J. Mycopathol. Res. 48(1) : 111—114 (2010)

First report of *Fusarium pallidoroseum* (Cooke) Sacc. causing wilt disease of *Chlorophytum nepalense* (Lindley) Baker

TANAY BOSE*, SUMITA GHOSH AND S. N. GHOSH
*Advanced Mycology and Plant Pathology Laboratory, Post Graduate Department of Botany, Presidency College, Kolkata-700073, West Bengal, * E-mail : tanaybose15@gmail.com*

Chlorophytum nepalense (Lindley) Baker is an endemic plant found in the Eastern Himalayas and adjoining areas. Till date there has been no report on the occurrence of any disease from this locality. However we observed the wilted plants of *C. nepalense* at the experimental garden of Presidency College, Kolkata. The pathogen was isolated from the diseased plant and its pathogenocity was confirmed. The causal organism of this wilt disease was identified to be *Fusarium pallidoroseum* (Cooke) Sacc. Studies regarding the symptoms, both external and internal of the host and the pathogen were made. Host plants exhibited leaf chlorosis, followed by yellowing and drooping of leaves which ultimately turned brown in colour. Vascular discoloration of aerial part and its death was ultimate. Isolations were made from the discolored leaf tissue on potato dextrose agar (PDA). *F pallidoroseum* was the only fungus isolated and appeared from most of the diseased fragments. Macro and micro morphology of the fungus was studied in detail. All isolates obtained were identified as *F. pallidoroseum* due to production of characteristic color of the colony which is light pink (rose pink) in color and a orangish tan colouration around the colony from reverse. Particular fusarial species showed presence of two types of macroconidia. Those borne in the aerial mycelium are mostly straight, 3-5 septate. Macroconidia borne in sporodochia are curved, possess a hyaline foot cell, 3-7 septate. Ellipsoid microconidia borne on short simple conidiophores and chlamydospores were observed in old cultures.

Key words: *Chlorophytum repalense, Fusarium pallidoroseum*, histopathology, Koch's postulate, safed moosli, soil borne

INTRODUCTION

There is no report of any disease in *Chlorophytum nepalense* (Lindley) Baker (Family: Liliaceae), but several diseases have been recorded from its closest relative *Chlorophytum borivilianum* (Safed moosli) (Chandra and Tandon, 1965; Sattar *et al.,* 2006; which have an immense ethnobotanical uses as a sex tonic. *C. nepalense* is endemic to region of forest margins, grassy slopes, and rocky places along valleys; at an altitude 1300-2800 m above the sea level (Hooker, 1892) in Guizhou, Sichuan, Xizang, Yunnan (China), Bhutan, NE India, Myanmar, Nepal, Sikkim and from Runctia Forest; Sherpur district, Bangladesh, (Afroz *et al,* 2008). The extensive use of the leaves of *C nepalense* as food in the rurual areas of Nepal is the main reason for its inclusion in the list of endangerd plant species of the world (IUCN Red Data Book, 2009; Joshi *et al,* 2007); this plant has also been noted for its ethnomedicinal values among the Tharu tribe members residing at Chitwan District of Nepal (Dangol and Gurung, 1991). Though it has been reported to possess unknown medicinal value (Basu and Jha, 2008) but it has immense potentiality of cureing several human diseases like chest infection, head ache, gastrointestinal problems, liver ailments etc (Dangol and Gurung, 1991).

Fusarium pallidoroseum (Cooke) Sacc. is a soil borne fusarial species that too has quit less evidences of causing disease in plants, though it has been reported to cause wilt disease in banana in Uganda (Kangine and Rutherford 2001). *F. pallidoroseum* has also been recorded from stored maize sample in Cameroon (Tagne *et al.,* 2003). This particular pathogen has been used as a biocontrolling agent against water hyacinth [*Eichhornia crassipes* (Mart) Solm], (Naseema *et al.,* 2006) in India.

MATERIALS AND METHODS

The diseased plant of *C. nepalense* was collected from the experimental garden of Presidency College, Kolkata, and the soil samples for the analysis was also collected from the same source.

Histopathological study of the diseased plant

Histopathology was carried on diseased host as well as on healthy host (control). A thin vertical section (V.S) of the leaf, transverse section (T.S) of the stem disc was performed along with longitudinal section (L.S) of the stem disc. The sections were observed under compound light microscope stained with cotton blue dissolved in lacto phenol and also under unstained condition.

Isolation of pathogen

A portion of leaf blade and stem disc of the infected plant was surface sterilized with 0.1% (w/v) mercuic chloride solution and was placed separately into sterilized culture tubes containing Potato Dextrose Agar (PDA) medium with streptomycin sulfate (2.5 µg/ml) for the isolation of pathogen.

Analysis of soil samples

Serial dilution (10^{-6} to 10^{-2}) of the soil samples, collected from the rhizosphere region of the infected plant was made, which was followed by platting it on the surface of sterilized PDA medium in Petriplates.

Koch's postulate

The spore suspension (1×10^{6} spores/ml) of *F. pallidoroseum* was prepared and the root region of a healthy plant was submerged into the suspension for 24 hrs at 28°C–30°C, with relative humidity of 90%. The artificially inoculated plant was then planted in earthen pots containing sterilized soil (sterilization was done in an autoclave at 121°C for 20 minutes for 3 consecutive days each time allowing the soil to cool down before the next sterilization) and was allowed to grow under adequate atmospheric condition of the experimental garden.

RESULT

Description of the pathogen (Fusarium pallidoroseum)

Macroscopic features

Fusarium pallidoroseum grew rapidly on Potato Dextrose Agar (PDA) medium at 25°C-28°C and produced rose pick colored woolly to cottony, flat, spreading colonies when mature. The mass of the mycelium was very compact in nature. From the front, the colour of the colony was rose pink in color. From the reverse it gave an orangish tan colored appearance around the colony region in PDA medium (Fig. 1, h).

Microscopic features

Hyaline septate hyphae with slightly bulged compartments; conidiophores, phialides, macro conidia, and micro conidia were observed microscopically. In addition to these basic elements, chlamydospores were also produced. Phialides cylindrical with a small collaret, solitary or produced as a component of a complex branching system. Monophialides and polyphialides were observed. Macro conidia (3-8 × 11-70 µm) were produced from phialides on unbranched or branched conidiophores. They were 2- or more celled, thick-walled, smooth, and cylindrical or sickle- (canoe-) shaped. Macroconidia were of two types. Those borne in the aerial mycelium were mostly straight, 3-5 septate, and measure 7.5-35 × 2.5-4 µm. Macroconidia borne in sporodochia were curved, possess a foot cell, 3-5 septate, and measure 20-46 × 3-5.5 µm. Micro conidia (2-4 × 4-8 µm), on the other hand, were formed on long or short simple conidiophores. They were 1-celled (occasionally 2- or 3- celled), smooth, hyaline, ovoid to cylindrical, and arranged in balls (occasionally occurring in chains). Chlamydospores were sparse, present in pairs, clumps or chains. They were thick-walled, hyaline, intercalary or terminal, (5-8 × 8-10 µm). (Figs i, j)

Systematic positon Kingdom: Fungi, Division: Eumycota, Sub-Division: Deuteromycotina, Class: Hyphomycetes, Order: Moniliales, Family: Tuberculariaceae, Genus: *Fusarium,* Specific epithet: *F. pallidoroseum* (Cooke) Sacc. (1886).

Symptoms

In August 2008 wilted plants of *Chlorophytum nepalense* (Lindley) Baker (Family: Liliaceae) were observed in the experimental garden of the Presidency College. The mean rainfall during this

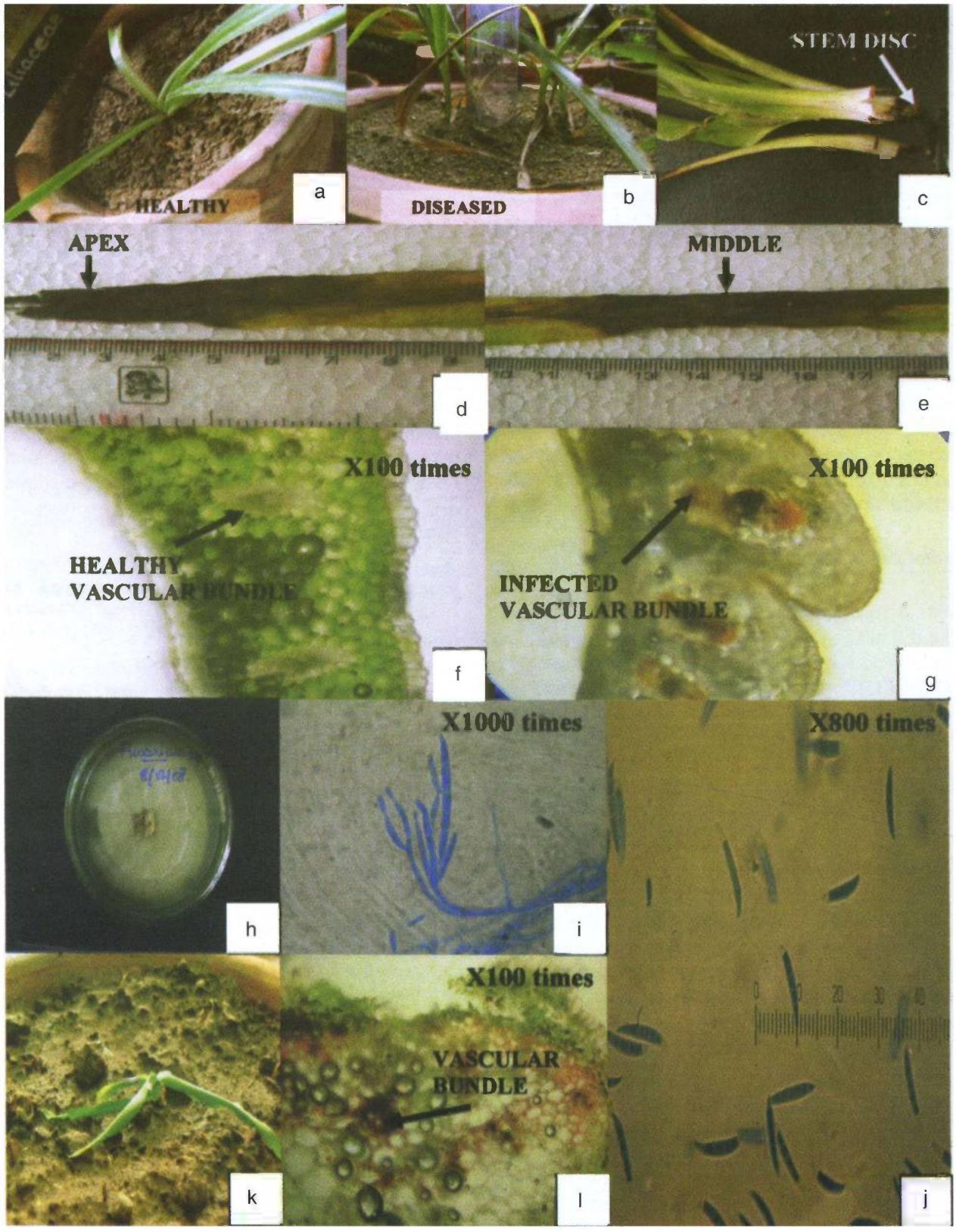

Fig. 1: (a) Healthy plant of *Chlorophytum nepalense;* (b) Diseased plant of *C nepalense* (host); (c) Stem disc; (d) Leaf apex; (e) Leaf middle, symptoms on the diseased host; (f) V. S through the leaf of a healthy host (Control) (X400times); (g) V. S through the leaf of a diseased host (X400 times); (h) Pure culture of *F pallidoroseaum;* (i) Conidiophore of *F pallidoroseum* (X1000 times); (j) Macroconidia of *F pallidoroseum* (X80 times); (k) Symptoms on a young plant of *C nepalense,* 48 hrs after inoculation with spore suspension of *F pallidoroseum;* (l) V.S through the leaf of an reinoculated host (X100 times).

period was about 150-200 mm, the temperature ranged between 20°C-30°C, with high relative humidity of 85%-95%, The plants showed typical wilt symptoms. The leaves had dried with shriveled appearance, brown apex and margins in matured leaf blades and chlorosis was observed along the margins in young leaves. Sheathing leaf bases had very fine reddish brown marks along the long axis of the leaf. Complete destruction of the root system was observed. (Figs. 1, a-e).

Histopathological study of the diseased plant

Vertical section (V.S), transverse section (T.S), longitudinal section (L.S) of the infected leaf revealed the presence of scattered vascular bundle which were dark red in colour and a gelatinous substance was found to clog the vessel, which were distorted to some extent. The infected stem disc showed similar symptoms (Figs. F-G).

Isolation and Identification of the pathogen

After 3 days of incubation in PDA medium it was found that the fungal mycelia were emerging out of the infected leaf portions. This was further sub-cultured for the generation of pure culture and was send to Indian Agricultural Research Instutute (IARI), New Delhi for identification. The pathogen was identified as *Fusarium pallidoroseum* (Cooke) Sacc. under the Reference number: 3245 and ID No. 7277.08, dated 19th November 2008.

Koch's postulate

The artifically inoculated plant showed the disease symptoms after 5 days of replantation, which was similar to the original symptoms observed on *C. nepalense*. The artificially infected plant was used for reisolation of the pathogen for comparative studies and the histopathological study of the reinfected plant was carried out. (Figs. k, l).

Analysis of soil samples

Isolation of soil borne microorganisms by serial dilution of soil samples revealed the presence on *F. pallidoroseum* in rhizospheric soil along with other soil borne microbes at concentrations 10^{-6}, 10^{-4} and 10^{-3}.

DISSUSSION

There is no earlier report of any disease of *C. nepalense* growing in different regions of Eastern Himalayas and adjoining areas till date. The wilt disease has been observed in plants growing at the experimental garden of Presidency College, Kolkata. It may have occurred because the plant was forced to grow under climatic condition very different from its place of origin which might have led to decrease in the defense mechanism of the plant making it susceptible to the wilt disease.

ACKNOWLEDGEMENT

The authors are grateful to Prof. Timir Baran Jha, Head, Department of Botany, Presidency College for his help, and Dr. T. Prameela Devi, Senior Scientist, IARI, New Delhi for identifying the fungal cultures.

REFERENCES

Afroz, S., Turul, E., Zashim Uddin, Md. and Hasan Md., 2008. *Chlorophytum nepalense* (Lindley) Baker (Liliacease)- A New Angiosperms record from Bangladesh. *Bangladesh J. Bot.* **37(2):** 193-194.

Basu, S., Jha, and T.B, 2008. *Chlorophytum nepalense* plant from *Chlorophytum borivilianum* still now has no medicinal use; Curr Sci, **95(4):** 439.

Chandra, S. and Tandon R.N, 1965. *Colletotrichum chlorophytumi* on *Chlorophytum* sp. *Curr. Sci* **34:** 565-566.

Dangol, D.R. and Gurung, S.B, 1991. Ethnobotany of the Tharu tribe Of Chitwan District Nepal; *Pharmaceutical Biology,* **3:** 203-209.

Hooker, J.D, 1892. *"The Flora of British India"*, L Reeve & Co Ltd., Kent, England. **6:** 333-335.

Joshi, N.; Kehlenbeck, K., and Maass, B. L, 2007. Conference on International agricultural Research and Development.

Kangine, A. and Rutherford, M. 2001. Wilt like disorder of banana in Uganda; *CABI Bioscience, Musa Disease Fact sheet* **No. 10.**

Naseema, A., Praveena, R., Nair, R. R and Peethambaram, R. K. 2006. Control of Water Hyacinth by *Fusarium pallidoroseum,* Curr. Sci. **86(6):** 770-771.

Sattar, A.; Alam, M.; Khaliq. K.; Sukla, R.S and Khanuja, S.P.S, 2005. First Report of Leaf Blight Disease Of *Chlorophytum borivilianum* caused by *Collectotrichum capsici* in Northern India; New Disease Reports, British Society for Plant Pathology, **11** (online version).

Tange A.; Kongsdal O.; Ngoko, Z.; The, C. and Mathur, S.B., 2003. *Fusarium pallidoroseum* in maize samples of three agro-ecological zones of Cameroon; *Journal of Stored Prodeuct Research,* **39(4):** 367-374.

(Accepted for publication September 15, 2009)