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Original Research Article

A New record of leaf spot caused by *Xanthomonas campestris* in *Tinospora cordifolia* in India

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K e y w o r d s

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A B S T R A C T

Tinospora cordifolia is an important medicinal plant extensively used in the traditional systems of medicine. A new leaf spot disease in *T. cordifolia* caused by *Xanthomonas campestris* has been observed in Bhadra Wildlife Sanctuary, Karnataka, India. The disease affected stem, leaf lamina, and midrib and occurred, particularly, during the post-monsoon months (October-November).

Introduction

Tinospora cordifolia (Willd.) Hook. f. & Thoms (Menispermaceae) is a valuable medicinal climber species and finds its application in the treatment of asthma, blood pressure, boils, bronchitis, cardiac problems, diabetes, fever, herpes, itching, malaria, sore, venereal diseases, wound, jaundice, rheumatism, urinary disorders, skin diseases, diabetes, anaemia, inflammation and allergic conditions in Ayurveda, homeopathy and traditional systems of medicine (Rajakumar and Shivanna, 2009; Shivanna and Rajakumar, 2010; Rajakumar and Shivanna, 2010; Ahmad *et al*., 2010; Shivanna and Rajakumar, 2011).

Materials and Methods

During the survey in Bhadra Wildlife Sanctuary, Karnataka, India for the foliar diseases in medicinally important climbers, *T. cordifolia* was found to be infected with irregular black spots with yellow halo on leaf lamina as well as on midrib (Fig.1), veins and in severe cases on petioles, and finally defoliation. The incubation of infected plant parts on moist blotters failed to yield any fungal fruiting bodies. Subsequently, it was presumed to be caused by bacterial pathogen. Hence, specimen samples were tested for the presence of pathogenic bacterial species as described in the bacterial identification manual (Schaad, 1980).

The infected leaves with black spots were segmented and surface disinfected with 0.1% Sodium hypochlorite solution for three minutes and washed with sterile water. The spots were cut at the center and

the ooze was taken into a droplet of water in sterilized Petri dish using a flame sterilized needle. The bacterial suspension thus obtained was used for other experiments.

Identification of genera and species

Since the bacterial pathogen could belong to any one of five common plant pathogenic bacterial genera (*Corynebacterium*, *Agrobacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*), it was identified by its colony growth on the differential medium (Schaad, 1980).

The media used for colony growth include Nutrient Glucose Agar (NGA: beef extract 3.0g/L; peptone 5.0g/L; glucose 2.5g/L; agar 15.0g/L), Yeast extract Dextrose-$CaCo_2$ agar (YDC: yeast extract 10.0g/L; dextrose 20.0g/L; calcium carbonate 20.0g/L; agar 15.0g/L), Nutrient Broth Yeast extract agar (NBY: nutrient broth 1L; yeast extract 2.0g/L; potassium phosphate 0.5g/L; di-potassium phosphate 2.0g/L; agar 15.0g/L), King's Medium 'B' agar (KB: proteose peptone 20.0g/L; di-potassium phosphate 1.5g/L; magnesium sulphate 1.5g/L; agar 15.0g/L; glycerol 15.0 ml/L), and D-1 agar medium (mannitol 15.0g/L; sodium nitrate 5.0g/L; lithium chloride 6.0g/L; calcium nitrate 0.002 g/L; di-potassium phosphate 2.0g/L; magnesium sulphate 1.5g/L; bromothymol blue 0.1g/L; agar 15.0g/L). The bacterial culture was also subjected to gram staining. The anaerobic test was done in the High and Leifson anaerobic growth media specified by Schaad (1980).

For species identification, diagnostic tests like colony growth at 35°C, protein digestion, mucoid growth, gelatin liquefaction, urease production and growth on specific media were employed. The growth test was determined in YS (Yeast Salt) broth media (ammonium phosphate 0.5 g/L; potassium phosphate 0.5 g/L; magnesium sulphate 0.2 g/L; sodium chloride 5.0 g/L; yeast extract 5.0 g/L) at 35°C for 10-12 days. The protein digestion was determined by streaking test on powdered skim milk added with 0.004% bromocresol purple. Mucoid growth was determined on YDC medium, while the gelatin liquefaction was determined using the gelatin medium (beef extract 3 g/L; peptone 5 g/L; gelatin 120 g/L) contained in test tube for a period of 7 to 14 days. Modified YS broth (ammonium phosphate 0.5 g/L; potassium phosphate 0.5 g/L; magnesium sulphate 0.2 g/L; sodium chloride 5.0 g/L; yeast extract 1.0 g/L; cresol red 0.016 g/L) was used to confirm the urease production. Finally, the species identification was confirmed by growing bacteria on the selective medium (SX agar - starch 10.0 g/L; beef extract 1.0 g/L; ammonium chloride 5.0 g/L; di-potassium phosphate 2.0 g/L; methyl violet 1.0 ml (in 20% ethanol); methyl green 2.0 ml; agar 15.0 g/L).

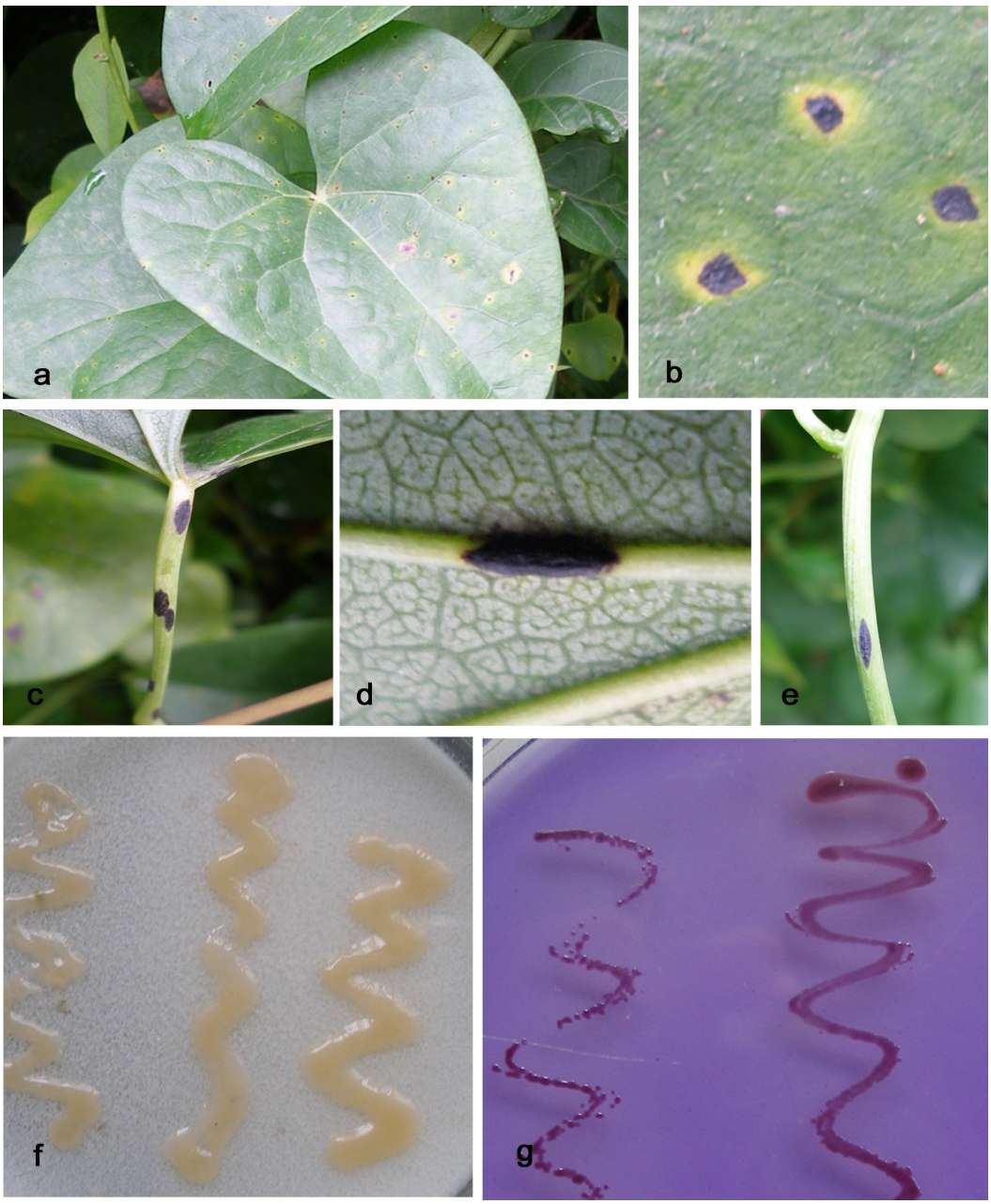
The bacterial suspension in nutrient broth was prepared and the cell load was adjusted to the turbidity of 0.08-0.1 OD at 640ηm (ELICO SL 159 UV-Vis Spectrophotometer). The apparently healthy plant parts like leaves and petioles of plants potted on sterile soil were pricked and spray inoculated with bacterial suspension. The inoculated plant was incubated in a poly-propylene cover moistened with water in dark for 48 h.

Result and Discussion

Identification of genera

The bacterial growth was observed on NGA, YDC and NBY, but not on KB and

Figure 1: Leaf spot caused by *Xanthomonas campestris* in *Tinospora cordifolia*



- **a.** *Tinospora cordifolia* leaf showing bacterial leaf spot. Note the enlarging spots
- **b.** Enlarged bacterial leaf spot
- **c.** Spots on petiole due to bacterial infection
- **d.** Midrib of leaf (under surface) showing bacterial infection
- **e.** Spots on stem due to bacterial infection
- **f.** Mucoid growth of bacterial pathogen on YDC medium
- **g.** Colony growth of *Xanthomonas* on SX agar medium confirming the identification of the pathogen as *X. campestris*

D-1 agar. The test bacterium was gram negative and hence it was not *Corynebacterium*, but could be the one among the other four bacteria. Since the test bacterium produced yellow colony on YDC medium it could be either *Erwinia* and *Xanthomonas* and not *Agrobacterium* and *Pseudomonas*, as the latter two failed to produce the yellow colony. Since the test bacterium was aerobic, the identity was confirmed as *Xanthomonas* and not *Erwinia* as latter was aerobic bacteria. The above tests confirmed that the bacterial pathogen could be *Xanthomonas*.

Identification of species

The growth test determined on YS (Yeast Salt) broth at 35°C for 10-12 days, the bacterial ability to digest casein in skimmed milk powder, mucoid growth on YDC medium (Fig. 1f) and gelatin liquefaction confirmed the identity of *Xanthomonas campestris* (Pammel) Dowson (1939).

Since *X. campestris* is negative to urease production ability in the modified YS broth media, the bacterium was presumed identified as *X.campestris*. Further confirmation of the species was determined by growing the bacterium on SX medium, a selective medium for *X. campestris*.

Pathogenicity test

The development of symptom of infection at the point of prick inoculation in plants suggested that the bacterial pathogen *X. campestris* is pathogenic capable of causing disease symptom similar to that in nature. *Xanthomonas* species are Gram-negative rod-shaped plant pathogenic bacteria reported in at least 124 monocot and 268 dicot plant hosts (Poplawsky et al., 2000). The pathovars *of Xanthomonas campestris* are reported to cause blight disease in a number of crop plants (Agrios, 2005). The bacterial leaf spot disease caused by *Xanthomonas campestris* in *T. cordifolia* is reported for the first time from India.

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References

Agrios, G. N. 2005. Plant Pathology. 5th Edition. Elsevier Academic Press, Burlington, USA

Ahmad, F., Ali, M., Alam, P. 2010. New phytoconstituents from the stem bark of Tinospora cordifolia Miers. Natural product research. 24: 926-34. Available at: http://www.ncbi. nlm.nih. gov/pubmed/20496230 [Accessed May 1, 2011].

Rajakumar, N., and Shivanna, M.B. 2010. Traditional herbal medicinal knowledge in Sagar taluk of Shimoga District, Karnataka, India. Indian J.Natural Product. Resour.1: 102-108.

Rajakumar, N., and Shivanna, M.B. 2009. Ethno-medicinal application of plants in the eastern region of Shimoga district. J. Ethnopharmacol.. 126: 64-73..

Schaad, N.W. 1980. Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society, Minnesota

Shivanna, M.B., and Rajakumar, N. 2010. Ethno-medico-botanical knowledge of rural folk in Bhadravathi taluk of Shimoga district, Karnataka. Indian J. Traditional Knowl. 9 : 158-162.

Shivanna, M.B., and Rajakumar, N. 2011. Traditional medico-botanical knowledge of local communities in Hosanagara Taluk of Shimoga District in Karnataka, India. J. Herbs.Spices Medicinal Plants. 17:291-317.