	Greyish white, fluffy thick, rough, regular with zonation	Greyish white, flat thick, rough, slightly irregular with zonation	Greyish white, fluffy thick, rough, regular with zonation	Greyish white, Sparse, flat, rough, slightly irregular with zonation	Greyish white, flat, smooth, slightly irregular with zonation

A12	Dark Olive green, fluffy thick, smooth, regular with zonation	Greyish white, fluffy thick, rough, regular without zonation	Olive green, fluffy thick, smooth, regular without zonation	Olive green, sparse, flat, smooth, regular with zonation	Olive green, Sparse, flat, smooth, regular with zonation	Centrally back, greyish white margin, flat, smooth, irregular with violet pigmentation, with zonation

Conclusion

The isolates exhibited variation in colony colour and growth pattern. PDA was found best to culture *Alternaria* spp. and it was followed by OMA. V8 medium was found not suitable for culturing *Alternaria*. Isolate A1 recorded maximum mean radial mycelial growth and isolate A7 recorded least mean radial growth as compare to the rest of the isolates.

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