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LEAF SPOT OF BETELVINE IN PAKISTAN

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Abstract

During a three year survey (1996-99) of betelvine fields in Karachi and Thatta districts of Sindh and Hub area of Balochistan, 23 species belonging to 12 genera of fungi were isolated from leaves of betelvine. Of these, 13 fungi appear to be new records on betelvine leaf in Pakistan.

Artificial inoculation of betelvine leaves or leaf discs with *Alternaria alternata, Bipolaris australiensis, B. hawaiiensis, B. papendorfii, Botryodiplodia theobromae Curvularia lunata, C. pallescens, C. tuberculata, Exserohilum rostratum, Fusarium semitectum, F. solani*, and *F. sporotrichoides* produced necrotic spots. *Bipolaris australiensis, B. hawaiiensis, B. papendorfii, Botryodiplodia theobromae, Curvularia pallescens, C. tuberculata, F. sporotrichoides*, and *Fusarium solani* appeared to be new leaf spot pathogens of betelvine not hitherto reported. Use of Benomyl or Topsin-M showed significant reduction in leaf spot disease.

Introduction

Leaves of betelvine (*Piper betle* L.), are chewed along with areca nut as a masticator in many parts of the world. Betel chewing is considered to be a good and cheap source of dietary calcium. Pakistan imports a major portion of betel leaves for its consumers from countries like Bangladesh, India, Sri Lanka and Thailand spending more than Rupees 200 million in foreign exchange (Anon., 1990). The cultivation of betelvine started in Karachi about 21 years ago and now it is grown on a commercial scale in Karachi, Thatta districts of Sindh and Hub region of Balochistan.

The betelvine is grown in conservatories under shady and humid conditions necessary for the growth of plant. This shady and moist atmosphere also favours the development of many diseases specially leaf-spot disease (Chattopadhayay & Maiti, 1990) that greatly affect the growth of plants and produce heavy losses to the farmers. The present report describes the results on the occurrence and control of leaf-spot disease of betelvine carried out during a survey from June 1996 to July, 1999.

Materials and Methods

1.Isolation of fungi from leaves: A survey of betelvine fields in Karachi, Thatta and Hub areas was carried out where a total of 400 diseased specimens were collected from 75 fields (35 in Thatta, 25 in Karachi and 15 in Hub). Infected parts were cut into 1 cm long pieces which after surface sterilization with 1% $Ca(OCl)_2$ were placed in Petri plates containing Potato Dextrose Agar (PDA) (Potato 200 g, Dextrose 20 g, Agar 20 g per, Penicillin 100,000 units and Streptomycin 0.2 g per litre). The Petri plates were incubated at 28°C for 5-7 days and microorganisms growing on leaf pieces were identi-

fied after reference to Barnett (1960), Ellis (1971, 1976), Booth (1971), Nelson *et al.*, (1983), Thom & Raper (1945), and Domsch *et al.*, (1980). Frequency of colonization of plant parts by different fungi was recorded using the following formula:

$$\text{Colonization}\% = \frac{\text{Total no. of pieces colonized by the pathogen}}{\text{Total number of pieces assessed}} \times 100$$

2.Pathogenicity test:The isolates of fungi viz., *Alternaria alternata, Bipolaris australiensis, B. hawaiiensis, B. papendorfii, Botryodiplodia theobromae Curvularia lunata, C. pallescens, C. tuberculata, Exserohilum rostratum, Fusarium semitectum, F. solani*, and *F. sporotrichoides* were grown on PDA. Conidial suspensions of test fungi were prepared in sterilized distilled water (SDW) and pathogenicity was examined by leaf disc and petiole inoculation methods.

a) Leaf disc method: Betelvine leaf was surface sterilized with 1% $Ca(OCl)_2$, cut into 3 cm diam., discs and floated on SDW in a 9 cm diam., Petri plate. A drop of conidial suspension of a pathogen was placed in the centre of each disc. A drop of SDW was added to each disc in the control treatment. There were 3 replicates for each treatment. Petri plates were incubated at room temperature and development of necrosis was observed. Leaf discs showing necrotic symptoms were surface sterilized with 1% $Ca(OCl)_2$ and placed on PDA for reisolation of the pathogen.

b) Petiole inoculation method: A drop of conidial suspension of a pathogen was injected into the petiole of a betelvine leaf which was then placed in a 9 cm diam., Petri plate containing SDW. Leaves in control were injected with a drop of SDW. There were 3 replicates for each treatment. Petri plates were incubated at room temperature and development of necrosis was observed. Necrotic portion of leaves were cut into 1 cm^2 pieces, surface sterilized with 1% $Ca(OCl)_2$ and placed on PDA for confirmation of pathogenicity.

3. Effect of fungicides on leaf-spot disease: Plant were sprayed with 0.25 and 0.5% solution of Benomyl at 0-day and after 15 days of first application. Non-treated plants served as control. Data on disease intensity were recorded after 21 days of second application. Experiments on the control of leaf spot disease were conducted at Thatta (2 fields in 1996-97, one each in 1997-98 and 1998-99), Karachi (one field in each year) and Hub (one field in 1996-97. During 1998, Topsin-M was also used @ 0.25 and 0.5% for the control of leaf-spot in one field in Thatta. Experiments were carried out in a Randomized Complete Block design. The results were analyzed by One-Way Analysis of Variance and the means were separated by Duncan's Multiple Range Test.

Results

1.Survey of betelvine fields: A total of 23 fungi were isolated from infected leaves of betelvine (Table 1). Of these, 13 fungi (marked with asterisk are new records on betelvine leaves from Pakistan (Ghafoor & Khan, 1976; Mirza & Qureshi, 1978; Doosani *et al.*, 1992; Shahzad & Ghaffar, 1995). *Alternaria alternata, Bipolaris australiensis, Botryodiplodia theobromae, Curvularia lunata, Fusarium semitectum* and *F. solani* were more frequently isolated as compared to other fungi. The extent of damage varied

with the age of the field since 3 year old plants were more severely affected as compared to young ones. In severe cases, more than 50% of the field was affected whereas in young plantings the damage was negligible.

Table 1. Frequency of colonization of leaves of betelvine by fungi.

Fungus	Colonization %	Locality#
Alternaria alternata	5-50	1,2,3
**Aspergillus flavus*	1-5	1,2,3
**A. niger*	5-16	1,2,3
Bipolaris australiensis	5-50	1,2,3
**B. hawaiiensis*	1-3	1,3
**B. papendorfii*	5-8	1,2
B spicifera	1-5	1,2
Botryodiplodia theobromae	4-35	1,2,3
**Cephalosporium acremonium*	5-13	1,2
**Chaetomium globosum*	1-3	1,2
**Circiniella* sp.	2-3	2
Cladosporium cladosporioides	3-10	1,2
Colletotrichum capsici	3-15	1,2,3
**C. gloeosporioides*	2-10	1
Curvularia lunata	5-20	1,2,3
**C. pallescens*	3-10	1,2
C. tuberculata	3-8	1,2,3
**Exserohilum rostratum*	1-5	1,2,3
Fusarium moniliforme	1-5	1,2,3
F. semitectum	5-22	1,2,3
F. solani	5-35	1,2,3
**F. sporotrichoides*	3-10	1,2,3
**Rhizopus* sp.	3-5	1,2,3

1= Karachi, 2= Thatta, 3= Hub.

*New records on betelvine leaves in Pakistan.

It was surprising to note that the farmers carry out heavy application of chemical pesticides on betelvine plants where a mixture of insecticides and fungicides is sprayed at 10-15 days interval. In some cases, the leaves were picked up after 2-3 days of spray and chemicals were visible on the leaves ready to send to the market.

2. Pathogenicity test: *Alternaria alternata, Bipolaris australiensis, B. hawaiiensis, B. papendorfii, Botryodiplodia theobromae Curvularia lunata, C. pallescens, C. tuberculata, Exserohilum rostratum, Fusarium semitectum, F. solani* and *F. sporotrichoides* produced necrosis on betelvine leaf discs and petiole of inoculated leaves. Reisolation of inoculated fungi from the necrotic tissues confirmed their pathogenicity.

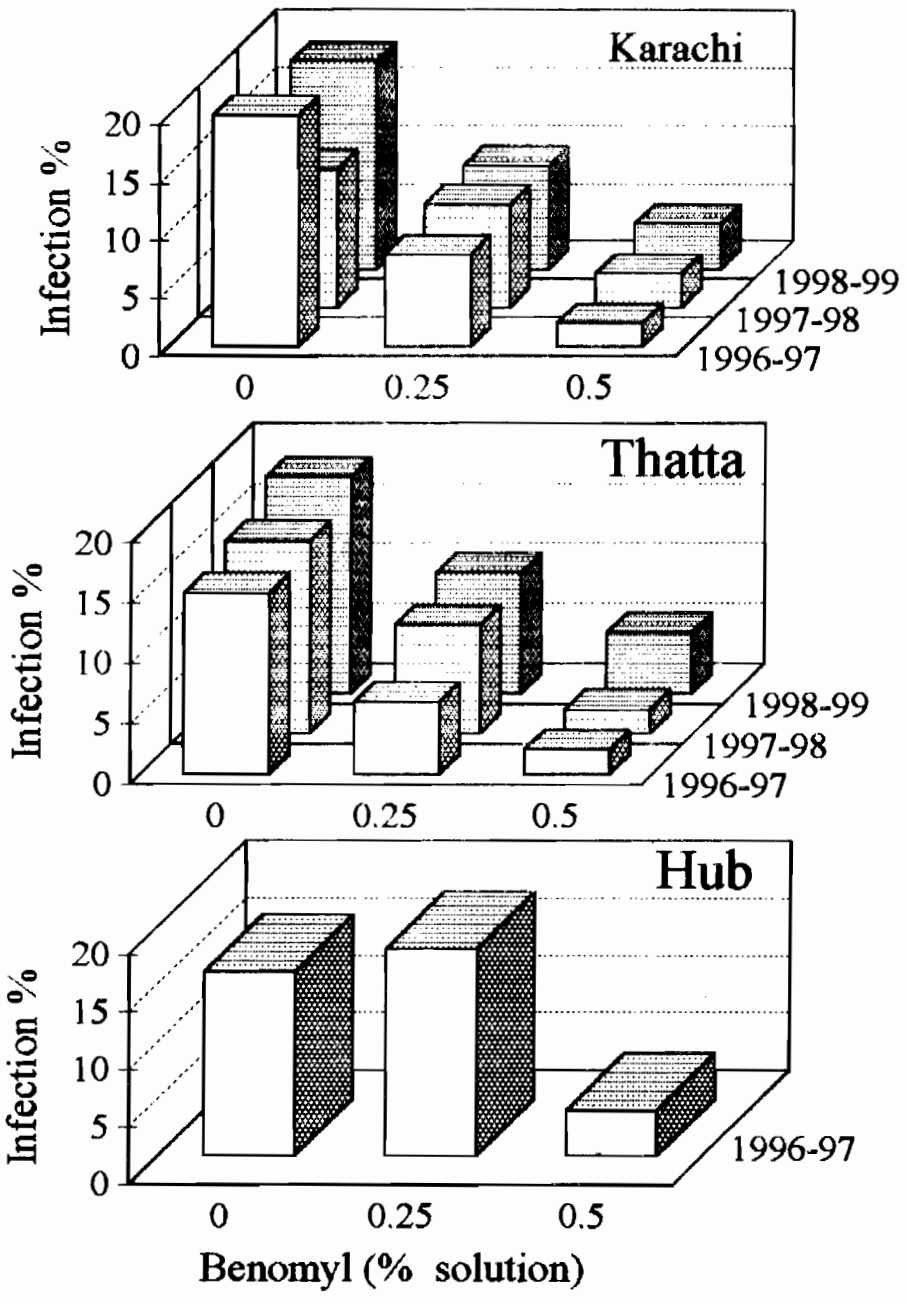

Fig. 1. Effect of Benomyl on the severity of leaf-spot disease of betelvine.

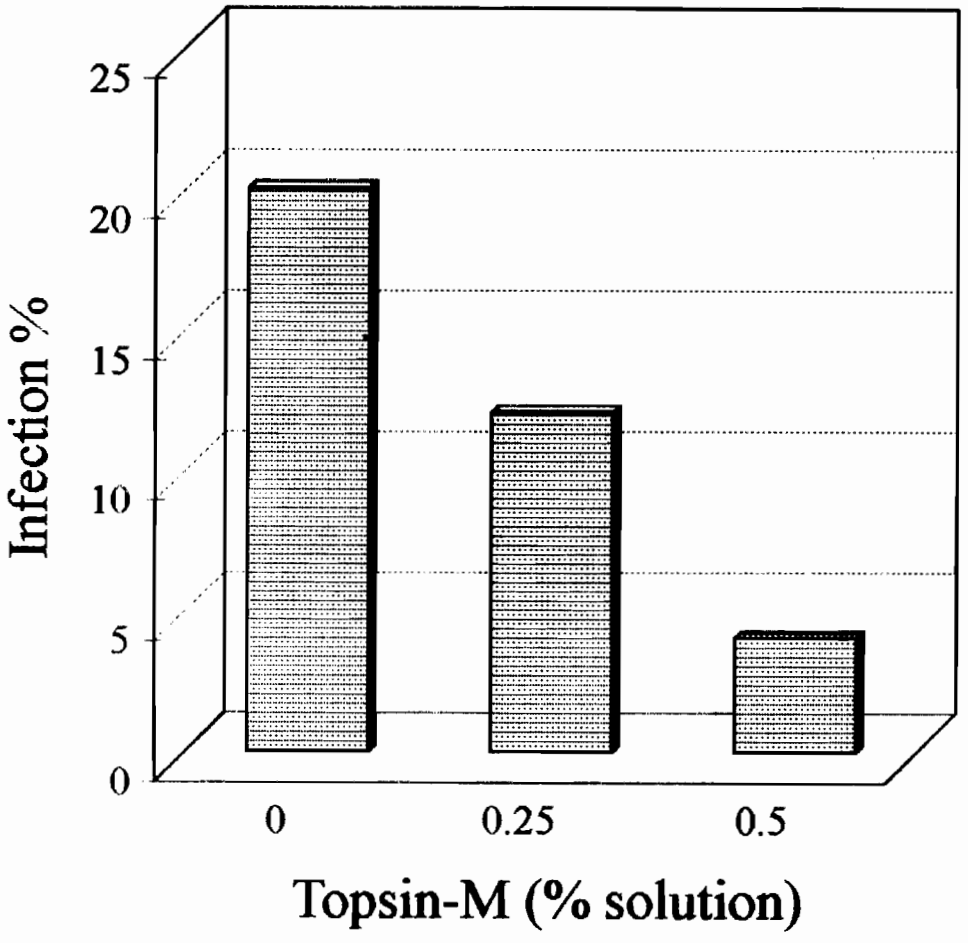

Fig. 2. Effect of Topsin-M on the severity of leaf-spot disease of betelvine.

3. Control of Leaf spot disease: During 1997-99, foliar application of Benomyl showed significant suppression ($p < 0.05$) in leaf spot symptoms at all the field locations. Use of Benomyl @ 0.5% solution gave the highest suppression (Fig. 1). Topsin-M used as foliar spray also gave significant suppression in leaf spot symptoms (Fig. 2).

Discussion

Of the fungi isolated from betelvine leaves during the present studies, *F. semitectum* has been reported to cause leaf spot on betelvine in India (Chattopadhyay & Seengupta, 1955). Similarly, *Drechslera rostrata* [*Exserohilum rostratum*] was found to cause leaf-spot in Madhya Pradesh, India (Singh & Joshi, 1974), whereas, *Alternaria*

alternata and *Curvularia lunata* have also been reported to produce leaf-spot on betelvine (Ojha *et al.*, 1988). Leaf rot caused by *Cephalosporium acremonium* was first reported from Jabalpur, India by Singh & Joshi (1973). *Fusarium solani, F. sporotrichoides, Bipolaris australiensis, B. hawaiiensis, B. papendorfii, Curvularia pallescens, C. tuberculata* and *Botryodiplodia theobromae* which were found to produce leaf necrosis on betelvine during our pathogenicity tests appeared to be new leaf spot pathogens of betelvine not hitherto reported.

Use of Benomyl and Topsin-M has shown promising control of leaf disease of betelvine during the present studies. Similar reports have been made by Maiti *et al.*, (1978) where Benomyl was found to be quite effective in the control of anthracnose of betelvine. Application of chemical fungicides is, no doubt, an effective method of plant disease control. However, indiscriminate use of fungicides could produce environmental and health hazards especially on a crop like betelvine because the leaves are chewed directly by human beings. It was surprising to note that no care is taken by betelvine farmers and the leaves sprayed with fungicides and insecticides are sent to the market with visible deposits of pesticides on the surface. There is, therefore, need to evaluate the pesticide residue in leaves and the time period require to reduce the residue level below the permissible limits before human consumption.

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