



## FIRST REPORT OF *ULOCLADIUM CHARTARUM* IN GERBERA CAUSING LEAF SPOT DISEASE

Praveen, N. M., Reshmy Vijayaraghavan and Beena, S.

Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Kerala, India-680 656

\*Corresponding author email: drreshmydhanesh@gmail.com

### ABSTRACT

A purposive sampling survey was conducted with an objective to catalogue and document fungal diseases of gerbera in Thrissur district which revealed the existence of a distinct leaf spot disease. The disease was observed in all the three seasons established where the crop was grown under polyhouse. Per cent disease severity of leaf spot during the three seasons was found to be 9.2, 6.0 and 19.4 respectively. From the diseased samples collected during the survey, the pathogen was isolated and pathogenicity of the isolate was proved by Mycelial Bit Inoculation Method (MBIM) and Mycelial Droplet Inoculation Technique (MDIT). Under natural condition, symptoms appeared as circular, pale to dark brown necrotic spots with definite borders scattered over the leaf lamina. Each individual spot appeared as deep sunken necrotic spots, delimited by major veins and further coalesced to form large blighted areas. The pathogen in PDA produced pure white mycelial growth which later transformed into typical yellow to dark orange pigmentation. Spores obovoid, non-beaked and produced olivaceous to dark brown coloured conidia having dimensions ranging from 26.54-51.54  $\mu\text{m}$  x 15.16-40.24  $\mu\text{m}$ . Hence, with the cultural and morphological characters, the pathogen was identified as *Ulocladium* sp. Species level identification was carried out from National Center for Fungal Taxonomy (NCFT), New Delhi and reported as *U. chartarum* which was found to be the first report in gerbera causing leaf spot disease in gerbera.

**KEYWORDS:** gerbera, leaf spot, *Ulocladium charatarum*.

### INTRODUCTION

*Gerbera jamesonii* Bolus, a very attractive, commercial cut flower crop ranks fourth in the international cut flower trade (Sujatha *et al.*, 2002). These plants are grown throughout the world in a wide range of climatic conditions and are in great demand in the floral industry as cut flower as well as potted plant due to its beauty, colour, long vase life and ability to rehydrate after long transportation. The flowers being hardy, can withstand rigorous transportation and a long shelf life and the production quality is favoured under protected conditions as these require partial shade (Singh, 2006). However, over the past few years several important diseases have gained considerable importance and pose serious threat to the cultivation and production of gerbera in India. Since gerbera flourishes well under the warm humid tropical climate of Kerala, the crop is prone to infection by different fungal pathogens which thrive well under such conditions. *Alternaria tenuis*, *Alternaria citrii*, *Alternaria tenuissima*, *Colletotrichum gloeosporioides*, *Colletotrichum capsici*, *Colletotrichum dematium*, *Colletotrichum coffeanum* and *Curvularia clavata* were the major pathogens previously reported to be infectious on gerbera causing leaf blight, leaf spot and anthracnose symptoms. The present study revealed the existence of a new pathogen infecting gerbera.

### MATERIALS & METHODS

Sampling survey and assessment of percent disease incidence and percent disease severity: A purposive sampling survey was carried out on the occurrence of leaf

spot disease in Thrissur district of Kerala. Disease incidence and disease severity of the leaf spot disease were recorded during three seasons viz., rainy (July-August), winter (November-December) and summer season (March-April).

#### Symptomatology of the disease

Symptomatology of leaf spot disease was carried out under natural and artificial conditions. The survey conducted in the three districts facilitated in recording the disease occurrence and symptom development of the leaf spot disease.

#### Isolation and pathogenicity test

Diseased specimens collected during the sampling survey were washed under running tap water and cut into small bits consisting of both healthy and infected portions using a sterile blade and were disinfected with sodium hypochlorite (1%) for one minute and subsequently three washings were given using sterilized distilled water and the excess moisture in the sample bit was dried with sterilized blotting paper. Such surface sterilized bits were then placed aseptically on solidified Potato Dextrose Agar (PDA) medium in sterile Petri dishes. The plates were then incubated at room temperature ( $26 \pm 2^\circ\text{C}$ ). Pathogenicity of the isolate was proved by artificial inoculation of cultures on healthy plants or plant parts by Mycelial Bit Inoculation Method (MBIM) (Rocha *et al.*, 1998) and Micro Droplet Inoculation Technique (MDIT) (Munaut *et al.*, 1997) to observe whether the isolates are capable of reiterating typical symptoms under artificial conditions.

#### Characterization and identification of pathogen

The cultural characters exhibited by the pathogen were recorded by visual observation of the cultures grown in PDA media. A variation in colony characteristics, pigmentation, growth pattern and growth rate of the isolate was studied. Morphological characters were studied by slide culture technique (Riddle, 1950). The slide was observed for various fungal structures viz., type of mycelium, branching pattern, type of spores, their shape, size and presence of sexual structures if any. Microphotographs and measurements of fungal structures were taken assisted by the software Ultrascope. For further confirmation, the pathogen was sent to NCFT, New Delhi where the culture was also deposited with an accession number.

## RESULTS & DISCUSSION

Assessment of disease incidence and disease severity: Leaf spot disease was observed in gerbera cultivated under protected conditions. The disease was noticed in all the

three seasons with highest disease incidence and severity during summer season. Per cent disease incidence (PDI) and per cent disease severity (PDS) ranged from 45.0-78.2 and 6.0-19.4 per cent respectively.

### Symptomatology of disease

Disease symptom was initiated by development of small yellow spots which later appeared as circular, pale to dark brown necrotic spots with definite borders scattered over the leaf lamina. Each individual spot appeared as deep sunken necrotic spots, delimited by major veins and further coalesced to form large blighted areas (Plate 1). The leaf spot pathogen caused extensive damage on leaves showing circular, pale to dark brown necrotic spots with definite borders scattered on leaf lamina and the latter showed typical symptoms as small, yellow-brown flecks, often with a light green halo on the upper surface of leaves which later turned into circular to oval chlorotic irregular patches.



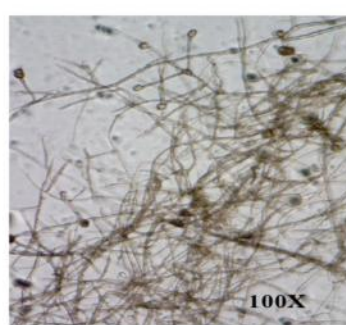
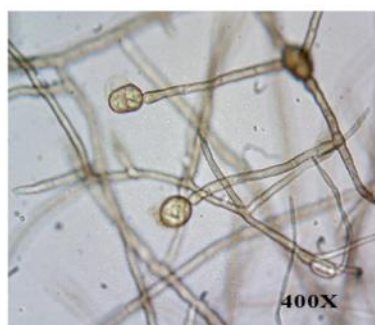
**Plate 1. Natural infection of *Ulocladium chartarum* under field condition**



**Plate 2. Development of lesion on artificially inoculated leaf**



**Plate 3. Cultural growth of the pathogen in PDA media  
A. Upper surface B. Reverse side of the Petri plate**



**Plate 4. Conidia of *Ulocladium chartarum* under 400X and 100 X**

### Isolation and pathogenicity test

Fungal pathogen responsible for leaf spot disease was isolated by standard isolation protocol and grown in PDA media and for testing pathogenicity, MBIM and MDIT were employed. Mycelial disc of the pathogen was inoculated on detached leaf of gerbera. Symptoms appeared on the fourth day of inoculation as typical circular spots on leaf lamina. Similar symptoms were produced on three month old gerbera plants when plants were inoculated by MDIT method. Pathogenicity test of *Ulocladium* sp. isolated from gerbera by artificial inoculation proved the pathogenic nature of the isolates (Plate 2). Reports of *Ulocladium* sp. as a disease causing agent was unusual among agricultural crops, though, Zitter and Hsu (1990) could isolate and prove the pathogenic nature of *Ulocladium* sp. from cucumber causing leaf spot disease.

### Characterisation of pathogen

The pathogen on PDA appeared as pure white mycelial growth which later transformed into typical yellow to dark orange brown pigmentation with greyish white mycelial growth (Plate 3). Spores obovoid, non-beaked and produced olivaceous to dark brown coloured conidia having dimensions ranging from 26.54-51.54 µm x 15.16-40.24 µm (Plate 4). These characteristics confirm the identity as *Ulocladium* sp. and species level identification was carried out from National Center for Fungal Taxonomy (NCFT), New Delhi as *Ulocladium chartarum* with accession number 7945.15. The occurrence of *Ulocladium* sp. on gerbera has not been reported so far and thus, it needs further detailed investigation to ascertain the extent of damage caused by the pathogen. Vannini *et al.* (2000) and Zarandi and Sharzei (2015) reported the causal agent of leaf spot disease of *Quercus pubescens* and lemon verbena leaf spot by *Ulocladium chartarum*. These characteristics confirmed the identity as *Ulocladium chartarum*. It is worthwhile to mention that this is the first report of *Ulocladium chartarum* in gerbera.

### ACKNOWLEDGEMENT

The authors would like to thank Kerala Agricultural University for the assistance provided for the successful conduct of the research work.

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