

Research Article

A New Flat Stem Disease of *Tinospora cordifolia* caused by Phytoplasma

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Abstract: *Tinospora cordifolia* is an important medicinal plant used in the treatment of several diseases and disorders which grow naturally in the Western Ghats of Karnataka. A new phytoplasma disease in *T. cordifolia* has been observed in Bhadra Wildlife Sanctuary, Karnataka, India for three consecutive years. The disease affected all the branches of infected plant, particularly, during winter season.

Keywords: Bhadra Wildlife Sanctuary, Dienes' stain, histochemical.

INTRODUCTION

Tinospora cordifolia (Willd) Hook f. & Thoms, belonging to Menispermaceae is an important medicinal plant species which finds application in the treatment of asthma, blood pressure, boils, bronchitis, cardiac problems, diabetes, fever, herpes, itching, malaria, sore, venereal diseases and wound [1, 2, 3]. The plant species has been studied for antiarthritic, antidiabetic, anticancer, antileprotic, antimalarial, antimicrobial, antineoplastic, antispasmodic, antiulcer, diuretic, free radical scavenging, hepatoprotective, immune-stimulating, immunomodulatory, radio protective and many other activities [4, 5, 6, 7]. During the survey for diseases in medicinal plants in Bhadra Wildlife Sanctuary, Karnataka, an interesting flat stem disease was observed in young growing regions of *Tinospora cordifolia*. The stem tip was transformed completely into a green flat ribbon with numerous small leaves. Based on the morphology of the malformed stem, the disease appears to be caused by phytoplasma. The similar kind of flattened stem disease was reported in petunia from Korea [8]. The flat stem is a common disease observed in *Manilkara zapota* (L.) P. Royen. Plants [9]. In this report, the flat stem disease in *T. Cordifolia* has been described. An attempt was also made to detect the actual causal organism of the flat stem disease in *T. cordifolia* by the histochemical study using Dienes' stain as suggested by Nath [10]

MATERIAL AND METHODS

The plant specimens both healthy and with flat stem symptom were collected from Kemmannugundi forest region of Bhadra Wildlife Sanctuary during July 2008. The plant materials were brought to the laboratory and plant characterized based on the manuals/flora [11, 12]. The symptoms of disease were

recorded. The flat stem specimen was fixed immediately in FAA (formalin: glacial acetic acid: alcohol, 10:05:85 v/v).

Dienes's stain (0.2%) was prepared by dissolving methylene blue (2.5g), azure II (1.25g), maltose (10g) and sodium carbonate (0.25g) in 100 ml of distilled water. The transverse sections (6-10µm thick hand sections) of both healthy and flat stem were stained with Dienes' stain for about 10 min, excess stain was removed by washing with distilled water and mounted in glycerin (1% in water), and observed under light microscope (45X) (OLYMPUS CX-31 Trinocular Microscope). Micro-photographs of the sections of flat stems were taken using OLYMPUS SP-350 digital camera.

RESULT AND DISCUSSION

The flat stem appeared in the form of a twisted green ribbon with small leaves arising from the flat regions of the stem. The leaves arose from the nodal regions at the same region. The internode region was short, as a result of which the leaves appeared to arise from a laterally extended flat rosette axis. The leaves were normal but smaller in size positioned on short petioles. A careful observation of the stem region which deviated from the normal stem revealed that the stem started showing flattening symptom at the base which continued as the stem tip grew into a green ribbon with an increasing area towards the tip. The leaves were few in number in old flat stems, and as the stem area in the flattened region expanded, more number of leaves was produced at the same region perpendicular to the flattened stem axis. The flat stem tip had as many as 20-30 young leaves arising from the same node like region. The flat stem tip appeared like the hood of a snake.

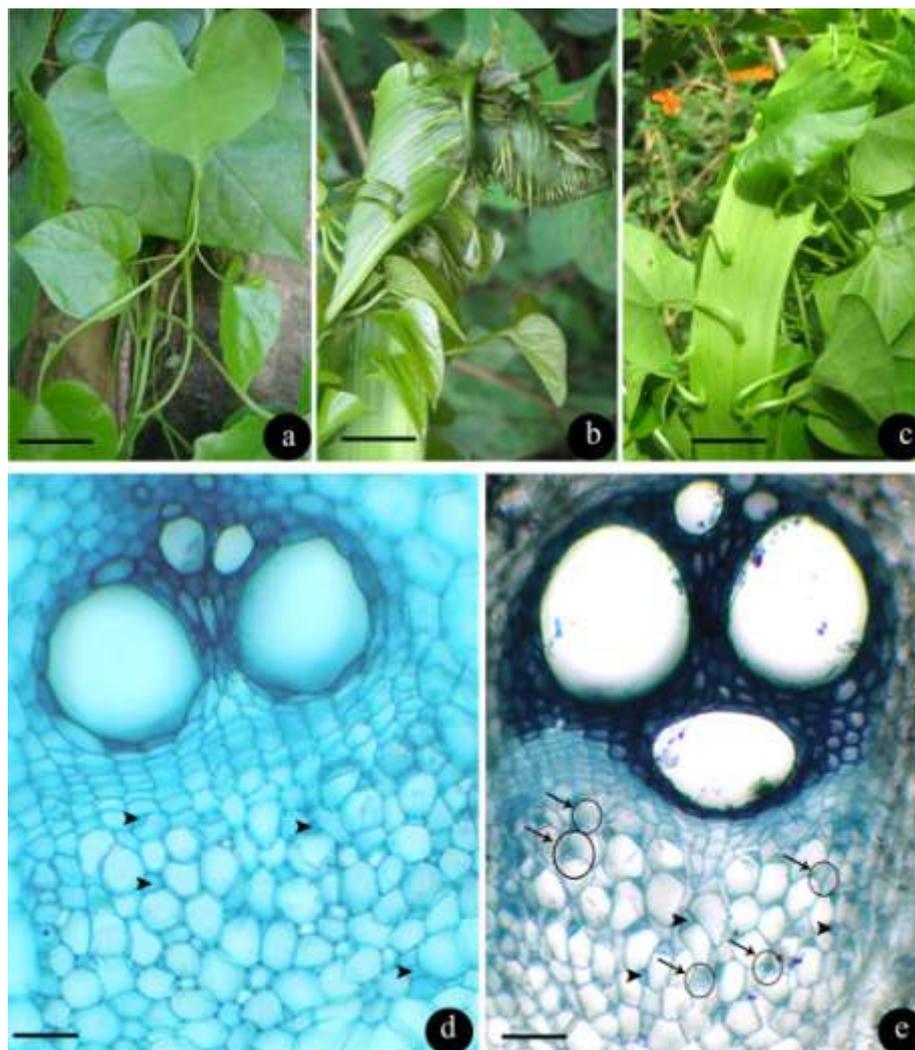


Fig 1: Flat stem disease caused by phytoplasma in *Tinospora cordifolia*

a. Healthy plant; b. flat stem symptom, note the multiple shoot tips; c. irregular arrangement of leaves on old flat stem; d. T.S. of healthy stem through vascular bundles; e. T.S. of flat stem through vascular bundles; Arrow head indicate the companion cells; arrow with circle indicate the deep blue stain in sieve cells containing phytoplasma (bar scale for a, b, c : 5cm; for d and e: 10µm)

Phytoplasmas are wall-less, minute, pleomorphic prokaryotes that inhabit phloem elements in plants [13]. Identification of the association of phytoplasma in the infected tissues by light microscopy is the fast and less expensive technique as compared to others. Since the nucleus is absent in sieve cells of phloem, it is possible to use nucleic acid specific stains to identify the localization of phytoplasma in phloem elements [14]. Dienes' stain is reported to be specific to phytoplasma in sieve elements. The presence of phytoplasma was characterized by deep blue patches of stain in the sieve elements upon Dienes' staining [10]; the xylem vessels took up turquoise and cortex light blue colour [14]. In the present study, similar discolouration of xylem, and phloem elements and cortex regions were observed (Fig 1). However, the deep blue staining of companion cells of both healthy and infected materials is because of the presence of nucleus in the companion cells as the Dienes' stains is a

nuclear stain. The deep blue patches that were detected in sections of flat stem were not observed in sections of healthy stem. This suggested that phytoplasma might be the causal organisms of the flat stem disease in *T. cordifolia*. The phytoplasma is reported to cause flat stem disease in *Lilium Oriental* hybrids [15], *Dendranthema morifolium* [16], *Crotalaria spectabilis* [17], *Euphorbia pulcherrima* [18], and *Senna surattensis* [19].

CONCLUSION

A thorough survey of literature indicated that there is no disease reported in *T. cordifolia* caused by phytoplasma and hence is the first report of the description of phytoplasma flat stem disease in *T. cordifolia*.

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