Indian J. Applied & Pure Bio. Vol. 35(2), 293-297 (2020). **ISSN: 0970-2091**

Leaf spot disease caused by *Nimbya alternantherae* in *Alternanthera philoxeroides* (Mart.) Grisb.: A new report from India

Anoop K.[1]* and Abhijnya J.[2]

[1&2]Department of Botany, The Zamorin's Guruvayurappan College, Kozhikode-673014 (India)
*email: anoopkuttiyil@gmail.com

Abstract

Alternanthera philoxeroides, commonly known as 'alligator weed', is an aquatic perennial herb belongs to the family Amaranthaceae. It is a weed found in temperate and tropical climates of the world. The leaf spot disease in this plant was noted in Kozhikode district of Kerala, India. The infected material was subjected to analysis and the symptoms were noted. The disease was characterized by numerous circular purple-reddish colored spots of varying sizes. The spots were found in greyish-brown necrotic centres with purple to red colored borders. Irregular shaped purple spots and streaks were present on internodal region of the stem. Chlorosis was prominent in advanced stages of the disease followed by premature leaf fall. The pathogenicity of the isolate was proved by detached leaf method. Based on the morphological characters, the pathogen was identified as *Nimbya alternanthera*. This is the first report of this pathogen as leaf spot pathogen of *A. philoxeroides* in India.

Alternanthera philoxeroides, commonly known as 'alligator weed', is an aquatic perennial herb belongs to the family Amaranthaceae. It is a weed found in aquatic and riparian regions of temperate and tropical climates of the world[2]. In India, it is found in Assam, Bihar, West Bengal, Tripura, Manipur, Andhra Pradesh, Karnataka, Maharashtra, Delhi and the state of Punjab[10]. The leaves are dark green, elliptic, glabrous and opposite. Mature aquatic plants have hollow stems that form thick interwoven mats throughout the water body and emerge up to out of the water when the plant flowers. Inflorescences are white, terminal and axillary on a short stalk. In New Zealand, Bassett *et al.,*[3] reported that an increasing cover of this weed decreased the cover of native plant species, resulting in loss of native species in the long term. It is a threat to internal water bodies and provides optimal condition for mosquito breeding. In India, Chatterjee and Dewanji[4] reported the reduction in the macrophyte species richness up to 30% during the high infestation by *A. philoxeroides*. Leaf spot disease in *A. philoxeroides* caused by *Alternaria alternantherae* was reported

early in 1976 [7]. Holcomb[8] reported wide host range of this pathogen infecting many members of the family Amaranthaceae. The pathogen was later identified as *Nimbya alternantherae* based on cultural character[11]. Xiang *et al.*,[13] reported *N. alternantherae* on *A. philoxeroides* in China for the first time in 1998. Barreto and Torres[2] reported *N. alternantherae* along with *Cercospora alternantherae* on alligator weed from Brazil. Gilbert *et al.*,[6] claim a mismatch between the early reported isolate and the latest one. The characters like conidial morphology, beak length, body length, width, shape, septation and ornamentation, the fungi reported by Gilbert *et al.*,[6] is regarded as the first report of *N. alternantherae* in Australia. Akhtar *et al.*,[1] severe leaf and stem necrosis disease of *A. philoxeroides* caused by *N. alternantherae* for the first time in Pakistan. No reports are available about *N. alternantherae* pathogenic to *A. philoxeroides* in India till the date.

Collection and isolation of pathogen :

The diseased leaves of *A. philoxeroides* were collected from Kozhikode District, Kerala. The diseased samples collected brought into laboratory were thoroughly studied. The symptoms noted on the diseased specimen were recorded. Microscopic observations and measurements were taken using digital camera attached to stereo microscope with the help of software 'IS Capture' Version 2.1. The diseased leaves were washed thoroughly with tap water to remove dust particles. Pieces of leaves from the advancing lesion were surface sterilized with 0.1% $HgCl_2$ for 3 min followed by washing in sterile distilled water for three times. The surface sterilized pieces were kept on sterile blotting paper to remove excess water. It was then transferred onto sterilized Petri plates containing Potato Dextrose Agar medium (PDA). It was incubated at room temperature for 3 days. The fungal hyphal tips grown after three days were subcultured and used for further studies.

Pathogenicity and identification of pathogen :

The organism isolated from *A. philoxeroides* was used to prove the pathogenicity by Koch's postulates. 'Detached leaf method' was followed to prove the pathogenicity of the isolate. Healthy leaves of *A. philoxeroides* were detached from the plant and cleaned with sterile distilled water. The conidia of the fungal isolate were collected by flooding and scraping the culture plate with sterile distilled water. The fungal mycelia were removed by filtering the suspension using double layered cheese cloth. The leaves were blotted with sterile filter paper and incubated in sterile Petri plates containing pre-wetted filter paper with sterile distilled water. The detached leaves before incubation were dipped in conidial suspension containing $1x10^5$ conidia/ mL for 5 min. The leaves dipped in sterile distilled water was kept as control. The Petri plates were incubated at room temperature for 7 days. The inoculated leaves were observed regularly for the symptoms of the disease. The part of the leaf which showed symptoms of the disease was cut and inoculated onto PDA for reisolation of pathogen as per the methods mentioned before.

The sporulation of the isolate was tested on different media and the cultural

characteristics were recorded. Agar discs of 5mm size cut off from the actively growing pathogen were inoculated at the centre of the 90 mm Petri plates containing media like carrot agar (CA) and potato-carrot agar (PCA). The plates were incubated at room temperature and regularly observed for 15 days at 3 days interval. The fungal hyphae taken onto glass slide under sterile condition were stained with lactophenol cotton blue stain for microscopic analysis. The microphotographs and measurements were taken using the software as per the procedure explained above. The culture characters of the pathogen were recorded. The characters were compared with characters mentioned by Zhao and Zhang[15].

The diseased leaf samples collected from the field showed brown necrotic lesions surrounded by reddish-purple border (Fig.1a). The diseased specimen had typical symptoms of leaf spot disease of *A. philoxeroides* mentioned in the earlier reports. The disease was characterized by numerous circular purple-reddish colored spots of about 0.1 to 0.8 mm size in diameter (Fig.1b). The advanced stage of the disease was characterized by irregular spots of varying sizes. The spots size up to 12mm were seen. The spots were found in greyish-brown necrotic centers and purple-red colored borders. The necrotic lesions in most of the leaf area coalesced to form large necrotic areas (Fig.1c). The lesions were often found along the mid-rib and also on different parts of the stem. Irregular shaped purple spots and streaks were present on internodal region. Chlorosis was prominent in advanced stages of the disease followed by premature leaf fall (Fig. 1c). Complete damage of the leaf was noted at the advanced stage of the disease (Fig. 1c).

The isolate was proved to be pathogenic in pathogenicity test by detached leaf method. The lesions were noted on the leaves in which the conidial suspension was inoculated. The lesions produced were similar to the symptoms of leaf spot disease found in natural condition. The infection started as small yellowish spots and then turned into purple spots after 7 days of inoculation. The leaves in control plates developed no lesion even after 7 days. The isolation and morphological characterization of the isolate from the infected leaves of pathogenicity test showed similar characteristics of the fungus that was isolated from infected leaves collected from the field.

Among the different media tested, conidia formation was noted in PCA after 10 days of inoculation. The fungal colony was fluffy grey and dark brown at the upper and reverse side of the culture plates respectively. The reverse of the Petri plate showed dark brown colored metabolites inside the medium (Fig. 1f&g). Conidia were acrogenous, pale brown. It consisted of 4 -16 cells and a long slender beak (Fig.1d&e). The cells towards the base was swollen and apical cell was slightly rostrate. The length of conidium ranged from 82–124µm (average 90.2 µm). The width of the cell ranged from 13–18µm (average 16.9µm). The overall length of the conidia averaged 235 µm. The characters were found similar to the descriptions of Akhtar *et al.*,[1] and Zhao and Zhang[15]. It also coincides with the description of *Nimbya alternantherae* by Holcomb & Antonopoulos[7] and Simmons[12].

PLATE 1

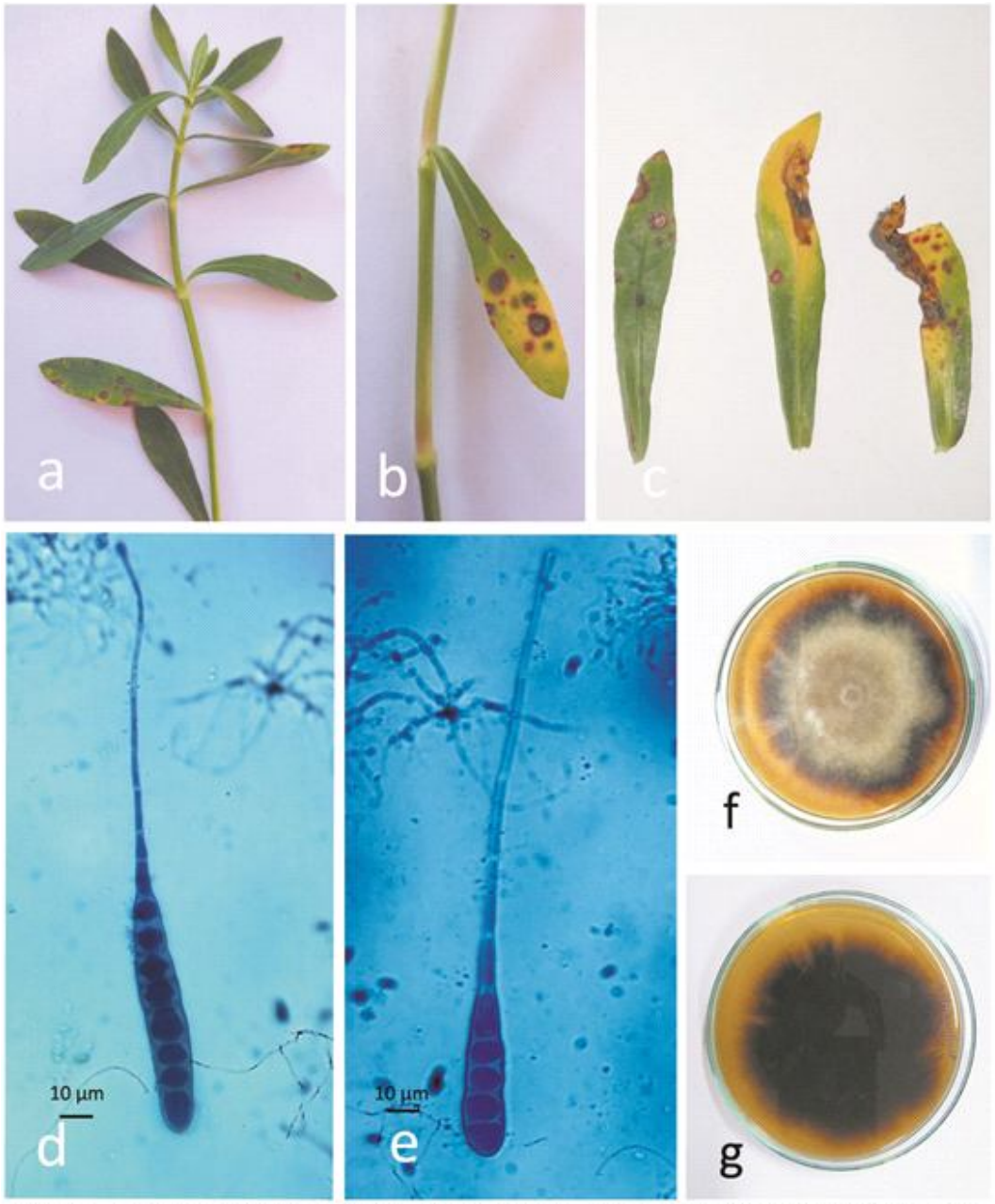

Figure 1. a. & b. Leaf spot disease in *A. philoxeroides* c. Different stages of leaf spot disease.
d. & e. Conidia of *N. alternantherae*
f. & g. Upper and lower view of culture plates of *N. alternantherae*

There for the isolate is confirmed as *N. alternantherae*.

The reports of *N. alternantherae* as leaf spot pathogen of *A. philoxeroides* are reported earlier from different parts of the world. This is the first report of *N. alternantherae* as leaf spot pathogen of *A. philoxeroides* in India. The pathogen shares its identity to the isolates reported in earlier work[1,2,13]. The results of the present study coincide with the study of Akhtar *et al*.[1]. The symptoms noticed while proving Koch's postulates, were similar to the results reported by Gilbert *et al.*,[6]. The formation of conidia was found more in PCA during the present study. This is in support with the findings of Xiang *et al.*,[13] who reported the highest productivity of the conidia on PCA. Meimei *et al.*,[9] have reported herbicidal activity of metabolite produced by *N. allternantherae*. Demuner *et al.*,[5] and Yi-Pin *et al.*,[14] have identified the phytotoxins from this pathogen. All these reports have emphasized the importance of using this pathogen or its phytotoxic compounds as biocontrol agents or weedicides. Hence, the present study also opens a new area of research on this pathogen or its bioactive compounds for the management of the plant diseases and weed management in India.

References :

1. Akhtar, K. P., N. Sarwar and Imran-Ul-Haq. (2012). *Tropical Plant Pathology, 37:* 428-430.
2. Barreto, R. W. and A. N. L. Torres (1999). *Australasian Plant Pathology, 28:* 103–107.
3. Bassett, I., Q. Paynter, R. Hankin and J. R. Beggs (2012). *New Zealand Journal of Ecology, 36:* 216–222.
4. Chatterjee, A., and A. Dewanji (2014). *Aquatic Invasions, 9:* 343-355.
5. Demuner, A. J., L.C.A. Barbosa, T.A.M. Veiga, R. W. Barreto, B. King-Diaz, and B. Lotina-Hennsen (2006). *Biochemical Systematics and Ecology, 34:* 790-795.
6. Gilbert, R. L., B. A. Auld, and B.R. Hennecke (2005). *Plant Pathology, 54:* 585.
7. Holcomb, Gordon, E. and A.A. Antonopoulos (1976). *Mycologia, 68:* 1125–1129.
8. Holcomb, G. E. (1978). *Phytopathology, 68:* 265–266.
9. Meimei, X., H. Pingan, J. Zhihai, O. Xuelian and W. Lianhua (2002). *Yunnan Nong Ye Da Xue Xue Bao, Journal of Yunnan Agricultural University, 17:* 352–355.
10. Pramod, K., M. Sanjay, and N. Satya (2008). *Journal of Indian Botanical Society, 87:* 285–286.
11. Simmons, E. G. (1989). *Sydowia, 41:* 314–329.
12. Simmons, Emory G., *et al*., (1995). *Mycotaxon, 55:* 55–163.
13. Xiang, M., R. Liu and Y. Zeng (1998). *Mycosystema, 17:* 283.
14. Yi-Pin, Z., X. Mei-Mei, J. Zi-De, L. Hua-Ping, S. Wei, L. Hai-Lin, and F. Huai-Zhong (2006). *Chemical Journal of Chinese Universities-Chinese, 27:* 1485–1487.
15. Zhao, G. Z., and T. Y. Zhang, (2005). *Fungal Divers, 19:* 201–215.