MORPHOLOGICAL AND BIOCHEMICAL VARIATION OF SOME
ALTERNARIA SPECIES INFECTED ON DIFFERENT
FLORICULTURAL PLANTS

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KEYWORDS

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INTRODUCTION

The genus Alternaria includes nearly 100 species of dematiaceous hyphomycetes, it causes a range of economically important leaf spot diseases on a variety of fruits, vegetable, field crops, ornaments and flowers. Many reports on the existence of variability among different Alternaria species from different hosts have been reported by earlier workers (Pryor and Gilbertson, 2002; Pryor and Michailides, 2002; Quayyum et al., 2005; Kumar et al., 2008). The genus is characterized by the production of dark colored conidia with longitudinal and transverse septa which is the key taxonomic feature. Within the genus Alternaria species are defined primarily on conidium characteristics including size, septation, presence and or size of a beak and pattern of catenation. Because of the diversity of Alternaria species, several sub-generic groups have been proposed. Alternaria spp. exhibit considerable morphological plasticity that is dependent on cultural condition of substrate, temperature, light and humidity. In addition, within any culture, there is considerable range of variation in conidium morphology in regard to size, shape, septation, color and ornamentation. In spite of the diversity in the species and the capacity of the fungus to infect a wide range of economically important plant, no comprehensive information about the occurrence of the pathogen in flower plants are available like tuberose, rose, marigold and gladiolus. The comprehensive understanding of this causal organism with reference to morphological, cultural and molecular variability within the four different host cultivars in this region which can contribute a preliminary idea to develop an effective programme for further detailed study for future breeding programme.

MATERIALS AND METHODS

Collection and isolation of the fungus: Leaves of different floricultural plants showing typical symptoms of dark blight lesions were collected and isolated by following technique below.

The infected leaf along with healthy portions cut into small bits and surface sterilized using 2% HgCl₂ for 30 sec. The bits were washed thoroughly in sterile distilled water for three times to remove all traces of HgCl₂. The surface sterilized leaf were placed on PDA medium and incubated at 27 ± 1°C for 7 days. Apparently pure culture developed from PDA slants.

Koch’s Postulates has proved the four floricultural plants tuberose, marigold, rose and gladiolus were raised in pots (Elwakil et al., 2009; Jain et al., 2005). One month old plants inoculated with spore 5×10⁴ spores/mL suspension using an atomizer. Disease symptom was appeared after 10-12 days on identified on external symptoms.

Morphological studies of the fungus

Media used for maintenance and cultural studies of pathogen 1. Czapek’s Dox Agar 2. PDA- Potato Dextrose Agar 3. Carrot Agar 4. Oatmeal Agar 5. Potato Carrot Agar were prepared as suggested by “Ainsworth and Bisby’s Dictionary of the fungi” by Ainsworth (1961). 20mm of each medium listed above was poured into 90mm diameter Petri plates. After solidification 5mm discs from 5-7 days old cultures
of *Alternaria* spp. were cut by using a cork borer and were placed at the centre of the plate. Each set of the experiment was replicated thrice and the plates were incubated at 27 ± 1°C for 9 days. The colony diameter, color of the colony, nature of colony margin and the zonation of the colony were recorded.

Morphological characters of the fungal pathogen infecting floricultural plants were studied from the culture growth on Carrot Agar media. The slides of the selected fungal cultures or colony were prepared in order to study the fungal morphology such as conidial length, breadth, number of septations and length of the beak. The prepared slides were observed under Phase-contrast microscope. The photographs of the observed conidia are taken and the micrometric measurements of the conidia are done. Ocular micrometer was calibrated and by use of micrometry (Meena et al., 2005)

**Studies on Iso-enzyme and gel Electrophoresis of Esterase Iso-enzyme**

To study this, different isolates of *Alternaria* spp. were grown in 250mL conical flask containing 50mL potato dextrose broth.
Three flasks were used for each isolate. Each flask was inoculated with 2 discs each of 5mm diameter cut from the periphery of actively growing 5 day old culture grown on PDA. The inoculated flasks were incubated at 28±ºC for 10 days. Electrophoresis of esterase isoenzyme was done in 7.5% gel according to the method proposed by Kahler and Allard (1970).

The Rm (Relative mobility) value of band(s) in gel was estimated.

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Rm \text{ value} = \frac{\text{Distance of the band from origin}}{\text{Distance of buffer front}}
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Estimation of Phenol and total carbohydrate of the fungal mat was estimated by the method of Mahadevan and Sridhar (1982) and total sugars were determined by using anthrone reagent (Hedge and Hofreiter). Prepare a standard curve using different concentrations of catechol for phenol. Standard curve was prepared with different concentration of dextrose for sugar. The color intensity was measured at 630 nm in a spectrophotometer.

RESULTS AND DISCUSSION

The morphological characters were different among the 30 observations taken within the same host (Table 1). In case of tuberose, length of conidia with beak varied between 17.28-64.30μm, length of conidia without beak varied between 14.44-46.98μm, beak length varied between 0.0-17.32μm, width of conidia varied between 4.68-11.71μm. Horizontal septation varied between 2-7, vertical septation varied between 0-2 and oblique septation varied between 0-1. The marigold isolate showed a varied length of conidia with beak varied between 21.45-44.42μm, length of conidia without beak varied between 16.41-43.76μm, beak length varied between 0.0-10.26μm, width of conidia varied between 7.93-32.30μm, number of horizontal septation varied between 1-5, vertical septation varied between 0-1 and oblique septation varied between 0-1. Similarly, in gladiolus, length of conidia with beak varied between 15.96-41.16μm, length of conidia without beak varied between 13.85-36.02μm, beak length varied between 0.0-9.19μm, width of conidia varied between 3.23-9.43μm. Horizontal septation varied between 2-8, vertical septation 0-4 and oblique septation was observed. So, the result noted that the Alternaria spp. effect on different host produced different type of morphological variability. Muthulakhsmi (1990) and Cuervo-Parra et al. (2011) reported A. alternata produced both beaked and unbeaked conidia. This conidial morphology of Alternaria spp. isolates in different hosts was in accordance with those described by Simmons (1952) in rose and Keisuke et al. (2000) on marigold. However, Kaul and Saxena (1989) also reported that spore dimensions are not useful in distinguishing the Alternaria spp. strains. The cluster analysis of morphological characters showed that tuberose and gladiolus were in similar cluster and nearer to marigold but rose is in different cluster. Fungi secure food and energy from substrate upon which

| Table 5: Cultural characteristics of the isolates of Alternaria polyanthi from tuberose on different media |
|---|---|---|---|---|---|---|
| Day | Media | Colony size (Diameter)(mm) | Colony colour | Mycelial growth | Margin of colony | Zonation |
| 1d-9d | Czapek’s dox | 7.33 -90 | Whitish | Thin - mycelium-Thin mycelium | Roundish | No zonation |
|   | Potato Dextrose Agar | 7.66 -90 | Whitish | Cotty - Fluffy | Roundish | No zonation |
|   | Carrot Agar | 8 -90 | Whitish | Fluffy - Fluffy | Roundish | No zonation |
|   | Oat Meal Agar | 7 -90 | Whitish | Fluffy - Fluffy | Roundish | No zonation |
|   | Potato Carrot Agar | 7.33 -90 | Whitish | Fluffy - Fluffy | Roundish | No zonation |

| Table 6: Cultural characteristics of the isolates of Alternaria fasciculatus from gladiolus on different media |
|---|---|---|---|---|---|---|
| Day | Media | Colony size (Diameter) (mm) | Colony colour | Mycelial growth | Margin of colony | Zonation |
| 1d-9d | Czapek’s dox | 8 - 45.66 | Whitish - Dull white | Cotty | Roundish - Irregular with white margin | No zonation |
|   | Potato Dextrose Agar | 14.66 -77.33 | Whitish - Whitish | Cotty | Roundish | No zonation |
|   | Carrot Agar | 12.66 - 62.66 | Whitish - Blackish with white margin | Cotty | Roundish | No zonation |
|   | Oat Meal Agar | 8.33 - 90 | Whitish - Whitish | Thin mycelium | Roundish | No zonation |
|   | Potato Carrot Agar | 6.66 -38 | Whitish - Whitish | Cotty | Roundish | No zonation |

| Table 7: Relative mobility (Rm) values of â- esterase isozyme in different isolates of Alternaria spp. |
|---|---|---|---|---|---|---|
| Sr. No. | Isolates | Rm value | No. of bands |
| 1 | Tuberose | 0.7185 | 6 |
| 2 | Rose | 0.625 | 7 |
| 3 | Gladiolus | 0.4375 | 4 |
| 4 | Marigold | 0.4375 | 2 |
**Dendrogram**

Figure 1: Morphological dendrogram of different *Alternaria* spp

Figure 2: Dendrogram of â-esterase isomers of different isolate of *Alternaria* spp.

**Hierarchical Cluster Analysis**

Dendrogram using Average Linkage (Between Groups)

Figure 3: Dendrogram of phenol isomers of different isolate of *Alternaria* spp.

they live in nature. They furnish nutrient from culture medium for their growth. All medium are not equally good for all fungi, nor there can be universal artificial substrate upon which they grow. Studies of liquid media revealed that *Alternaria solani* growth was best on Potato Dextrose Broth followed by Czapek’s medium and sporulation was maximum on Potato Dextrose Agar (Somappa et al. 2013). So, the cultural variability also showed different characters among the four isolates grown on different media like Potato Dextrose agar, Czapek’s Dox, Carrot agar, Oatmeal agar and Potato Carrot agar. The size of colony was increased with increase of incubation period. Rose isolate showed maximum colony...
Figure 4: Dendrogram of Carbohydrate isomers of different isolate of Alternaria spp.

Plate 1: Cultures of Alternaria spp. in different media
diameter on potato dextrose agar/carrot agar/oat meal agar (Table 3), marigold isolate on potato dextrose agar (Table 4) and tuberose isolate produced maximum colony diameter in all the five media (Table 5). Whereas gladiolus isolate showed maximum growth on oat meal agar at 9 days after incubation among the five media (Table 6). Color of the colony showed different colors on different media particularly whitish, greyish and brownish which was changed to black with increase in the age of the fungal cultures in every media. Gladiolus isolate produced cottony growth in every media with a few exceptions whereas tuberose produced fluffy growth. Isolates of rose and marigold produced cottony growth with few exception of thin mycelium growth of rose and fluffy growth of marigold on some media few days after incubation. Different media showed same margin of the colony i.e. roundish of different isolates. These four Alternaria spp. produces no zonation in all the media with a few exception or substrate by the four isolates from 24 hours after incubation (Plate 1). Generally Alternaria species produce some toxin on the culture media which was noted by a zonation around the growth of mycelia of the fungus. To found out any difference in toxin production on different media zonation of the four isolates were observed and no zonation was noted within the four media by the four isolates with a few exception. This result indicate that the tuberose and gladiolus isolate does not able to produce any toxin within the five media used upto 9 days after incubation. Whereas other two isolates i.e. rose and marigold able to produce toxin on czapek’s dox and potato carrot agar media within 9 days after incubation.

Relative mobility (Rm) values -Esterase isozyme in different isolates of Alternaria spp. In case of -esterase, tuberose produces six bands, rose produces seven bands and gladiolus produces four bands and marigold produces two bands. The biochemical analysis of phenol shows that the highest amount of total phenol was found in gladiolus isolate (2.347 mg/g) followed by tuberose isolate (2.176 mg/g) and rose isolate (2.101 mg/g) and minimum in marigold isolate (1.924 mg/g) among the four isolates tested. The biochemical analysis of carbohydrate showed that the highest amount of carbohydrate was in tuberose isolate (141.20 mg/g) followed by rose isolate (132.25 mg/g) and gladiolus isolate (124.5 mg/g) and minimum in marigold isolate (112.54 mg/g) among the four isolates tested. The dendrogram of these four isolate produce two clusters in which tuberose and marigold were similar and both of them were nearer to gladiolus isolate and rose came in under separate cluster. So, it can be concluded from this study that Alternaria spp. of four host cultivars exists high morphological variability and biochemical variability among the isolates themselves. So, the direct and reliable technique is analysis of DNA. It has many strains, quite different to each other in physiological, ecological and pathological characters.

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