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ISOLATION, CHARACTERISATION AND *IN VITRO* MANAGEMENT OF LEAF BLIGHT PATHOGEN OF GERBERA

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ABSTRACT
Gerbera is one of the most demanding cut flower crop which is extensively affected by leaf blight disease throughout the tropical, subtropical and temperate climatic conditions. To study about the disease and disease causing pathogen, a purposive sampling survey has been scheduled in Wayanad district of Kerala during three seasons *viz.*, rainy, winter and summer season. Leaf blight disease was observed during all the three seasons where highest per cent disease incidence of 72.0 and per cent disease severity of 17.3 per cent was recorded. The disease was observed under open field condition where the symptom appeared as small, circular brown necrotic, concentric spots in leaf lamina formed near the margin which later coalesced resulting in withering, extensive drying and shedding of leaves. The disease causing pathogen was isolated and the cultural and morphological studies revealed the identity of the pathogen as *Alternaria tenuissima. A. tenuissima* appeared in PDA medium as olive-green to black colonies in PDA. Olivaceous to dark brown coloured conidia of 23.86 to 48·69 µm long and 7.34 to 16.32 µm wide with horizontal and vertical septations were observed. *In vitro* management of *A. tenuissima* with chemical fungicides led to cent per cent inhibition of the pathogen using carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), triazoles *viz.*, hexaconazole 5EC (Contaf), difenoconazole 25EC (Score) and tebuconazole 250EC (Folicur). *Trichoderma viride* and *Pseudomonas fluorescens* while evaluated against the pathogen showed that the fungal antagonist out performed than the bacterial antagonist.

KEYWORD: Gerbera, leaf blight, *Alternaria tenuissima, Trichoderma viride, Pseudomonas fluorescens.*

INTRODUCTION
Gerbera, a commercial cut flower grown throughout the world occupies the fourth place among cut flowers in the International market (Sujatha *et al*., 2002). It is known for its wide spectrum of colours, shapes, excellent vase life and handling. According to the global trend in floriculture, gerbera fetches an excellent price in the market and contributes greatly to the export earnings of our country because of its graceful appearance, hardiness and long shelf life (Aswath and Rao, 2006). It grows well in open tropical and subtropical regions, however, under temperate climatic conditions; it is protected from frost and thereby cultivated in green houses. Inspite of favourable environmental conditions for gerbera cultivation, the crop is affected by various fungal, bacterial and viral diseases (Moorman, 1995). Among these diseases, fungal diseases are the most detrimental under Kerala climatic conditions and the leaf blight disease is one among them which reduces the production quality of the cut flowers. Hence, a deliberate attempt has been made in the present study to ponder on the leaf blight disease, its causative agent and *in vitro* management of the pathogen.

MATERIALS & METHODS
Survey and diseased sample collection: A purposive sampling survey was conducted to observe the occurrence of leaf blight disease prevailing in Wayanad district of Kerala. The survey was carried out during three seasons *viz.*, rainy (July-August), winter (November-December) and summer (March-April) where diseased samples were collected for isolation of the pathogen causing the leaf blight disease. Typical symptoms exhibited under natural conditions were also noticed during the survey. Isolation, pathogenicity and symptomatology of pathogen: The diseased samples collected during the survey were brought into laboratory and subjected to isolation of pathogens under aseptic conditions. Washed, blot dried sample bits were surface sterilized with 1 per cent sodium hypochlorite for one minute followed by three wash of sterile water and the excess moisture in the sample bit was dried with sterilized blotting paper. The bits were then placed on autoclaved, solidified Potato Dextrose Agar (PDA) medium in sterile Petri plates and incubated at room temperature (26 $\pm 2^{o}$C). Pathogenicity of the isolate was employed through Mycelial Bit Inoculation Method (MBIM) (Rocha *et al.*, 1998) and Micro Droplet Inoculation Technique (MDIT) (Munaut *et al.*, 1997).

Characterisation and identification of pathogen
The cultural characters of the isolated, leaf blight pathogen grown in PDA media was observed and recorded visually. Colony characteristics, pigmentation, growth pattern and growth rate of the isolate was studied. Slide culture technique (Riddle, 1950) was used for the study regarding various fungal structures *viz.*, type of mycelium, branching pattern, type of spores, their shape, size, L/B ratio, septal distance and presence of sexual structures if any. Microphotographs and measurements of fungal structures were taken assisted by the software Ultrascope.

Management of leaf blight disease: Chemical fungicides consisting of contact, systemic and new molecules and bioagents *viz.*, *Trichoderma viride* and *Pseudomonas fluorescens* (KAU reference cultures) were used for the *in vitro* study of leaf blight disease (Table 1).

TABLE 1. *In vitro* evaluation of fungicides against *Alternaria tenuissima*

Sl. No.	Chemicals	Concentration (%)	Percent inhibition
1.	Bordeaux mixture	0.15	100 (10)[a]
		0.2	100 (10)[a]
		0.25	100 (10)[a]
2.	Copper hydroxide 77WP	0.15	100 (10)[a]
		0.2	100 (10)[a]
		0.25	100 (10) [a]
3.	Hexaconazole 5EC	0.25	79.99 (8.94)[d]
		0.3	82.77 (9.09)[b]
		0.35	100 (10)[a]
4.	Propineb 70WP	0.05	73.33 (8.56)[i]
		0.1	78.32 (8.85)[e]
		0.15	81.11 (9.01)[c]
5.	Difenoconazole 25EC	0.15	73.88(8.60)[h]
		0.2	76.44 (8.74)[g]
		0.25	78.32 (8.85)[f]
6.	Carbendazim12%+Mancozeb 63% WP	0.05	100 (10)[a]
		0.1	100 (10)[a]
		0.15	100 (10)[a]
7.	Cymoxanil 8% + Mancozeb 64% WP	0.1	100 (10)[a]
		0.15	100 (10)[a]
		0.2	100 (10) [a]
8.	Tebuconazole 250EC	0.02	100 (10) [a]
		0.05	100 (10)[a]
		0.1	100 (10)[a]
9.	Pyraclostrobin 20WG	0.5	57.77 (7.60)[l]
		1	61.66 (7.85)[k]
		1.5	66.10 (8.13)[j]
	CD (0.05)		0.79

RESULTS & DISCUSSION

Assessment of disease incidence and disease severity: Leaf blight disease was observed in Wayanad district of Kerala where the crop was grown under open field condition and the disease was observed during all the three seasons. The disease recorded a per cent disease incidence of 72.0, 62.1 and 67.4 respectively during rainy, winter and summer season whereas per cent disease severity of 17.1, 15.8 and 17.3 %.

Symptomatology

Leaf blight symptom was found initiated with small, circular brown necrotic, concentric spots in leaf lamina formed near the margin which later coalesced resulting in withering, extensive drying and shedding of leaves (Plate 1). The above depiction on symptomatology of *Alternaria* causing leaf blight was in agreement with the findings of Honda *et al.* (2001) who noticed *Alternaria tenuissima* leaf spot in broad bean.

Isolation, characterisation of pathogen: Isolation of leaf blight pathogen from the diseased samples collected during the survey produced aerial hyphae with greyish white colonies which later turned olive-green to black in PDA (Plate 2). Microscopic view of the culture revealed that the size of conidia varied from 23.86 to 48·69 µm long and 7.34 to 16.32 µm wide with olivaceous to dark brown colour. Horizontal and vertical septations of conidia varied from 1-8 and 0-2 respectively (Plate 3). Based on these characters the isolate was identified as *Alternaria* sp. Genus level identification of the isolate was carried out by National Centre for Fungal Taxonomy (NCFT), New Delhi as *Alternaria tenuissima.* Nagrale *et al.* (2012) made similar observations in morphological characters of the pathogen which was found in accordance with the present study.

Management of leaf blight pathogen

The pathogen, *Alternaria tenuissima* causing leaf blight disease in gerbera was evaluated against nine chemical fungicides under *in vitro* conditions (Table 1). It was observed that the two combination fungicides *viz.*, carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8) along with three triazoles *viz.*, hexaconazole 5EC, difenoconazole 25EC (Score) and tebuconazole 250EC (Folicur) recorded cent per cent inhibition whereas strobilurin, pyraclostrobin

20WG showed maximum per cent inhibition of 81.11 per cent at 0.15 per cent respectively. Percent inhibition of 57.77 % was recorded with Bordeaux mixture compared to copper hydroxide 77 WP (Kocide) which showed a superior performance of 70 to 80 per cent. The efficacy of triazoles in the present study is in agreement with previous studies who evaluated the chemicals against *Alternaria* spp. (Arunkumar, 2006; Patel and Choudhary, 2010 and Parveen *et al.*, 2013). Roopa *et al.* (2014) compared the efficacy of propineb 70WP, hexaconazole 5EC and difenoconazole 25EC against *Alternaria* sp. and observed that least inhibition was shown by propineb and the maximum with hexaconazole 5EC (0.1%) which is found in corroboration with the present study.

The reponse of *Trichoderma viride* to the fungal pathogen showed better control of the pathogen, *A. tenuissima* by overgrowth mechanism of inhibition. The fungal antagonist, *T. viride* inhibited the pathogen by 63.3 per cent whereas *P. fluorescens* inhibited the pathogen by 57.7 per cent showing a low per cent of inhibition than the fungal antagonist. However, contradictory to the above result, Ambuse *et al.* (2012) reported 80 per cent inhibition of *Alternaria tenuissima* with *Trichoderma viride.* Similar results were noticed by Taj and Kumar (2012) and Roopa *et al.* (2014). Moreover, Manjunatha *et al.* (2012) noticed very less inhibition of 20 per cent of *Alternaria sesami* with *Pseudomonas fluorescens.*

PLATE 1. Marginal blightening symptom under field conditions

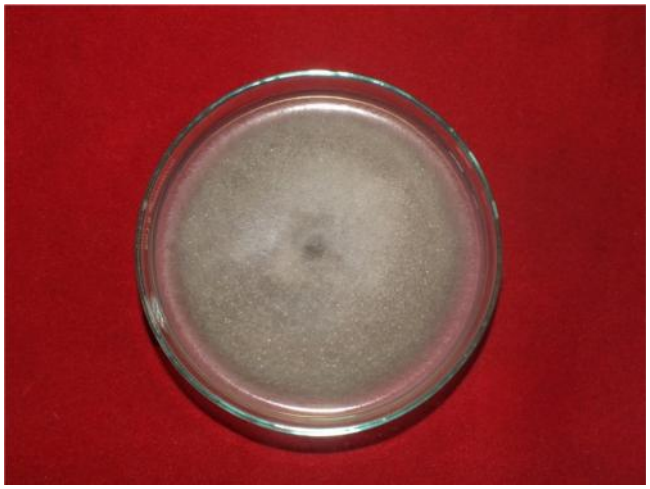

PLATE 2. Leaf blight pathogen,*A. tenuissima* grown in PDA medium

PLATE 3. Chain of conidia of *Alternaria tenuissima*

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